Protoplasma (2015) 252:1263–1273
DOI 10.1007/s00709-015-0758-9

ORIGINAL ARTICLE

Membrane water permeability of maize root cells under two levels of oxidative stress

G. A. Velikanov · T. A. Sibgatullin · L. P. Belova · I. F. Ionenko

Received: 21 October 2014 / Accepted: 2 January 2015 / Published online: 18 January 2015

Abstract Changes in the total water permeability of two cell membranes (plasmalemma and tonoplast), estimated by the effective diffusion coefficient of water (D_{ef}), were controlled using the NMR method. The time dynamics of D_{ef} in maize (*Zea mays* L.) root cells was studied in response to (i) root excision from seedling and the following 6-h incubation in the growth medium (wound stress) and (ii) the superposition of wound stress plus paraquat, which induces the excess of reactive oxygen species (ROS). The dynamics of lipid peroxidation, oxygen consumption, and heat production was studied to estimate general levels of oxidative stress in two variants of experiments. Under wound stress (the weak oxidative stress), the reversible by dithiothreitol increase in cell membrane water permeability was observed. The applicability of mercury test to aquaporin activity in our experiments was verified. The results of wound stress effect, obtained using this test, are discussed in terms of oxidative upregulation of aquaporin activity by ROS. The increase of oxidative stress in cells (wound–paraquat stress), contrary to wound stress, was accompanied by downregulation of membrane water permeability. In this case, ROS is supposed to affect the aquaporins not directly but via such processes as peroxidation of lipids, inactivation of some intracellular proteins, and relocalization of aquaporins in cells.

Keywords Aquaporins · Membrane permeability · Nuclear magnetic resonance · Oxidative regulation · Reactive oxygen species · *Zea mays*

Handling Editor: Friedrich W. Bentrup

G. A. Velikanov · T. A. Sibgatullin · L. P. Belova · I. F. Ionenko (✉)
Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, P.O. Box 30, Kazan, Russia 420111
e-mail: ionenko@kibb.knc.ru

Abbreviations

D_{ef}	Effective diffusion coefficient of water
DD	Diffusional decay
t_d	Diffusion time
ROS	Reactive oxygen species
POL	Peroxidation of lipids

Introduction

The processes of transmembrane water transfer in plant cells attract the increasing attention of researchers due to the discovery in cell membranes of the large family of integral proteins—aquaporins, which can regulate the passive transfer of water and some low molecular electroneutral compounds (Maurel 1997, 2007; Johansson et al. 2000; Verkman and Mitra 2000; Chaumont and Tyerman 2014). The wide range of selectivity profiles and regulation properties allows the aquaporins to participate in many processes of plant development and adaptation to variable environmental conditions (Maurel et al. 2008). Under stress, the contribution of the transmembrane pathway to the total water flow through the plant might become considerable (Steudle and Herzler 1995).

Various molecular mechanisms are involved into the regulation of plant aquaporin activity. These mechanisms have been studied poorly, though a number of aquaporin amino acid residues were identified to be responsible for sensitivity of water permeability of these integral proteins to phosphorylation–dephosphorylation, to their oligomerization in the membrane, and to the cytoplasmic level of pH and Ca^{2+} (Chaumont et al. 2005; Zhao et al. 2008; Bienert et al. 2012; Prado et al. 2013; Chaumont and Tyerman 2014). Some other intracellular and external factors, such as heavy metals, nutrients, temperature, cell turgor, solute gradients, and reactive oxygen species (ROS), were shown to be able to modify

aquaporin activity (Preston et al. 1993; Daniels et al. 1996; Clarkson et al. 2000; Henzler and Steudle 2000; Niemietz and Tyerman 2002; Lee et al. 2004; Chaumont et al. 2005; Boursiac et al. 2008; Chaumont and Tyerman 2014).

Accumulation of the excess ROS in plant cells resulting from the disturbance of the normal equilibrium is one of the earliest responses to any biotic and abiotic stress (Minibayeva et al. 1998; Hernandes et al. 2001). Under stress conditions, the production of free oxygen radicals by mitochondria increases, and first of all, there is accumulated superoxide anion radical, then the product of its dismutation—hydrogen peroxide, and finally, the most toxic hydroxyl radical. The increase in ROS production in plants under stress can also result from the activation of the plasmalemma NADPH oxidase (Sagi and Fluhr 2006) and/or extracellular peroxidase (Minibayeva et al. 2001; Garrido et al. 2012).

Proteins are one of the most ROS-sensitive targets in cells (Stadtman 1992), which can bind from 50 to 75 % of oxygen radicals. The X-ray structural analysis of the glycerol transporter from bacteria (Fu et al. 2000) and of erythrocyte membrane water channel (Murata et al. 2000) showed the large degree of similarity of molecular structure of all aquaporins. The aquaporin topographic model based on these data includes six transmembrane domains which form a kind of a keg in the membrane due to inter-domain loops consisting of amino acid residues. Two submersed into the lipid bilayer inter-domain loops, containing highly conserved asparagine–proline–alanine (NPA) motifs, and participated directly in the formation of a water channel. These two loops are folded into the bilayer from the opposite sides of the membrane and create inside the membrane an hourglass-like structure. This transmembrane domain forms a water pore (Daniels et al. 1996; Maurel 2007). For most aquaporins, in particular tonoplast aquaporins, a conserved cysteine residue containing a thiol group is located immediately at the water pore zone. This residue is believed to be in charge of aquaporin activity and aquaporin sensitivity to mercury compounds (Preston et al. 1993; Hachez and Chaumont 2010). Highly conserved cysteine residues with thiol groups are also located in other structural sites of aquaporins, including hydrophilic loops on both sides of a membrane (Daniels et al. 1996; Kukulski et al. 2005; Zhao et al. 2008; Bienert et al. 2012). From analysis of the literature data, a complex picture of the relationship between cysteine residues and mercury response in plant aquaporins emerges (Daniels et al. 1996; Frick et al. 2013). Mercury binds to three out of four cysteine residues (Frick et al. 2013). Thus, there is admitted the existence of one more way of aquaporin functional state regulation, based on changes in thiol group redox status of these structural sites. It is likely that some questions concerning the effect of oxidative stress and ROS on aquaporin functional activity should be related to redox processes of thiol groups of these proteins. The high conservatism of cysteine residues points to the possibility of their participation in aquaporin activity regulation by affecting the level of aquaporin oligomerization in membranes and/or transitions between open–closed states of water channels via formation of inter- or intra-molecular disulfide bonds, correspondingly (Bienert et al. 2012).

Currently, the reversible dithiol-disulfide transitions in proteins are actively studied, since the redox regulation is assigned to have a leading role in endogenous regulation mechanisms of vital processes (Berczi and Moller 2000; Gelhaye et al. 2005; Minibayeva et al. 2009, 2012; Bienert et al. 2012). Earlier, the attempts were made in experiments in vitro to confirm the role of such transitions in transmembrane water transport via aquaporins (Ampilogova et al. 2006; Zhestkova et al. 2009). In order to simulate the effect of endogenous redox regulators, the authors of these papers studied the effect of exogenous oxidizing or reducing thiol group agents (diamide, dithiothreitol, tributylphosphine) on the process of osmotic shrinkage of plasma membrane vesicles. The data obtained from in vitro experiments (Ampilogova et al. 2006; Zhestkova et al. 2009) testify in favor of the possibility of aquaporin water permeability regulation due to changes in the status of their thiol groups by endogenous ROS.

