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Effect of temperature on water transport through aquaporins

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Abstract

The mean effective water self-diffusion coefficient in maize root segments under the effect of aquaporin blocker (mercuric chloride, 0.1 mM) was measured using the spin-echo NMR method with pulsed magnetic field gradient within the temperature range from 10 to 35 °C. $HgCl_2$ caused the reduction in water diffusion by 30 % as compared to the control samples. Temperature dependences of water self-diffusion coefficients showed two linear regions with different values of Q_{10} and activation energy, E_a. As the temperature reduced from 20 to 10 °C, E_a values calculated from the Arrhenius plots were close to those of bulk water (20 ± 3 kJ mol^{-1}) and slightly changed for the sample pretreated $HgCl_2$. Within the temperature range from 25 to 35 °C the slope of temperature dependences became steeper and E_a values were 31 ± 3 kJ mol^{-1} for the control and 40 ± 4 kJ mol^{-1} for the treated sample. In the vicinity of 20 °C, the temperature dependence of water diffusion *via* the mercury-sensitive water channels showed extreme value. In the region, the specific area of the mercury-sensitive aquaporins was 0.004 % of the total cell surface area. The data indicate that water transfer *via* aquaporins is sensitive to temperature, and the contributions of the transmembrane pathways (aquaporins, lipid bilayer) differ in different temperature ranges.

Additional key words: activation energy, nuclear magnetic resonance, permeability, transmembrane transfer, *Zea mays.*

Introduction

The important role of water channels (aquaporins) in the regulation of transmembrane water transfer in plant cells is generally accepted. Aquaporins function as narrow protein pores, which facilitate essentially passive movement of water molecules. It has been estimated that as much as 70 - 90 % of water moving from cell to cell passes *via* these pores (Henzler and Steudle 1995, Tazawa *et al.* 1997, Zhang and Tyerman 1999, Maurel and Chrispeels 2001). To date a large number of aquaporin genes are expressed in a wide variety of plants and plant parts. At least 31 aquaporin homologues are expressed in maize (Chaumont *et al.* 2001). A high level of expression of tonoplast aquaporin (ZmTIPI) in the endodermis and xylem parenchyma (Barrieu *et al.* 1998) and of two plasmalemma aquaporins (ZmPIP2;1 and ZmPIP2;5) in the exodermis and endodermis (Hachez *et al.* 2006) have been demonstrated in maize roots.

Conditions for opening-closure of water conducting channels are not yet studied sufficiently, but there are data that their activity is controlled by phosphorylation (Maurel *et al.* 1995, Johansson *et al.* 1998, Azad *et al.* 2004), pH changes (Tournaire-Roux *et al.* 2003, Sutka *et al.* 2005), and by calcium content (Gerbeau *et al.* 2002, Alleva *et al.* 2006). Rather fast changes in membrane water permeability under water and salt stresses, nutrient deficiency, hypoxia, heavy metals, mechanical stimuli and temperature (Zhang and Tyerman 1999, Steudle 2000, Wan *et al.* 2004, Lee *et al.* 2005a,b, Azad *et al.* 2004, Melkonian *et al.* 2004, Aroca *et al.* 2005, Ermawati *et al.* 2009) are probably connected with the aquaporin functioning.

It is generally accepted that the transport of water *via* channels is less temperature dependent and has a lower activation energy E_a than transport *via* the lipid bilayer (Finkelstein 1987, Chrispeels and Agre 1994). Low values of E_a for water transport in plant cells are considered to be one of the criteria of the aquaporin presence and participation in transmembrane water transfer. It is known from literature (Hertel and Steudle 1997, Wan and Zwiazek 1999, Gerbeau *et al.* 2002, Lee *et al.* 2005a) that E_a values for plant membranes vary within the range 17 - 25 kJ mol^{-1} and differ significantly from E_a values

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Abbreviations: D_{ef} - effective diffusion coefficient of water; DD - diffusional decay; P_d - coefficient of diffusion water permeability of membranes; R - relative echo amplitude; t_d - diffusion time.
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for the water flow through a membrane lipid bilayer which is 45 - 60 kJ mol^{-1}. An inhibition of the water transport *via* aquaporins by mercurial compounds, which react with sulfhydryl groups of channel proteins resulting in the closure of the channels, increases E_a to the level of that for transport through the lipid bilayer (Henzler and Steudle 1995, Niemietz and Tyerman 1997, Schütz and Tyerman 1997). This fact was used in the present work to find the temperature dependence of water transport *via* aquaporins of maize root cell membranes.

