

NMR study of pressure effect on water diffusional transfer in maize roots

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ABSTRACT

Response of the hydrodynamic root system was studied in maize *Zea mays L.* seedlings after the treatment with hydrostatic pressure created by compressed air in a pressure bomb and gradient pressure created by centrifugal force, by detecting diffusional water transfer with the use of pulsed NMR. In order to establish the contribution of water transfer through aquaporins, the transmembrane water transport was detected in roots treated with aquaporin blocker. Changes in diffusional water transfer after the pressure impact were shown to depend on its intensity and the number of cycles of pressure treatment, and varied for different diffusion times. It is proposed that maize root response to the pressure impact lies in the unequal changes in water permeability of the plasmalemma and tonoplast resulting from the changes in aquaporin activity and perhaps in the escalation of water transfer along the cell vacuole. The authors develop the idea that pressure might act as a factor of physiological function regulation in plants.

KEYWORDS: aquaporins, membrane permeability, nuclear magnetic resonance, pressure impact, *Zea mays*

ABBREVIATIONS

D_{ef} - effective self-diffusion coefficient of water,
DD - diffusional decay, R - relative echo amplitude,
 t_d - diffusion time

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INTRODUCTION

Pressure effects on plant organisms arouse the interest of many investigators mainly from the point of view of improvement of the technology of biological product preservation and storage, and also stimulation of growth processes, particularly, seed germination. In this context, high pressure effects in the range of 100-1000 atm were discussed in a number of papers [1, 2, etc.]. However there is very little data available on the pressure effect within the interval of physiological values [3, 4]. The latter seems astonishing since the role of pressure (osmotic, gravitational, transpirational, turgor, etc.) in providing a driving force for water solution transfer and growth processes in plants is well known. Pressure pulses that can be spread in water medium at the velocity up to 1500 m/s also might play a role in plant signaling. Christmann *et al.* (2007) showed the role of pressure drops in generation and rapid root-to-shoot transmission of hydraulic signals [5].

Primary sites of pressure impact and other external factors are cell membranes [1]. It is proposed that the application of pressure of several atmospheres increases the phase order of membrane lipids, particularly in the proximity of membrane-associated proteins [6]. The studies of pressure effect in connection with determination of regulation mechanisms of water-conducting activity of aquaporins (AQPs) are of great interest [3]. AQPs are widely believed to be important in the conduction and regulation of water movement through plant membranes [7]. A possible role for AQPs as an osmotic or turgor sensor has also been suggested [8]. The impact of turgor pressure

pulses on the membrane permeability was demonstrated in studies, carried out on plant cells with the aid of a pressure probe [3, 4]. The authors proposed two models for the mechanical inhibition of AQPs by pressure pulses: 1 - the transfer of kinetic energy from the water to the channel constrictions (energy-input model), 2 - the creation of water thread tension at the channel constrictions (a cohesion-tension model). As a result, the conformational changes in transport proteins may occur, and these are accompanied by the inhibition of aquaporin activity (their closure). The mechanosensitivity of water channels analogical to that proposed for ion channels is discussed [4].

Therefore, the hydrodynamic pressure changes can be supposed to be regulation factors of cell-to-cell water transport. Moreover, considering the tissue and organism levels, it should be noted that the plant symplast, as an uninterrupted continuum, can be the most appropriate channel for the pressure pulse transmission. In general, there is little data on the water transport regulation by pressure impact. It can be connected with difficulties in detecting water transfer immediately after pressure supply. Such opportunity is provided by the spin-echo NMR with pulsed magnetic field gradient.

The aim of the present paper was to investigate the diffusional water transport in maize roots by the pulsed NMR after the treatment with hydrostatic pressure created by compressed air in a pressure bomb, and gradient pressure created by centrifugal force, and to establish the contribution of transmembrane water exchange via AQPs to the early response of roots to the pressure impact.