To date, there are numerous studies of changes in root hydraulic conductivity under exogenous factors, which are able to cause ROS accumulation (e.g., Boursiac et al. 2008; Ehlert et al. 2009). Using a cell pressure probe, hydraulic properties (half-time of water exchange, inversely proportional to water permeability) of parenchyma cells in the midrib tissue of maize leaves have been measured (Kim and Steudle 2007). This methodical approach was also used to estimate the effect of exogenous ROS on cell hydraulic permeability. Results were discussed in terms of an oxidative gating of aquaporins by ROS (Kim and Steudle 2009). The contribution of aquaporin-mediated water transport in the experiments in vivo is estimated mostly using mercuric inhibitors (Javot and Maurel 2002; Bramley et al. 2009; Hachez and Chaumont 2010). To date, however, there are studies demanding caution while using mercury test to estimate the aquaporin role in water membrane permeability regulation (Frick et al. 2013; Chaumont and Tyerman 2014).

The NMR method with a pulsed magnetic field gradient proved to be successful in studies of water diffusional transport in biological samples, such as cells and tissues (Anisimov et al. 1998; Cho et al. 2003; Duval et al. 2005; Van As 2007). This method allows the non-invasive registration of water self-diffusion and separation of contributions of different water transport pathways in plant tissues (Ishida et al. 2000; Anisimov et al. 2004; Velikanov 2007; Ionenko et al. 2012). It was applied for plant roots to study mercury-induced changes in the permeability of cell membranes in intact tissue (Ionenko et al. 2003, 2012; Volobueva et al. 2004; Ionenko and Anisimov 2007).

In the present study, the changes in the total water permeability of cell membranes (plasmalemma and tonoplast) in maize seedling roots under two levels of oxidative stress were analyzed by the NMR method.

Materials and methods

Plant growth conditions and preparation of samples

Maize (*Zea mays* L., cv. Mashuk) seedlings were grown hydroponically in 0.25 mM $CaCl_2$ (pH 6.3) at 22 °C for 5 days under the 16-h photoperiod (irradiance of 15 W m^{-2}) and relative humidity of 60 %. Ten-millimeter-long root segments from the elongation zone, cut with a blade, were used in the experiments. The root segments (300 mg) were then incubated for 1 to 6 h with moderated shaking (gentle aeration) in (i) 0.25 mM $CaCl_2$ (6 ml, pH 6.3), wound stress variant, and (ii) 0.25 mM $CaCl_2$ plus 100 μM paraquat (6 ml, pH 6.3), wound–paraquat stress variant. In all experiments, the incubation of roots was started directly after excision. The excision of roots from seedlings (wound stress) and the subsequent incubation activated root cells to produce oxygen radicals (Minibayeva et al. 1998, 2001, 2009, 2012).

Diffusion measurements by pulsed field gradient NMR

After incubation, the root segments were gently wiped with a filter paper. Forty-five to 50 arranged in parallel segments were placed into a test tube for NMR measurements. Experiments were carried out at 25 °C on the time domain ^{1}H NMR analyzer "Spin Track" (Resonance Systems Ltd., Yoshkar-Ola, Russia) operating at 19.1 MHz and equipped with an electromagnet (Bruker, Karlsruhe, Germany). Pulsed field gradient sequences were used to measure the translational diffusion coefficients (Stejskal and Tanner 1965). The pulsed magnetic field gradient was applied perpendicularly to the test tube. It allowed the observation of water self-diffusion in a cross direction of the root. It is in the radial direction that the main water transporting role of aquaporins occurs (Maurel et al. 2008).

During the experiments, we registered diffusional decays (DD) of spin echo signals as a function of parameters of the pulse sequence: the amplitude of magnetic field gradient pulses (g), pulse duration (δ), and interval between pulses (t_d), conventionally called the diffusion time. DD of the echo is expressed as

$$R = \exp\left[-\gamma^2\delta^2 g^2\left(t_d - \delta/3\right)D\right] \quad (1)$$

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 proton gyromagnetic ratio (the constant is equal to 2.67×10^8 T^{-1} s^{-1} for protons); and D is the self-diffusion coefficient.

Diffusional decays were obtained while changing the values of g up to 3 T m^{-1} with fixed values of δ of 350 μs and t_d of 700 ms. An initial part of DD (at $g \rightarrow 0$) was fitted with Eq. 1, resulting in an average over a sample value of water diffusion coefficient.

For quantitative description of the experimental results, we employed the formalism of effective coefficient of water diffusion (D_{ef}) (Cooper et al. 1974; Anisimov et al. 1998). The formalism D_{ef} is based on the fact that cell membranes significantly restrict the mobility of water molecules when the distances travelled by molecules become comparable with the cell size. As a result, the measured value of D_{ef} is less than the self-diffusion coefficient of bulk water. The decrement of D_{ef} directly correlates with the decrement of water permeability of cell membranes, which restrict the diffusional process (Cooper et al. 1974; Anisimov et al. 1998). The longer is the diffusion time, the stronger is the effect of the decrease in D_{ef}.

The relaxation time of water molecule magnetic induction in root tissue is a factor that determines the maximum possible, in the NMR experiment, time t_d. In plant tissues, the longitudinal (along the steady-state magnetic field) relaxation time (T_1) of magnetization is larger than the transverse one (T_2). We used the three-pulsed sequence (Tanner 1970) for diffusion measurements because in this case, the limitation of t_d is determined by T_1. The heterogeneity of a plant cell on a microlevel is the reason for differences in relaxation times of water molecules which are located in different cell compartments. For mature cells of higher plants, these differences are most contrast between an apoplast and a large central vacuole. The fast component of the magnetic induction multiexponential decay was attributed to the apoplast water, and the slowest component with the maximum population was attributed to the cell vacuole water (Van Dusschoten et al. 1995; Van der Weerd et al. 2001, 2002; Velikanov 2007; Sibgatullin et al. 2007; Ionenko et al. 2012). Eighty to 85 % of a cross section area of root segments used in the experiments consists of large (with a diameter of about 40 μm) strongly vacuolated cortical cells (Fig. 1). At t_d equal to 700 ms, the echo signal of the root is formed mostly by water molecules of the central vacuoles of cortical cells (Sibgatullin et al. 2007; Velikanov 2007; Ionenko et al. 2012), and the displacement of water molecules becomes comparable with the diameter of cortical cells. As a result, the measured D_{ef} directly correlates with the overall water permeability of a barrier between vacuoles of adjacent cells, namely, the tonoplast, plasmalemma, and thin cytoplasm layer (Fig. 1b). At short t_d (1–2 ms), the water self-diffusion in the cytoplasm does not differ from the bulk water self-diffusion, and at long fixed t_d (700 ms), changes in D_{ef} might be caused only by membrane barrier properties (Crick 1970). Additional