Up to now there are only few papers where E_a of water transmembrane flow have been investigated in plant roots (Wan and Zwiazek 1999). It is largely due to the lack of methods allowing the quantitative estimation of water flow through aquaporins since it is difficult to differentiate the contributions of different pathways of water transfer (transmembrane, symplastic and apoplastic) to the total water flow (Steudle 1997, 2000). Nuclear magnetic resonance (NMR) method is a nondestructive and informative way of measurement of membrane water permeability in intact tissue, and is widely used in the investigation of plant water transfer (*e.g.*, Van Dusschoten *et al.* 1995, Van der Weerd *et al.* 2001, Krishnan *et al.* 2004). It allows direct discriminating intra- and extracellular water signals in plant cells and tissues (Anisimov *et al.* 1998, 2004, Quigley *et al.* 2001).

The aim of the present work was to study temperature dependences of the water transport in maize root segments of control samples, and samples treated with the water channel blocker ($HgCl_2$) to detect the contribution of aquaporins to the transmembrane water transfer. One of the tasks was to estimate the specific area of aquaporin pores from the data of diffusion measurements using the NMR method with pulsed magnetic field gradient.

Materials and methods

Plant growth conditions and preparation of samples: Experiments were performed on primary roots (12 - 15 cm in length) of 7-d-old seedlings of maize (*Zea mays* L., cv. Donskaya 1) grown in hydroponic culture (¼ strength Hoagland-Arnon solution) with continuous aeration, under the 12-h photoperiod (irradiance of 200 μmol m^{-2} s^{-1}), temperature of 22 ± 2 °C and relative humidity of 60 %. The roots of intact maize seedlings were immersed for 15 min in the nutrient solution supplemented with aquaporin inhibitor 0.1 mM $HgCl_2$. Inhibitor concentration and the time of incubation were chosen according to Maggio and Joly (1995), Tazawa *et al.* (1997), Zhang and Tyerman (1999) and Ionenko and Anisimov (2007). It was shown that 0.1 mM $HgCl_2$ does not induce considerable side effects (Ionenko *et al.* 2006), is not toxic (Willmer *et al.* 1999), and does not damage membranes significantly (Wan and Zwiazek 1999).

For measuring the temperature dependence, 10 mm long segments (from the root elongation zone) of the control and previously $HgCl_2$-treated roots were used. About 20 segments were packed in a 10 mm diameter tube divided into two parts (for control and treated samples), then placed into the probe of the NMR diffusion-meter, and thermostated at 20 °C. All temperature measurements were made in two stages in order to take into account the possible changes in diffusion connected with the experiment duration. At first, water diffusion was measured at the temperatures changing from 20 to 10 °C (descend) and back to 35 °C (ascend) in 10 °C steps. Further diffusion measurements were carried out at the temperatures changing from 20 to 10 °C and back to 20 °C in 5 °C steps for one sample and from 20 to 35 °C in 5 °C steps for the other sample. The temperature in the NMR diffusion-meter probe was maintained with an accuracy of larger than ± 1 °C. The time required to adjust the temperature was 5 min. For each target temperature, the diffusion was measured at least 20 min after the temperature had stabilized. Thus, the time of water diffusion measurement of each sample was 2 - 2.5 h. Water diffusion was measured in the radial direction of the roots.

NMR measurement of water diffusion coefficients: Experiments were carried out on the spin echo NMR diffusion-meter at a frequency of 16 MHz with pulsed magnetic field gradient. Water diffusion was measured by the stimulated echo NMR technique (Tanner 1970). The specifics of the spin-echo NMR employment for the determination of water diffusion parameters were described previously in (Anisimov *et al.* 1998, Ionenko *et al.* 2006). This method is based on the recording of translational diffusive motion of water molecules over a certain diffusion time (t_d) in the sample volume marked with the magnetic field gradient. During the experiments we registered diffusion decays (DDs) of spin echo signals as a function of parameters of pulse sequence: the amplitude of magnetic field gradient pulses (g), pulse duration (δ), and the interval between pulses (t_d), conventionally called the diffusion time. For the quantitative estimation of water diffusion we determined the effective coefficient of water self-diffusion (D_{ef}). D_{ef} value was calculated as a tangent to the initial part of the DD curve using the equation:

$$R = \exp [-\gamma^2 \delta^2 g^2 (t_d - \delta/3) D_{ef}]$$

where R is the relative echo amplitude, which is equal to the ratio of echo amplitudes in the presence and absence of magnetic field gradient, A(g)/A(0); γ is the gyromagnetic ratio (the constant is equal to 2.67×10^8 T^{-1} s^{-1} for protons). The diffusion time, t_d, was chosen to be 300 ms taking into account the following reasons: *1*) to exclude the relaxational contribution to the diffusion decay of the apoplastic water, which is characterized by

short spin-lattice relaxation times (T_1 < 100 ms) and *2*) to reveal the region of hindered diffusion where the contribution of membrane permeability is significant. Moreover, previously it was shown (Ionenko *et al.* 2006, Ionenko and Anisimov 2007) that the inhibition of the diffusion by mercuric chloride was more pronounced at t_d > 100 ms.

Measurements of Q_{10} and E_a: Effective diffusion coefficients (D_{ef}) were continuously measured at the temperatures changing from 20 to 10 °C and back to 35 °C in 5 °C steps. The temperature coefficient (Q_{10}) was determined from the temperature dependence of D_{ef}. It represents the factor by which the rate of a process increases for every 10-degree rise in the temperature. In order to evaluate the activation energy (E_a) of water diffusion, the Arrhenius plots were obtained by plotting the logarithm of D_{ef} against the reciprocal of the absolute temperature (1/T). The activation energy represents the per mole difference in enthalpy of a molecule which is necessary to overcome transport barriers during its passage across the membrane. The absolute value of E_a for water flow through channels should depend on the structure of the channels and on interactions between water molecules moving along the channels, and the channel walls. In order to evaluate E_a from Q_{10} values, a modified equation (Hertel and Steudle 1997, Lee *et al.* 2005a) was used:

$$\ln Q_{10} = E_a/R \times [1/T - 1/(T+10)]$$

Determination of the aquaporin specific area: To calculate the specific aquaporin area the following relation (Holz and Finkelstein 1970) was used:

$$S = P_d L/D_p$$

where S is the pore area per 1 m^2 of the membrane surface, P_d is the coefficient of diffusion water permeability of the membrane, L is the membrane thickness, and D_p is the coefficient of water diffusion within a pore. The value of P_d is related to D_{ef} by the relation (Crick 1970):

$$1/D_{ef} = 1/D_0 + 1/(P_d \times a)$$

where D_0 is the diffusion coefficient of water in cells measured at the minimal feasible diffusion time t_d = 10 ms (1.2×10^{-9} m^2 s^{-1}), D_{ef} is the diffusion coefficient of water in cells measured at t_d = 300 ms, a is the cell diameter in transverse direction which is equal to about 25×10^{-6} m.

Statistics: All experiments were repeated for 3 - 5 samples. Each DD is an average of 5 - 7 measurements (accumulations of the echo signal amplitude). The statistic analysis was carried out by the *Origin 7.0* (*OriginLab Corporation*) for *Windows* software package. Differences between the control and variants with $HgCl_2$ were statistically significant ($P < 0.05$).

Results

The decays of the relative echo amplitude R plotted against the amplitude of gradient pulses g^2 (*i.e.*, diffusion decays, DDs) were non-exponential for maize root segments (Fig. 1). DD dynamics is inherent in the translational diffusion of water molecules restricted within compartments with permeable walls and also in the relaxational redistribution of contributions of various water fractions (apoplast, symplast) to the echo signal. The 15-min treatment of roots with 0.1 mM $HgCl_2$ slowed down the diffusion decay and decreased, correspondingly, the water diffusion transfer by about 30 % as compared to the control (Fig. 1). The inhibition of water diffusion was reversible by a 15 min exposure of $HgCl_2$-treated roots in 5 mM β-mercaptoethanol. In agreement with previous results (Maggio and Joly 1995, Maurel *et al.* 1997, Tyerman *et al*. 1999), the reversibility points to the absence of any essential side effects caused by the $HgCl_2$ treatment.