MATERIALS AND METHODS

Plant growth conditions and preparation of samples

Experiments were performed on the roots of 7-day-old maize (*Zea mays* L., cv. Donskaya 1) seedlings grown in hydroponic culture (¼ Hoagland-Arnon solution) with continuous aeration, under the 12-h photoperiod (irradiance of 15 W m^{-2}), at a temperature of $22 \pm 2^\circ\text{C}$. The excised primary roots of 20-25 seedlings were placed into a 10 mm diameter and 100 mm long

glass test tube and treated with the hydrostatic pressure (of 5-50 atm) created in the test tube by supplying compressed air (variant 1) or with the gradient pressure created by the centrifugal force of 450-700 g (that correspond to pressure of 2-3.5 atm) in the root apical direction (variant 2). Pressure was supplied either singly, or in several consequent cycles. Each cycle included the pressure delivery and discharge. The period of pressure treatment was 15 min. Water diffusion was measured in the root apical part (1 cm from the root apex) after pressure discharge in the test tube (var 1) or after centrifugal force termination (var 2). The test tube with the pressured sample was inserted into the probe of the NMR spectrometer and thermostated at 20°C . The water diffusion was measured in radial and axial directions of root. In order to evaluate the contribution of water transfer through aquaporins in response to pressure, the roots were treated with aquaporin blocker (0.1 mM HgCl_2 , 15 min) before and/or after pressure treatment. Mercuric chloride is a potent inhibitor of most aquaporins [9]. Its inhibiting effect is related to reversible changes in aquaporin conformation, induced by the interaction of HgCl_2 with SH-groups in the narrow part of the protein pore [10]. We have shown earlier that the inhibition level of water diffusion transfer in maize roots remained unaltered for the first 3 h after 0.1 mM HgCl_2 treatment and inhibition was removed by 5 mM mercaptoethanol [11, 12]. Latter indicates that the mercuric inhibition of root water flow was not due to metabolic inhibition.

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 [13]. The specifics of the spin-echo NMR employment for the determination of water diffusion parameters were described previously in [11, 14, 15]. 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

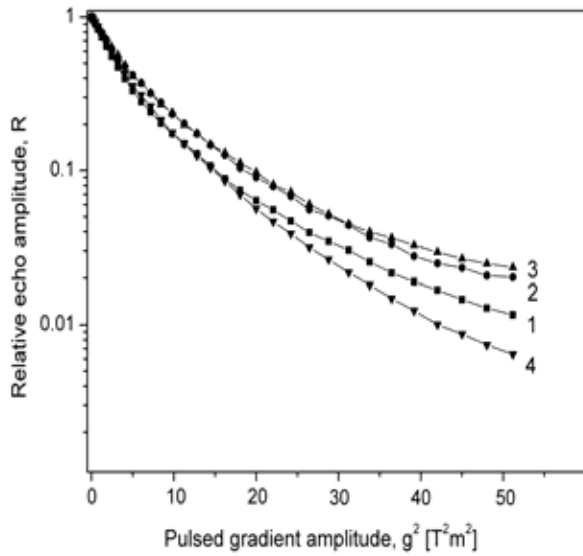


Fig. 1. Dependence of diffusional decays of the spin-echo amplitude on the intensity of hydrostatic pressure for maize roots ($t_d = 300$ ms): 1 - control; 2 - pressure 15 atm; 3 - pressure 15 atm + 0.1 mM HgCl_2 ; 4 - pressure 40 atm.

sequence: the amplitude of magnetic field gradient pulses (g), pulse duration (δ), and the interval between pulses (t_d), conventionally called the diffusion time. DD of the echo is expressed as:

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

where R is the relative echo amplitude, which is equal to the ratio of echo amplitudes in the presence and absence of the magnetic field gradient, $A(g)/A(0)$; γ is the proton gyromagnetic ratio; D is the self-diffusion coefficient.