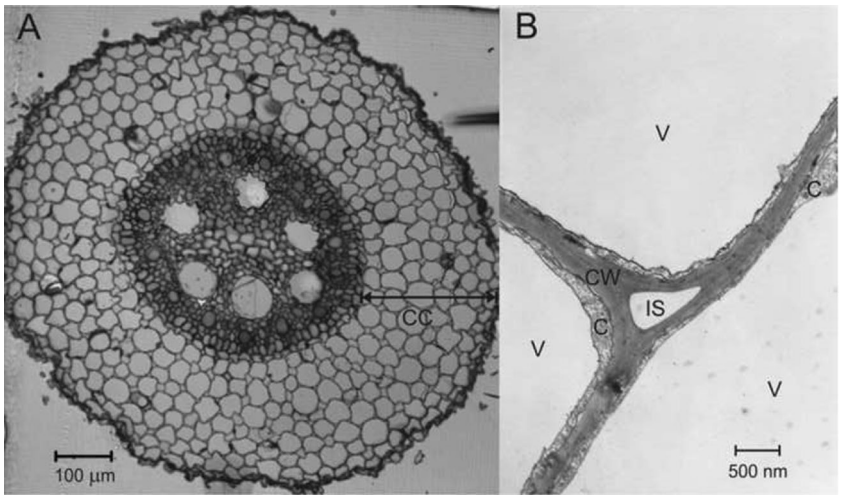

Fig. 1 A transverse section of maize root cuts from the elongation zone. Most of the transverse section area is occupied by cortex cells (**a**). A typical view of cortex cells at a higher magnification obtained by electron microscopy. Diffusion of water molecules between adjacent cell tonoplasts is restricted by two membranes (tonoplast and plasmalemma) and a thin layer of cytoplasm between them (**b**). Key to the lettering in the figures: *CC* cortex cells, *CW* cell walls, *V* vacuole, *C* cytoplasm, *IS* intercellular space

evidence in favor of correctness of the applied method is presented in the "Results" section.

Calorimetric measurements

Metabolic heat production of root segments was registered with differential dark microcalorimeter LKB-2277 (Bio Activity Monitor, Sweden). The root segments (50 mg) were submerged into 1 cm^3 of 0.25 mM $CaCl_2$ solution (wound stress variant) or 1 cm^3 of 0.25 mM $CaCl_2$ plus 100 μM paraquat solution (wound–paraquat stress variant) in the calorimetric test tube with the volume of 3 cm^3. The time of the sample temperature stabilization prior to the isothermal (25 °C) registration of heat production was 30 min.

Registration of oxygen consumption

Oxygen consumption was registered with manometric method in a Warburg apparatus (Myers and Matsen 1955). The excised root weights of 150 mg each were placed into Warburg vessels with 3 cm^3 of 0.25 mM $CaCl_2$ (wound stress variant) or with 3 cm^3 of 0.25 mM $CaCl_2$ plus 100 μM paraquat (wound–paraquat stress variant). After 10-min temperature stabilization, the oxygen consumption was registered every hour at the temperature of 25 °C.

Determination of lipid peroxidation level

The most general indicator of the development of oxidative stress caused by the accumulation of the excessive amount of ROS is the state of peroxidation of lipids (POL), which was judged by the amount of produced malondialdehyde (MDA). The contents of MDA were determined by a method (Heath and Packer 1968) based on the formation of a colored complex of MDA and thiobarbituric acid during heating. Root samples were fixed with liquid nitrogen. The frozen samples were stored at −84 °C before the measurements.

Electron microscopy

The conventional technique of chemical fixation of root segments described earlier was used (for details, see Velikanov et al. 2011). Preparations were examined in a JEM-1200EX electron microscope (JEOL Ltd., Japan).

Statistics

The NMR experiments were repeated for three to five samples. The time of registration of one DD in NMR measurements was determined by the number of accumulations of echo signals (four to five accumulations) and was about 10–15 min. Other experiments (determination of oxygen consumption, heat production, lipid peroxidation level) were performed in five recordings each. The statistical analysis was carried out using the OriginPro 7.0 (OriginLab Corp., Northampton, MA, USA) software.

Chemicals

β-Mercaptoethanol (β-ME), dithiothreitol (DTT), thiobarbituric acid, and paraquat were purchased from Sigma Chemicals, USA.

Results

The time dynamics of D_{ef} changes in response to the excision of maize roots from seedlings and following incubation in 0.25 mM $CaCl_2$ during 1, 2, 3, or 6 h is shown in Fig. 2 (wound stress variant). With the increase in incubation time, the value of D_{ef} first increased from the initial value at the moment of excision to the maximum by the second to the third hour of incubation and then reduced a little by the sixth hour. In order to verify whether the observed increase in D_{ef} is

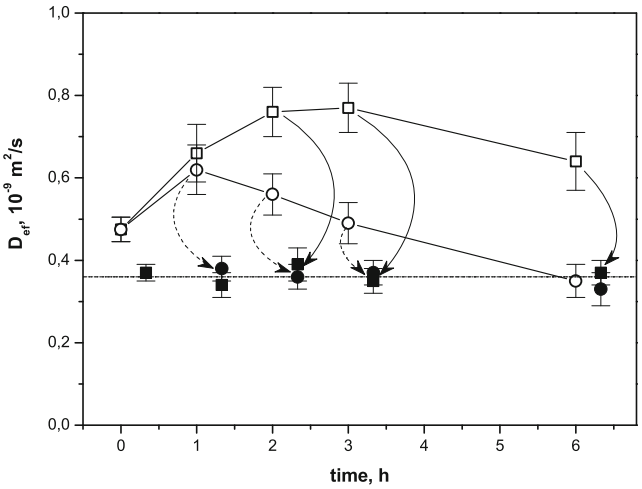

Fig. 2 Time course of effective water diffusion coefficient in response to wound stress (*white square*) and wound–paraquat stress (*white circle*). Stressed samples were treated with mercuric chloride (0.2 mM $HgCl_2$, 20 min) to inhibit water transport through aquaporins (*black square*, *black circle*, respectively). In both stress conditions, the mercuric chloride treatment resulted in the decrease in D_{ef} (*solid arrows* for wound stress and *dashed arrows* for wound–paraquat stress) to the same level (*horizontal dashed line*) independent of the duration of stress. *Bars* show SE (n=5)