Since the temperature measurements took a sufficiently long time, there were doubts that $HgCl_2$ might cause irreversible changes in the membrane structure (permeability). Therefore, firstly the dynamics of changes in diffusion coefficients for control and pretreated sample were measured (for 5 h). The relative value of $D_{ef\ Hg}/D_{ef\ contr}$ after the $HgCl_2$-treatment slightly varied for the first 3 h (by about 2 %) and after this period it increased by about 5 % compared to the initial inhibition (Fig. 2). To exclude possible changes in D_{ef} connected with the experiment duration, the temperature measurements were carried out in two stages (see Materials and methods). Since the samples differed in the absolute value of D_{ef}, we normalized D_{ef} values using the

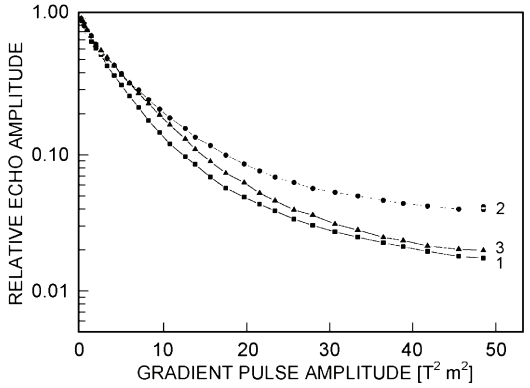

Fig. 1. Diffusional decays of relative echo signal amplitude, R, *versus* gradient pulse amplitude, g^2, for maize roots at diffusion time t_d = 300 ms for control (*1*) and sample treated by 0.1 mM $HgCl_2$ (*2*) and consecutively treated by 0.1 mM $HgCl_2$ and 5 mM mercaptoethanol (*3*). Water diffusion coefficients (D_{ef}) equal to 3.6×10^{-10} m^2 s^{-1} for control, 2.8×10^{-10} m^2 s^{-1} for sample treated by $HgCl_2$, 3.5×10^{-10} m^2 s^{-1} for sample consecutively treated by $HgCl_2$ and mercaptoethanol (the temperature was 20 °C). The curves are an average of 7 measurements of the echo signal amplitude.

D_{ef} of control samples at 20 °C as the reference value. This allowed the direct comparison of data for all measured samples.

For relatively narrow temperature intervals, where the measurements were carried out without cell damage (10 - 35 °C), D_{ef} values increased with the increase in temperature (Fig. 3). The Q_{10} values were obtained from non-logarithmic plots (Fig. 3*A*). The Arrhenius plots of the logarithm of D_{ef} versus 1/T of both control and $HgCl_2$-treated roots (Fig. 3*B*) had linear portions between 20 and 10 °C and between 25 and 35 °C. E_a values

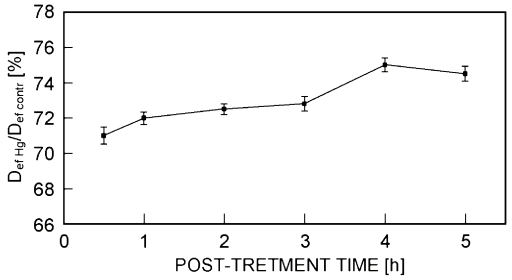

Fig. 2. Relative values of water diffusion coefficients, $D_{ef\ Hg}/D_{ef\ control}$, in maize roots as a function of time elapsed after $HgCl_2$ treatment (0.1 mM, 15 min) at the temperature of 20 °C. *Bars* show SE (n = 5).

Table 1. Q_{10} and E_a values of water diffusion in maize roots at various temperature intervals. Q_{10} values were obtained from non-logarithmic plots given in Fig. 3*A*. E_a values were calculated from the slopes of linear portions of the graphs in Fig. 3*B*.

Temperature [°C]	Sample	Q_{10}	E_a [kJ mol^{-1}]
20 - 10	control	1.3 ± 0.2	20 ± 3
	$HgCl_2$-treated	1.2 ± 0.1	16 ± 2
10 - 20	control	1.4 ± 0.2	25 ± 3
	$HgCl_2$-treated	1.3 ± 0.1	20 ±2
25 - 35	control	1.5 ± 0.2	31 ± 3
	$HgCl_2$-treated	1.6 ± 0.2	40 ± 3
	bulk water	1.25	18

obtained from the slopes of linear portions of the graphs differed in various temperature intervals (Table 1). The mean Q_{10} and E_a values of D_{ef} in the temperature range of 20 - 10 °C for the control and the $HgCl_2$-pretreated samples are similar to those for the bulk water and close to the literature data obtained for water passage across cell membranes (Hertel and Steudle 1997, Tyerman *et al.* 1999, 2002, Javot and Maurel 2002, Lee *et al.* 2005a). As the temperature increased from 25 to 35 °C, Q_{10} and E_a values of D_{ef} for root segments increased much more than those for the bulk water and varied for the control and the treated samples. The low and high temperature portions of the Arrhenius plot intersected at 20 °C. More appreciable change of the slope of the Arrhenius plot under the influence of the blocker was at temperatures from 20 to 25 °C.