Diffusional decays were obtained while varying the values of g (T/m) with fixed values of δ (μs) and t_d (ms). To quantify the experimental results we employed the formalism of the effective coefficient of water self-diffusion (D_{ef}) [15]. D_{ef} values were determined using relation (1) from the multiexponential dependence of DD $\ln R$ versus g^2 in the region of the slowly decaying exponential depending mainly on membrane permeability (Fig. 1). The dynamics of the behavior of DD is characteristic of the processes with restricted diffusion and exchange in biological systems, where the decrease of the decrement of decay as t_d increase results from the relaxational redistribution of contributions of the

fractions of apoplast, cytoplasm and vacuolar water to the echo signal. Van Dusschoten *et al.* (1995) pointed out the multi-exponentiality of the diffusional decay in apple parenchyma tissue and correlated the decay components with different water compartments.

The diffusion times, t_d , were chosen to be 300 and 700 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.

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.

RESULTS AND DISCUSSION

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). The largest changes under pressure effect were observed in the slope of the slow component of DD, which depends mainly on membrane (plasmalemma and tonoplast) permeability.

The steepness of DDs and, consequently, the values of water self-diffusion coefficients (D_{ef}), depended on the intensity and duration of the pressure treatment and diffusion time t_d . Single 15-min treatment of roots with hydrostatic pressure of 15 atm slowed down the diffusion decay (at $t_d = 300$ ms) and decreased, correspondingly, D_{ef} by about 20% as compared to the control (Fig. 1). The following treatment of these roots with aquaporin blocker left the value of D_{ef} unchanged. On the other hand the root treatment with pressure of 40 atm increased D_{ef} by 17%.

The increase in the number of cycles of pressure supply and discharge with consecutive increase in pressure intensity caused unequal response at different t_d (Fig. 2): two-stage (decrease-increase)

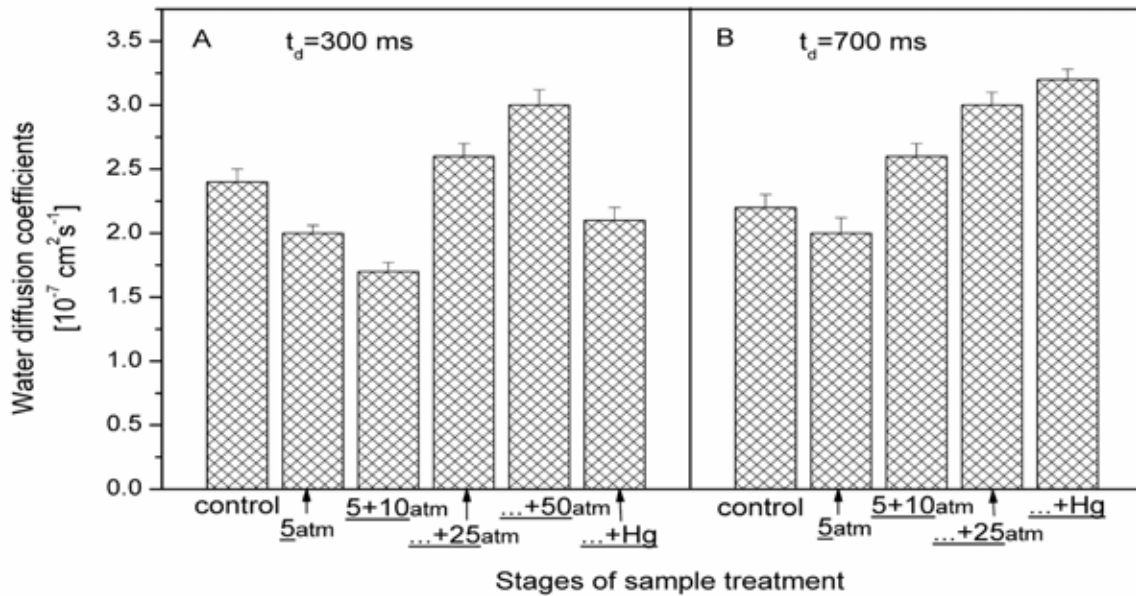


Fig. 2. Hydrostatic pressure effect on water diffusional transfer in maize roots at the different diffusion times (300 and 700 ms). Bars show SE ($n = 5$).