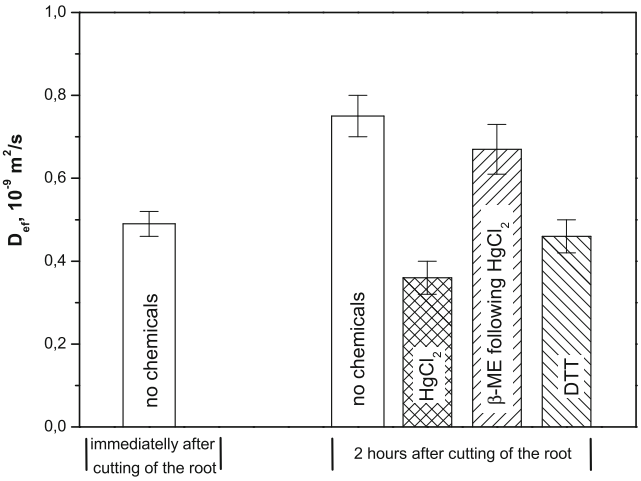

Fig. 3 Effect of $HgCl_2$, β-mercaptoethanol (*β-ME*), and dithiothreitol (*DTT*) on the effective water diffusion coefficient (D_{ef}) in cells of excised maize roots preliminary subjected to 2-h incubation in 0.25 mM $CaCl_2$ (wound stress). —no chemicals; —$HgCl_2$ (0.2 mM, 20 min); —β-mercaptoethanol (5 mM, 15 min) following $HgCl_2$ (0.2 mM, 20 min); —dithiothreitol (5 mM, 15 min)

related to membrane aquaporin activity, we tested the aquaporin mercury sensitivity. For this purpose, we added 0.2 mM $HgCl_2$ solution for 20 min after 2, 3, or 6 h of incubation (Fig. 2). Under the effect of mercuric chloride, the value of D_{ef} at the mentioned time points reduced (solid arrows) to a constant level (a horizontal dashed line) corresponding to the mercuric chloride effect on D_{ef} at the 0 time point, i.e., immediately after root excision. The inhibitory effect of mercury was mostly reversed by a 15-min exposure of $HgCl_2$-treated roots in 5 mM β-mercaptoethanol (Fig. 3). After the 2-h incubation of excised roots (wound stress), we added the reductant of thiol groups—dithiothreitol (DTT)—into the incubation medium (concentration of 5 mM) for 15 min. This resulted in the removal of the D_{ef} increase caused by 2-h wound stress (the value of D_{ef} returned to the value of D_{ef} immediately after excision) (Fig. 3).

As far as wound stress produces some excess of ROS (Orozco-Cárdenas et al. 2001; Ross et al. 2006; Garrido et al. 2012), we wondered whether D_{ef} changes if we artificially increase ROS production against the background of the initial response to wound stress. For this purpose, we used paraquat which is a well-known "generator" of superoxide radicals in plant cells (Radyukina et al. 2008; Lascano et al. 2012). Paraquat gradually eliminated D_{ef} response to wounding, and by the sixth hour of incubation, the resulting value of D_{ef} achieved a constant level, corresponding to the mercury effect in the wound stress variant (Fig. 2, wound–paraquat stress). Under the treatment with mercuric chloride (0.2 mM, 20 min) in the wound–paraquat variant, the value of D_{ef} after 1, 2, 3, or 6 h of incubation also reduced (dashed arrows) to a constant level, corresponding to the effect of mercuric chloride on D_{ef} at the 0 time point (Fig. 2).

The monitoring of cell membrane permeability using the NMR method is based on the theory of restricted diffusion (Tanner and Stejskal 1968). Water molecule diffusion between two compartments, contrast with respect to magnetization relaxation time (central vacuole and apoplast), in root cells is restricted by a thin layer of cytoplasm with two membranes—tonoplast and plasmalemma (Fig. 1). The degree of diffusion restriction (decrease in D_{ef} compared to bulk water diffusion coefficient) depends on the total permeability of these two membranes and the average vacuole size (Crick 1970). However, D_{ef} under the effect of mercuric chloride during both stresses (wound stress and wound–paraquat stress) reached plateau, corresponding to the effect of mercuric chloride on D_{ef} at the 0 time point (Fig. 2). It means that there occurred no significant changes in the vacuole size in the course of both stresses. Thus, for the studied samples, the increment of the overall water permeability of two membranes—tonoplast and plasmalemma—participated in the increment of D_{ef} under the applied effects. Therefore, in the following analysis of results, we shall directly relate the registered changes in D_{ef} to changes in the overall permeability of these two membranes. Under the transient conditions during cell volume changes, or artificially maintained unidirectional hydraulic flow, the role of plant membrane aquaporins can be partially fulfilled by potassium channels (Wayne and Tazawa 1990). Therefore, the lack of vacuole volume changes in our studies (the steady with respect to transmembrane water exchange conditions) prevented the possibility of potassium channel influence.

The excess ROS accumulation in cells presents an early event, accompanying the effect of actually all abiotic and biotic stresses in plants (Hernandes et al. 2001). The most general indicator of the development of oxidative stress caused by accumulation of the excess ROS is the peroxidation of lipids (POL) level. The amount of malondialdehyde (MDA), as a criterion of POL intensity, is shown in Fig. 4 for both stresses. In the course of wound stress, the amount of MDA gradually reduced or did not change. Under wound–paraquat stress, the amount of MDA increased by the first hour and then remained at a constant level. In the latter case, the amount of MDA at all time points reliably exceeded the amount of MDA at wound stress alone.

The disturbance of the balance between the ROS level and activity of antioxidant defense systems under stress is associated with the involvement of the complex of metabolic and physiological changes. So, we registered the oxygen consumption and metabolic heat production by root segments. The data on oxygen consumption are shown in Fig. 5. For wound–paraquat stress, there was observed a surplus of the amount of oxygen consumed by root segments compared to the wound stress variant for all time points. The corresponding data on heat production are shown in Fig. 6. In the case of wound–paraquat stress, the total level of heat production during the first 3 h was twice as large as the one for wound stress. But in the wound stress variant, after 3 h, there began the significant increase in heat production. In the wound–paraquat variant, the increase in heat production during this time period was not that drastic.

Discussion

Maize roots are widely used to study cell water self-diffusion using the NMR method (Anisimov et al. 1998; Ionenko et al. 2012). In our experiments, the maize roots were stressed by excising them from seedlings and subsequent incubating in growth medium for several hours. Earlier, this method of wound stress was used for wheat (Minibayeva et al. 1998) and pea (Karimova and Petrova 2007) seedling roots. It was shown that in this case, similarly to other kinds of stress factors, a complex of metabolic and physiological changes in cells was switched on (Minibayeva et al. 2009). In particular, wound stress activated root cells to produce oxygen radicals. The increase in production of superoxide anion radicals and a corresponding increase in peroxidase activity were shown (Minibayeva et al. 1998, 2001, 2009, 2012). It is well established that production of ROS is one of the universal responses of various plants to wounding (Orozco-Cárdenas et al. 2001; Ross et al. 2006). The mechanism of ROS production in excised roots was studied in detail by Garrido et al. (2012).