In order to separate the component of water transfer *via* aquaporins, the temperature dependence of the difference of two curves in Fig. 3*A* was plotted. While plotting this dependence (Fig. 4) we took into account that the mercuric inhibition of D_{ef} resulted from blocking of the water channels. The increase in the diffusion at the temperature of about 20 °C exceeded 1.5 times the increase in the diffusion of bulk water caused by the temperature increase (within the interval from 10 to

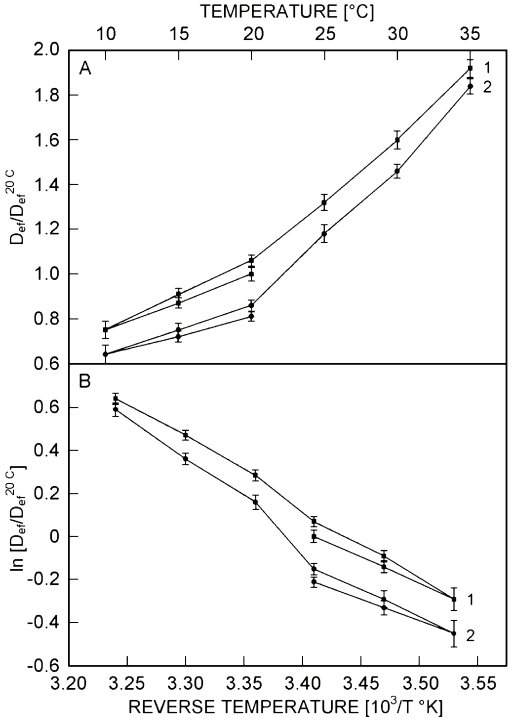

Fig. 3. Temperature dependences of water diffusion transfer for the control (*1*) and $HgCl_2$-pretreated (*2*) maize roots. D_{ef} was measured in temperatures descending to 10 °C followed by ascending temperatures to 35 °C. In *A*, D_{ef} values were normalized using the D_{ef} at 20 °C as the reference value. Q_{10} values were evaluated from plots given in *A*. From Arrhenius plots of normalized D_{ef} values (*B*), activation energies E_a were calculated. *Bars* show SE (n = 6).

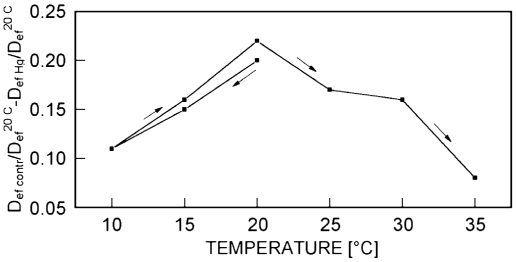

Fig. 4. Temperature dependence of water diffusional transport mediated by mercury-sensitive aquaporins. The dependence was obtained from the difference of two curves in Fig. 3*A*. *Arrows* show the direction of the temperature change.

20 °C the values of Q_{10} for water flow *via* aquaporins and for the bulk water diffusion equaled 2.0 and 1.25, respectively).

An estimation of the specific aquaporin area (S) was carried out from diffusion measurements at 20 °C where the maximum of water transfer *via* aquaporins was observed. We took into account the increment, ΔP_d (0.8×10^{-5} m s^{-1}), *i.e.*, the difference between the coefficients of the diffusion water permeability of membranes (P_d) of the control and the $HgCl_2$-treated samples. P_d values equal $2.2 \pm 0.2 \times 10^{-5}$ m s^{-1} for the control and $1.4 \pm 0.15 \times 10^{-5}$ m s^{-1} for $HgCl_2$-treated samples. Assuming that water within a pore has approximately the same diffusion coefficient as bulk water ($D_p = 2.2 \times 10^{-9}$ m^2 s^{-1}), and the membrane thickness, L, is approximately 10^{-8} m, then the area occupied by the $HgCl_2$-sensitive aquaporins per 1 m^2 of the membrane surface equals 3.6×10^{-5} m^2. This value is about 0.004 % of the total area of the cell surface. Actually, the area of water channels might be larger since the value obtained in calculations is related to the channels sensitive to $HgCl_2$.