response of D_{ef} at 300 ms and D_{ef} increase at 700 ms. Taking into account the fact of redistribution of contributions of the fractions of apoplast, cytoplasm and vacuolar water to the echo signal when t_d increase [15, 16] it can be concluded that at $t_d = 300$ ms the intracellular water contributed in DD and the apoplast water became nondetectable, since its relaxation time is less than 100 ms. At $t_d = 700$ ms mainly the vacuole water was detected and changes in the slow DD component result from the changes in the tonoplast permeability.

Hence the different effects of hydrostatic pressure at different t_d were connected most likely with unequal influence of pressure on plasmalemma (Fig. 2A) and tonoplast (Fig. 2B) permeability. Analogical changes in membrane permeability were observed by us early in maize roots under the effect of osmotic pressure of -0.5 MPa and were attributed to changes in aquaporin activity [15]. In order to reveal the role of aquaporins in responses to pressure effect, pressured roots were treated with aquaporin blocker. Two-phase response in membrane water permeability to pressure impact (Fig. 2A) may imply two types of aquaporin regulation (closing during the first phase and opening during the second phase). This is supported by the results on Hg-insensitivity of

pressured roots during the first phase and their Hg-sensitivity during the second phase. Wan *et al.* (2004) showed that pressure pulses from 0.1 to 0.2 MPa resulted in the reversible decrease in hydraulic permeability of maize root cell membranes, while the pressure pulses above 0.2 MPa - in the irreversible one. The authors suggest that the pressure causes the increase in the intensity of water flow through water channel and results in its structure changes and consequently the channel closure. D_{ef} increase with the increase in the intensity and the number of cycles of pressure supply and discharge (5+10+25+50 atm) at 300 and 700 ms was caused by different mechanisms. In the first case (Fig. 2A) D_{ef} increase appeared to be a result of aquaporin activity increase, since the following treatment of pressured roots with aquaporin blocker decreased D_{ef} , and in the second case (Fig. 2B) D_{ef} increase was caused by distortion of tonoplast permeability and/or escalation of water transfer along the cell vacuome (the sensitivity to blocker was absent).

Water diffusional transfer in maize roots after the effect of gradient pressure differed for variants of diffusion measurements in radial and axial directions of root main axis. For the radial direction (Fig. 3A), D_{ef} value increased with the increase in cycles of the centrifugal force impact,