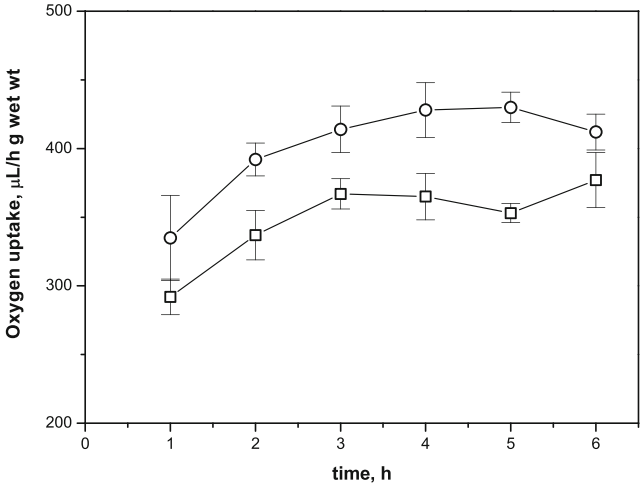

Fig. 5 The rate of oxygen uptake for excised roots during wound stress (*white square*) and wound–paraquat stress (*white circle*). *Bars* show SE (n=5)

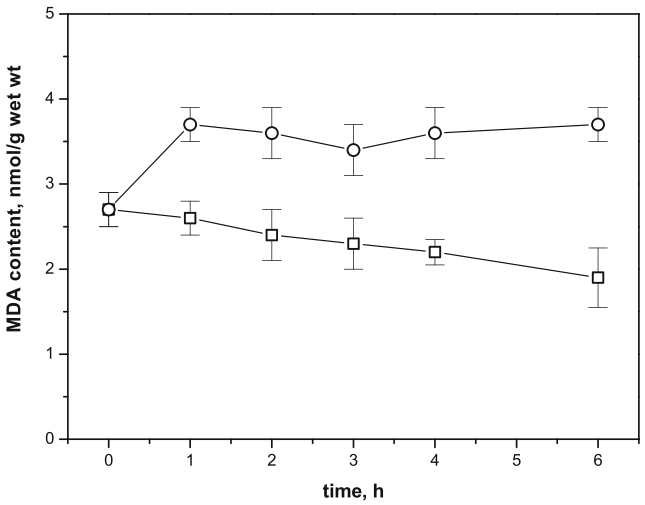

Fig. 4 MDA content as a measure of lipid peroxidation (POL) level during the development of wound stress (*white square*) and wound–paraquat stress (*white circle*). *Bars* show SE (n=5)

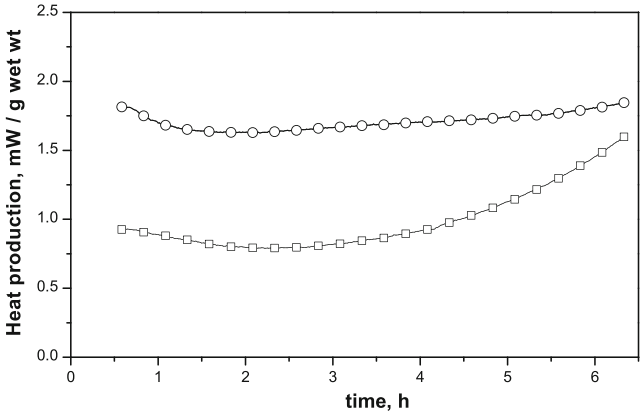

Fig. 6 Heat production of excised roots during wound stress (*white square*) and wound–paraquat stress (*white circle*). The level of heat production in the wound–paraquat variant during the first 3 h is twice larger compared to wound stress alone. Beginning with the third hour, a considerable increase in heat production is observed for wound stress. SE was less than 7 % (n=5)

During maize root response to wound stress (Fig. 2), there was observed the increase in D_{ef} from the initial value at the time point of excision to the maximum one by the second to the third hour of incubation. These changes in D_{ef} result from corresponding changes in the effective total water permeability of two membranes (tonoplast and plasmalemma) in excised root cells (see "Materials and methods"). However, the water uptake capacity of plant roots (i.e., their hydraulic conductivity) is known to be determined largely by aquaporins of the plasma membrane intrinsic protein (PIP) subfamily (Boursiac et al. 2008). The increase in D_{ef} mostly resulted from the increase in plasmalemma permeability.

The contribution of aquaporin-mediated water transport across the membrane is, as a rule, estimated using an inhibitor. Mercury is the most commonly applied inhibitor for aquaporins (Martre et al. 2001; Javot and Maurel 2002; Volkov et al. 2007; Bramley et al. 2009; Hachez and Chaumont 2010). Mercury can inhibit the channel activity of a hetero-oligomer composed of *Z. mays* PIP1;2 and PIP2;5 through its interaction with a cysteine residue located in the loop A of *Z. mays* PIP1;2, a residue involved in disulfide bond formation between PIP monomers (Bienert et al. 2012). The structure of the PIP2;1–mercury complex has been solved and reveals three binding cysteine residues for mercury, which could act on the channel gating (Frick et al. 2013). However, strangely, reconstitution of PIP2;1 in liposomes showed that mercury did not inhibit but increased their water channel activity in a cysteine-independent way, possibly through changes in the properties of the lipid bilayer (Frick et al. 2013). These authors considered it premature to apply the results, obtained with liposomes, to real plant membrane aquaporins and pointed to the need of special studies. Apparently, the mercury test for plant membranes should be applied with caution (Chaumont and Tyerman 2014).

In the case of wound stress, the addition of mercuric chloride after 2, 3, or 6 h of incubation resulted in the decrease in membrane water permeability to the constant level corresponding to the mercuric chloride effect at the 0 time point (Fig. 2, horizontal dashed line). Obviously, we failed to observe the abnormal response of the membrane water permeability to mercury effect, predicted by Frick et al. (2013). The inhibitory effect of mercury was mostly reversed after the addition of 5 mM β-mercaptoethanol in the incubation medium for 15 min (Fig. 3). This corresponds to the well-known response of aquaporins to the treatment with $HgCl_2$ and β-mercaptoethanol (Preston et al. 1993). After the 2-h wound stress, DTT was added into the incubation medium for 15 min, resulting in the removal of the D_{ef} increase caused by 2-h wound stress (Fig. 3). The increase in D_{ef} under wound stress and its subsequent removal by DTT appear to show the processes of oxidation and reduction of SH– groups of water permeating paths in membranes, respectively. In this case, the role of lipid bilayer can be excluded since mercury reduced D_{ef} to the constant level (Fig. 2) during the whole period of root response to the stress. One can suppose that the increase in membrane water permeability under wound stress is associated with aquaporin activation.

Wound stress was shown earlier to activate wheat root cells to produce oxygen radicals (Minibayeva et al. 1998, 2001, 2009, 2012; Ross et al. 2006). In addition, the superoxide production achieved maximum in 2 h after root excision and at the beginning of incubation (Minibayeva et al. 1998). One can suppose that aquaporin activation during wound stress results from redox processes of thiol groups of these proteins. In vitro studies (Ampilogova et al. 2006) gave the experimental evidence in favor of this concept. The isolated from pea roots plasmalemma responded adequately to the presence in the medium of agents that oxidize (diamide) or reduce (dithiothreitol, tributylphosphine) thiol groups by changing the balance in the number of SH– groups and S–S– bonds. Changes in this balance resulted in modification of plasmalemma osmotic water permeability in vitro: it increased with the increase in S–S– bonds and decreased with the increase in the number of SH– groups.