Discussion

In the present study, we used the spin-echo NMR method with pulsed magnetic field gradient to study the effects of $HgCl_2$-pretreatment on the diffusion of water, and temperature dependences of water movement in root segments of maize seedlings. The temperature dependence of the effective self-diffusion coefficient (D_{ef}) of water was measured in the temperature range from 20 to 10 °C and back to 35 °C in 5 °C steps. It should be noted that since the diffusion time, t_d, for temperature measurements was chosen to be 300 ms (see Materials and methods), D_{ef} values referred mainly to the intracellular water restricted by membrane structures. The obtained temperature dependences revealed the regions with high and low values of Q_{10} and E_a. In the range of (20 - 10 °C) these values were similar to those for the bulk water. A low E_a value (< 25 kJ mol^{-1}) for water transport is the typical feature of membranes with water-transporting pores (Finkelstein 1987, Chrispeels and Agree 1994, Maurel *et al.* 1997), while transport through the membrane lipid bilayer is associated with a high E_a value (> 40 kJ mol^{-1}). Low E_a values for water transport through the water channels have been interpreted by the fact that, during its passage through aquaporins, water molecules moves in a surrounding similar to bulk water (Hertel and Steudle 1997, Lee *et al.* 2005a). The formation and breakage of hydrogen bonds between water and polar groups of pore walls should require similar energy as between water molecules in bulk water. The difference in E_a values at different temperature intervals may be related to a variable contribution of the water channel pathway as compared with other parallel pathways with different E_a.

The region with high sensitivity of D_{ef} to temperature on Arrhenius plots differed for the control and $HgCl_2$-pretreated samples. The literature data indicate that the Hg can increase E_a of water transport facilitated by water channels (Wayne and Tazawa 1990, Henzler and Steudle 1995, Niemietz and Tyerman 1997). Under the influence of $HgCl_2$, the energy barrier to water movement increased to 40 kJ mol^{-1} in the temperature range 25 - 35 °C. A more pronounced temperature dependence for $HgCl_2$-pretreated roots (Q_{10} = 1.6) is characteristic for water transport through lipid bilayers and is connected to phase transitions of membrane lipids as the temperature increases (Lee *et al.* 2005a). The significant change in the slope of the Arrhenius plot on Fig. 3*B* in the range of 20 - 25 °C for $HgCl_2$-pretreated roots suggests that the water channels significantly contribute to water transport only within the optimum temperature region.

In the region of the temperature reduction from 20 to 10 °C, E_a values of control and pretreated roots were slightly different. This finding can be related to the fact that both the water transferred through membranes and the water in the symplast with low values of E_a contribute to the measured D_{ef}. The apoplast water does not contribute since its relaxation time is much less that 300 ms and by this time it becomes invisible in the diffusion measurements. In this connection it would be of great interest to separate the component of water movement particularly *via* aquaporins. The temperature dependence of water diffusion *via* the Hg-sensitive aquaporins (Fig. 4) indicates that the maximum of water transfer *via* water channels is at 20 °C. The reduced flow rate at low temperatures can be explained by the increase in viscosity of water within the channels. The dramatic suppression of the diffusion in the region from 30 to 35 °C is most likely to be connected to the water channel closure (changes in channel conformation).

The maximum diffusion at temperature of about 20 °C is of the most interest. The increase in the diffusion in this region exceeds by 1.5 times the increase in bulk water diffusion as the temperature increases. The enhanced water diffusion coefficient was observed earlier in the apoplast of maize roots within the temperature range from 18 to 20 °C (Anisimov *et al.* 2004) and it was related to the possibility of initiation of the compensatory water flow along the symplast. Anyway, the presence of maximum diffusion at optimum temperature points to the possibility of regulation of water transfer *via* $HgCl_2$-sensitive aquaporins by temperature. Presently there are a great number of data showing the possibility of regulation of aquaporin activity in response to water and salt stresses, low temperature, pressure pulses, ABA, *etc*. (Steudle 2000, Tyerman *et al.* 2002, Ye *et al.* 2004, Lee *et al.* 2005a,b, Wan *et al.* 2004). Possible mechanisms of regulation are discussed from the point of view of

changes in conformation of water channel proteins (Wan *et al*. 2004, Ye *et al*. 2004).

Thus, our results indicate that water diffusion *via* $HgCl_2$-sensitive aquaporins is temperature-sensitive. In different temperature intervals the contribution of concurrent membrane pathways to water transport (protein channels, lipid bilayer) changes. Within the region of optimum temperatures, the water transfer *via* aquaporins is preferential. At the higher temperatures (above 25 °C), the contribution of the transport through lipid bilayer increases.

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