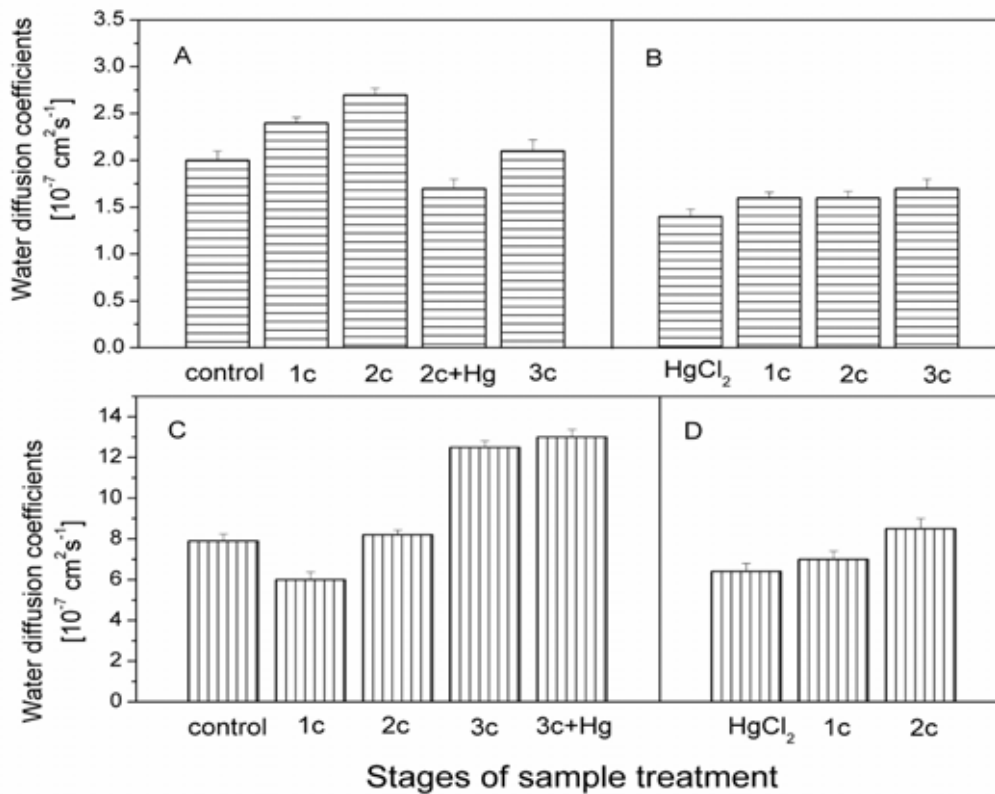


Fig. 3. Gradient pressure effect on water diffusional transfer in control (A, C) and HgCl₂-pretreated (B, D) maize roots in radial (A, B) and axial (C, D) direction of root: 1c, 2c, 3c - one, two, three centrifuging cycles. Bars show SE ($n = 5$).

i.e. additivity of pressure effect was observed. These roots kept the sensitivity to mercurial blocker (column 4), which testified to the maintenance of aquaporin functional activity. Centrifugation of Hg-pretreated roots did not result in D_{ef} increase (Fig. 3B). These data indicate that when diffusion was measured in the root radial direction, the D_{ef} increase after the effect of gradient pressure was caused by the membrane permeability increase due to the increase in aquaporin water-transporting activity.

In the experiments with the diffusion measurements in the root axial direction (Fig. 3C), D_{ef} values decreased after the first cycle and increased only after the third cycle of centrifugation. The following treatment of pressured roots with aquaporin blocker (column 5) left D_{ef} unchanged. For Hg-pretreated roots, little increase of D_{ef} was observed after the second cycle of centrifugation. The differences in the gradient pressure effect on D_{ef} in the variants (Fig. 3A and C) most likely

resulted from the inequality in the intensity of pressure exerted on periclinal and anticlinal cell walls and membranes (situated along and across the root main axis). Anticlinal walls suffered from the effect of the gradient pressure to a larger extent, therefore, three consecutive cycles of centrifugation resulted in the loss of sensitivity of D_{ef} to Hg-treatment. Water diffusional transfer in the axial direction (Fig. 3C, D) was measured at $t_d = 700$ ms and the echo signal was formed by the vacuole water. Therefore, the D_{ef} increase in this variant followed most probably, from the distortion of the tonoplast water permeability.

CONCLUSION

The pressure (hydrostatic and gradient) effect on diffusional water transfer in maize roots was studied by the pulsed NMR method. Changes in water transfer were shown to depend on the pressure intensity and the number of cycles of pressure delivery and discharge. In general a

conclusion can be drawn that maize root response to the pressure impact lies in the unequal changes in water permeability of plasmalemma and tonoplast resulting from the changes in aquaporin activity. Moreover, cell tonoplast suffers from the pressure effect to a larger extent, compared to plasmalemma. As a result of unequal changes in plasmalemma and tonoplast permeability, the redistribution of water flows along different pathways (transmembrane and symplast) can take place, which probably has the adaptive character.

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