Bienert et al. (2012) tested whether the loop A cysteine residue involved in disulfide bond formation between PIP monomers had an effect on oxidative or reductive treatments that might affect PIP gating. This hypothesis was investigated by measuring the osmotic water permeability coefficient (P_f) in oocytes expressing ZmPIP2;5 after treatment with oxidants (H_2O_2 or *t*-butylhydroperoxide) or reductants (2-mercaptoethanol or DTT). No significant differences were observed in the P_f values for non-treated, or oxidant-treated, or reductant-treated oocytes, indicating that the disulfide bonds, as well as other potential modifications of the cysteine thiol group, do not affect the activity of ZmPIP2;5 (Bienert et al. 2012). The same researchers showed that the loop A cysteine residue of ZmPIP1;2, but not that of ZmPIP2;5, is involved in mercury sensitivity. The latter does not exclude the possibility that PIP isoforms, differing from ZmPIP2;5, or some other cysteine residue can be involved in ROS regulation. Indeed, the research (Ampilogova et al. 2006) clearly demonstrated that a change in the ratio of thiol to disulfide bonds in plant plasma membrane proteins correlated with a change in water permeability. These researchers supposed that it is not the loop A cysteine residue, but two cysteine residues from CLGAIC sequence in the transmembrane domain, associated with the loop C of PIPs, that might be involved into the realization of the revealed regulation. The short distance between these cysteine residues allows the coupling and redox transitions between thiol groups of this pair of cysteine residues (Ampilogova et al. 2006). Apparently, the possibility of direct influence of ROS on aquaporin activity on the molecular level is not yet studied in detail. However, our data (in vivo) on regulation of membrane water permeability under wound stress agree nicely with the abovementioned data on

regulation of water permeability for the plasma membrane vesicles (Ampilogova et al. 2006).

In the wound–paraquat variant, the increment of D_{ef} inherent to wound stress appeared to reduce after the first hour, and by the fifth to the sixth hour of incubation, D_{ef} reached the value on the plateau (dashed line), which tallies with the blocked by mercury state of the membrane at the 0 time point. Under mercuric chloride effect, the value of D_{ef} at all time points of exposition (1, 2, 3, and 6 h) achieved a constant level corresponding to that for wound stress. This fact additionally indicates that in our experiments, mercury does not influence water permeability of the membrane lipid phase. We did not observe the abnormal effect of mercuric chloride (see aforesaid, Frick et al. 2013). The mercury test in the experiment with wound–paraquat stress proves that, in this case as well, the time course of membrane water permeability decrease might be determined by the corresponding aquaporin reaction.

By using the measurements of MDA, oxygen consumption, and heat production, as conventional factors of general oxidative stress, we showed the presence of real differences between two variants of the experiments. The development of wound–paraquat stress was characterized by the exceeding amount of oxygen consumed by root segments during all time points of exposition compared to the wound stress experiment (Fig. 5). In addition, the overall level of heat production during the first 3 h of simultaneous effect of stressors was twice as large as for the wound stress alone (Fig. 6). During the disturbance of coordination of processes occurring in the mitochondrial matrix, caused by stress impact, there increases a leakage in the electron transfer chain. The direct interaction of leaking electrons with oxygen is the principal pathway of superoxide synthesis during the paraquat treatment (Lascano et al. 2012). Comparing the data from Figs. 5 and 6, one can see that the increase in oxygen consumption during wound–paraquat stress is coupled with a large gain in heat production. Presumably, a large part of the exceeding amount of the consumed oxygen is spent for production of ROS, which activate exothermal reactions and appear to be one of the factors of the overall heat production increase. Apparently, the observed changes in energy metabolism imply that paraquat indeed fulfills the assigned function of the inductor of ROS excess in our experiments as well.

It should be noted that after 3 h of wound stress, a significant increase in heat production began. With the restricted energy resource in the excised root, the oxidative stress can induce a catabolic process of intracellular degradation of some macromolecules and organelles—autophagy (Minibayeva et al. 2012). This process is characterized by production of one- and two-membrane vesicles (autophagosomes), containing the fragments of degradation. Presumably, this exothermal process causes the abovementioned increase in heat production during wound stress. This kind of exothermal process can be considered as an independent proof of the presence of ROS excess (compared to the norm) during the initial hours of development of wound stress in our experiments.

The double excess of heat production during the first 3 h of the wound–paraquat stress provides a convincing evidence of the general increase in oxidative reaction intensity compared to that for wound stress alone. It is also indicated by POL intensification that is directly confirmed by a higher level of MDA in the wound–paraquat variant. Therefore, two variants of our experiments differed essentially in levels of general oxidative stress. At the same time, characteristics of the general oxidative stress allowed us to judge indirectly about both ROS production and the state of the membrane lipid phase under the effect of two stress factors.

The results of our experiments point to the counter-directed response of membrane water permeability under wound and wound–paraquat stresses (increase and decrease, respectively). There apparently exists an optimal level of oxidative stress (excess of ROS), against the background of antioxidant cell activity, to maximize water flow across root cells. In our experiments, wound stress, apparently, is associated with the weak oxidative stress and wound–paraquat stress—with the considerably increased ROS impact on the cells.

This explanation is supported by literature data. Thus, the hydraulic permeability of maize roots increases twice during the exogenous 1-h treatment with 100 μM hydrogen peroxide (Aroca et al. 2005). On the contrary, 1-h treatment with 2 mM hydrogen peroxide (the higher dose) resulted in inhibition of hydraulic permeability in the maize roots (Boursiac et al. 2008; Ehlert et al. 2009). Other authors measured hydraulic permeability (L_p) of parenchyma cells in the midrib tissue of maize leaves using a cell pressure probe (Kim and Steudle 2009). In response to low light intensity of 200 μmol m^{-2} s^{-1}, the cell L_p was increased. In contrast, high light intensities of 800 and 1800 μmol m^{-2} s^{-1} decreased the cell L_p. Together with it, the treatment of the tissue with oxidants (H_2O_2 and hydroxyl radical produced in the Fenton reaction) had an effect similar to high light, and the presence of the antioxidant (glutathione) tended to prevent the inhibition by high light.

The state of the membrane lipid phase during the wound–paraquat stress differed from that during the wound stress (Fig. 4). Therefore, changes in the lipid phase related to the intensification of lipid peroxidation can be supposed to affect directly the conformation and transport function of integral proteins—aquaporins, resulting in their downregulation. Under wound–paraquat stress, the proteins participating in the system of intracellular phosphorylation, for example, protein phosphatase of the tyrosine type, can experience destruction and inactivation by ROS (Karimova and Petrova 2007). Aquaporin traffic from the plasma membrane to the intracellular vesicular membrane structures can also provide the important mechanism of downregulation under this stress (Boursiac et al. 2008). However, for all three processes (peroxidation

of lipids, cell protein destruction, and relocalization of aquaporins), it is possible that ROS do not gate aquaporins through a direct oxidative mechanism (Boursiac et al. 2008).

In this paper, we used the NMR method of water self-diffusion measurement in maize root cells, allowing for express monitoring of cell membrane water permeability. Changes in membrane water permeability was studied in response to (i) root excision from seedling and the following 6-h incubation in the growth medium (wound stress) and (ii) the superposition of wound stress plus paraquat. The noticeable difference in the general level of the oxidative stress was shown for these variants of the experiments. Wound stress is associated with the weak oxidative stress, and wound–paraquat stress with the considerably increased ROS impact on the cells. Results of our experiments point to the counter-directed response of membrane water permeability under wound and wound–paraquat stresses (increase and decrease, respectively). There apparently exists an optimal level of oxidative stress (excess of ROS), against the background of antioxidant cell activity, to maximize water flow across root cells. We showed the reversible by dithiothreitol increase in cell membrane water permeability in maize roots under wound stress. The obtained data in our experiments for root cell membranes in vivo under this stress (weak oxidative stress) agree nicely with the data presented in literature about upregulation of water permeability of isolated plasma membrane vesicles, occurring under the conditions of changes in equilibrium between the number of SH– groups and S–S– bonds towards the latter. The possibility of direct influence of ROS on aquaporin activity on the molecular level is not yet studied in detail. In our experiments, the applicability of mercury test to aquaporin activity was verified. The results of wound stress effect, obtained using this test, are discussed in terms of oxidative upregulation of aquaporin activity by ROS. It is supposed that under wound–paraquat stress, ROS affected the aquaporins not directly, but via such processes as peroxidation of lipids, intracellular protein destruction, and relocalization of aquaporins in the cell. Results of our research might be important to cope with environmental factors.

Acknowledgments The authors are grateful to A.Yu. Alyab'ev, T.I. Ogorodnikova, and T.M. Il'ina for rendering assistance in the experiments. This research was supported by grants No. 13-04-01203 and No. 14-04-31606 from the Russian Foundation for Basic Research.

References

Ampilogova YN, Zhestkova IM, Trofimova MS (2006) Redox modulation of osmotic water permeability in plasma membranes isolated from roots and shoots of pea seedling. Rus J Plant Physiol 53:622–628

Anisimov AV, Sorokina NY, Dautova NR (1998) Water diffusion in biological porous systems: a NMR approach. Magn Reson Imaging 16: 565–568

Anisimov AV, Ionenko IF, Romanov AV (2004) Spin-echo NMR study of the translational water diffusion selectively along the apoplast and the cytoplasmic and vacuolar symplasts of plants. Biophysics 49: 816–821

Aroca R, Amodeo G, Fernandez-Illescas S, Herman EM, Chaumont F, Chrispeels MJ (2005) The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. Plant Physiol 137:341–353

Berczi A, Moller IM (2000) Redox enzymes in the plant plasma membrane and their possible roles. Plant Cell Environ 23:1287–1302

Bienert GP, Cavez D, Besserer A, Berny M, Gilis D, Rooman M, Chaumont F (2012) A conserved cysteine residue is involved in disulfide bond formation between plant plasma membrane aquaporin monomers. Biochem J 445:101–111

Boursiac Y, Boudet J, Postaire O, Luu DT, Tournaire-Roux C, Maurel C (2008) Stimulus-induced down-regulation of root water transport involves reactive oxygen species-activated cell signaling and plasma membrane intrinsic protein internalization. Plant J 56:207–218

Bramley H, Turner NC, Turner DW, Tyerman SD (2009) Roles of morphology, anatomy and aquaporins in determining contrasting hydraulic behavior of roots. Plant Physiol 150:348–364

Chaumont F, Tyerman S (2014) Aquaporins: highly regulated channels controlling plant water relations. Plant Physiol 164:1600–1618

Chaumont F, Moshelion M, Daniels MJ (2005) Regulation of plants aquaporin activity. Biol Cell 97:749–764

Cho CH, Hong YS, Kang K, Volkov VI, Skirda V, Lee CYJ, Lee CH (2003) Water self-diffusion in Chlorella sp. studied by pulse field gradient NMR. Magn Reson Imaging 21:1009–1017

Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E (2000) Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. J Exp Bot 51:61–70

Cooper RL, Chang DB, Young AC, Martin CJ, Ancker-Johnson B (1974) Restricted diffusion in biophysical systems. Biophys J 14:161–177

Crick F (1970) Diffusion in embryogenesis. Nature 225:420–422

Daniels MJ, Chaumont F, Mirkov TE, Chrispeels MJ (1996) Characterization of new vacuolar membrane aquaporin sensitive to mercury at unique site. Plant Cell 8:587–599

Duval FP, Cambert M, Mariette F (2005) NMR study of tomato pericarp tissue by spin-spin relaxation and water self-diffusion. Appl Magn Reson 28:29–40

Ehlert C, Maurel C, Tardieu F, Simonneau T (2009) Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. Plant Physiol 150:1093–1104

Frick A, Jarva M, Ekvall M, Uzdavinys P, Nyblon M, Ornroth-Horsefield ST (2013) Mercury increases water permeability of a plant aquaporin through a non-cysteine-related mechanism. Biochem J 454:491–499

Fu D, Libson A, Miercke LJW, Weitzman C, Nollert P, Krucinsky J, Stroud RM (2000) Structure of a glycerol-conducting channel and the basis of its selectivity. Science 290:481–486

Garrido I, Espinosa F, Alvarez-Tinaut MC (2012) Apoplastic superoxide production and peroxidase activity by intact and excised axenically grown seedling roots of sunflower. Protoplasma 249:1071–1080

Gelhaye E, Rouhier N, Navrot N, Jacquot JP (2005) The plant thioredoxin system. Cell Mol Life Sci 62:24–35

Hachez C, Chaumont F (2010) Aquaporins: a family of highly regulated multifunctional channels. In: Jahn ThP, Bienert GP (eds) MIPs and their role in the exchange of metalloids Springer series: Advances in experimental medicine and biology, 679 Landes Bioscience, Austin, pp 1–17

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: 1. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Henzler T, Steudle E (2000) Transport and metabolic degradation of hydrogen peroxide in *Chara coralline*: model calculations and

measurements with the pressure probe suggest transport of H_2O_2 across water channels. J Exp Bot 51:2053–2066

Hernandes JA, Ferrer MA, Jiménez A, Ros Barceló A, Sevilla F (2001) Antioxidant systems and $O_2^{\bullet-}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127:817–831

Ionenko IF, Anisimov AV (2007) Radial diffusion transport of water in various zones of maize root and its sensitivity to mercury chloride. Russ J Plant Physiol 54:224–229

Ionenko IF, Anisimov AV, Romanov AV (2003) Effect of water stress and mercuric chloride on the translational diffusion of water in maize seedling roots. Russ J Plant Physiol 50:79–83

Ionenko IF, Dautova NR, Anisimov AV (2012) Early changes of water diffusional transfer in maize roots under the influence of water stress. Environ Exp Bot 76:16–23

Ishida N, Koizumi M, Kano H (2000) The NMR microscope: a unique and promising tool for plant science. Ann Bot 86:259–278

Javot H, Maurel C (2002) The role of aquaporins in root water uptake. Ann Bot 90:301–313

Johansson I, Karlsson M, Johanson U, Larsson C, Kjellbom P (2000) The role of aquaporins in cellular and whole water balance. Biochem Biophys Acta 1465:324–342

Karimova FG, Petrova NV (2007) Effect of H_2O_2 on tyrosine phosphorylation of pea proteins. Russ J Plant Physiol 54:322–328

Kim YX, Steudle E (2007) Light and turgor affect the water permeability (aquaporins) of parenchyma cells in the midrib of leaves of Zea mays. J Exp Bot 58:4119–4129

Kim YX, Steudle E (2009) Gating of aquaporins by light and reactive oxygen species in leaf parenchyma cells of the midrib of Zea mays. J Exp Bot 60:547–556

Kukulski W, Schenk AD, Johanson U, Braun T, de Groot BL, Fotiadis D, Kjellbom P, Engel A (2005) The 5 A structure of heterologously expressed plant aquaporin SoPIP2;1. J Mol Biol 350:611–616

Lascano R, Mucoz N, Robert G, Rodriguez M, Melchiorre M, Trippi V, Quero G (2012) Paraquat: an oxidative stress inducer. In: Hasaneen MN (ed) Herbicides—properties, synthesis and control of weeds. InTech, pp 135–148. doi: 105772/32590

Lee SH, Singh AP, Chung GC, Ahn SJ, Noh EK, Steudle E (2004) Exposure of roots of cucumber (*Cucumber sativus*) to low temperature severely reduces root pressure, hydraulic conductivity and active transport of nutrients. Physiol Plantarum 120:413–420

Martre P, North GB, Nobel PS (2001) Hydraulic conductance and mercury sensitive water transport for roots of *Opuntia acanthocarpa* in relation to soil drying and rewetting. Plant Physiol 126:352–362

Maurel C (1997) Aquaporins and water permeability of plant membranes. Annu Rev Plant Physiol Plant Mol Biol 48:399–429

Maurel C (2007) Plant aquaporins: novel functions and regulation properties. FEBS Lett 581:2227–2236

Maurel C, Verdoucg L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. Annu Rev Plant Biol 59:595–624

Minibayeva FV, Kolesnikov OP, Gordon LK (1998) Contribution of a plasma membrane redox system to the superoxide production by wheat root cells. Protoplasma 205:101–106

Minibayeva FV, Gordon LK, Kolesnikov OP, Chasov AV (2001) Role of extracellular peroxide production by wheat root cells. Protoplasma 217:125–128

Minibayeva F, Kolesnikov O, Chasov A, Beckett RP, Luthje S, Vylegzhanina N, Buck F, Bottger M (2009) Wound-induced apoplastic peroxidase activities: their roles in the production and detoxification of reactive oxygen species. Plant Cell Environ 32: 497–508

Minibayeva F, Dmitrieva S, Ponomareva A, Ryabovol V (2012) Oxidative stress-induced autophagy in plants: the role of mitochondria. Plant Physiol Biochem 59:11–19

Murata K, Mitsuoaka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y (2000) Structural determinants of water permeation through aquaporin-1. Nature 407:599–605

Myers J, Matsen FA (1955) Kinetic characteristics of Warburg manometry. Arch Biochem Biophys 55:373–388

Niemietz CM, Tyerman SD (2002) New potent inhibitors of aquaporins: silver and gold compounds inhibit aquaporins of plant and human origin. FEBS Lett 531:443–447

Orozco-Cárdenas ML, Narvaez-Vasquez J, Ryan CA (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell 13:179–191

Prado K, Boursiac Y, Tournaire-Roux C, Monneuse J-M, Postaire O, Dalnes O, Schaffner AR, Hem S, Santoni V, Maurel C (2013) Regulation of Arabidopsis leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. Plant Cell 25:1029–1039

Preston GM, Jung JS, Guggino WB, Agre P (1993) The mercury-sensitive residue at cysteine 189 in CHIP28 water channel. J Biol Chem 268:17–20

Radyukina NL, Shashukova AV, Shevyakova NI, Kuznetsov VV (2008) Proline involvement in the common sage antioxidant system in the presence of NaCl and paraquat. Russ J Plant Physiol 55:649–656

Ross C, Küpper FC, Jacobs RS (2006) Involvement of reactive oxygen species and reactive nitrogen species in the wound response of *Dasycladus vermicularis*. Chem Biol 13:353–364

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 141:336–340

Sibgatullin TA, De Jager PA, Vergeldt FJ, Gerkema E, Anisimov AV, van As H (2007) Combined analysis of diffusion and relaxation behavior of water in apple parenchyma cells. Biophysics 52:196–203

Stadtman ER (1992) Protein oxidation and aging. Science 257:1220–1224

Stejskal EO, Tanner JE (1965) Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient. J Chem Phys 42: 288–292

Steudle E, Herzler T (1995) Water channels in plant: do basic concepts of water transport change. J Exp Bot 46:1067–1076

Tanner JE (1970) Use of the stimulated echo in NMR diffusion studies. J Chem Phys 52:2523–2526

Tanner JE, Stejskal EO (1968) Restricted self-diffusion of protons in colloidal systems by the pulse-gradient spin-echo method. J Chem Phys 49:1768–1777

Van As H (2007) Intact plant MRI for the study of cell water relations, membrane permeability, cell-to-cell and long distance water transport. J Exp Bot 58:743–756

Van der Weerd L, Claessens MMAE, Ruttink T, Vergeldt FJ, Schaafsma TJ, van As H (2001) Quantitative NMR microscopy of osmotic stress responses in maize and pearl millet. J Exp Bot 52:2333–2343

Van der Weerd L, Claessens MMAE, Efde C, van As H (2002) Nuclear magnetic resonance imaging of membrane permeability changes in plant during osmotic stress. Plant Cell Environ 25:1539–1549

Van Dusschoten D, de Jager PA, van As H (1995) Extraction diffusion constants from echo-time dependent PFG NMR data using relaxation-time information. J Magn Reson Series A 116:22–28

Velikanov GA (2007) Vacuolar symplast and methodological approach to monitoring water self-diffusion between vacuoles of contacting root cells. Rus J Plant Physiol 54:683–692

Velikanov GA, Ponomareva AA, Belova LP, Ilyina TM (2011) Stromule-like protrusions of plastid membrane envelope in root cells. Cell Tissue Biol 5:305–310

Verkman AS, Mitra AK (2000) Structure and function of aquaporin water channels. Am J Physiol 278:F13–F28

Volkov V, Hachez C, Moshelion M, Draye X, Chaumont F, Fricke W (2007) Water permeability differs between

growing and non-growing barley leaf tissues. J Exp Bot 58: 377–390

Volobueva OV, Velikanov GA, Balŭska F (2004) Regulation of intercellular water exchange in various zones of maize root under stresses. Russ J Plant Physiol 51:676–683

Wayne R, Tazawa M (1990) Nature of water channels in the intermodal cells of Nitellopsis. J Membr Biol 116:31–39

Zhao C-X, Shao H-B, Chu L-Y (2008) Aquaporin structure-function relationships: water flow through plant living cells. Colloids Surfaces B: Biointerfaces 62:163–172

Zhestkova IM, Ampilogova YN, Shevyreva TA, Trofimova MS (2009) Effect of chilling temperature on osmotic water permeability and aquaporin activity in the plasma membrane from pea roots. Russ J Plant Physiol 56:635–641