Water Self-Diffusion in the Roots of the Plant Effected by Stress Factor under Long-Continued NMR-Experiment

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Abstract. The water self-diffusion in the root segments of winter wheat plantlet after their cutting off from the intact plants during continued «adaptive aging» was investigated by PGSE technique. The diffusion decay of the stimulated echo relative amplitude under the diffusion times 10 ms, 155 ms, 350 ms may be described by the sum of three exponents. Strongly marked correlated dependences of diffusion coefficients and population of the diffusion decay components on the sample lifetime have been revealed. These dependences may be caused by the change of diffusion direction of water molecules running through the tonoplast aquaporin channels in the different cell compartments. This change of direction seems to be the important mechanism of the cell water homeostasis maintenance and it is considered as the part of the general stress roots reaction.

Keywords: NMR, roots, self-diffusion of water molecules, membranes, vacuole, cytoplasm.

Abbreviations: PGSE – pulse-field-gradient spin-echo technique; DD – diffusion decay of the spin-echo amplitude; SDC – self-diffusion coefficient.

Introduction
The processes of self-diffusion of water in segments of roots cut off from the whole plant; carry important information about metabolism of root cells and the effect of stress mechanisms on the living system [1]. To research these processes the PGSE technique is used [2-4]. Cutting off the roots as mechanical stress as well as other types of phytostress includes the whole complex of metabolic and physiological changes referred to as nonspecific adaptative syndrome, observed in the cells [5]. On the
first stages of the stressor’s effect these changes have the lifetime character, revealing itself in the adaptative reorganization of structural-functional characteristic of cells/tissues and the most complicated hierarchy of regulatory interrelations. In case the effect of damage factor continues, spare capacities inevitably exhaust and «defatigation phase» comes. This phase is characterized by intensive destruction, nonreversible metabolic disturbance and the death of cells. In respect to cut off roots this phenomena is known as «adaptative aging» or «survival» [1], and the understanding of its nature lies within the modern concept of stress and general principles of responsiveness of living system [5,6]. At the same time biophysical aspects of prolonged stress response of roots, particularly its relationship with the process of self-diffusion of water, need in the additional study and precise interpretation.

Thereby the aim of the present work is the research of dependence of self-diffusion of water molecules on «adaptative aging» time of vegetable patterns in the conditions of prolonged mechanical stress.

1. Methods and objects of research

1.1 Diffusion decay

Primary information about self-diffusion in PGSE technique is obtained due to DD analysis. Diffusion decay is known to be logarithm dependence with normalized amplitude of spin echo

\[ M(k^2) = \frac{A(k^2)}{A(0)} \]

on the square wave-vector \( k^2 = \gamma \delta g^2 \) and diffusion time \( t_d \). Here \( \gamma \) – gyromagnetic ratio of proton, \( \delta \) - duration and \( g \) – amplitude pulse of the gradient of magnetic field. DD have been registered by means of diffusiometer with computer process control and resonance frequency of protons equal to value 60 MHz. Peak amplitude of gradient pulse was equal \( \approx 30 \) T/m.

Measurements have been made by «stimulated echo» technique [6] with diffusion time \( t_d \approx 10, 155 \) and \( 350 \) ms. The registration time of one DD was determined by accumulation number (from 4 to 6) and was equal \( \approx 25 - 40 \) min. Measurements were taken at 23°C degree. The measurement error was equal \( \approx 10\% \) in each case.

![Figure 1. Diffusion decay in the segments of roots with \( t_d = 10 \) ms.](image)

Figure 1 shows DD in the research samples at \( t_d =10 \) ms. DD had the same nonexponential shape also at \( t_d =155 \) and \( 350 \) ms. In each case DD may be described by sum of three exponents

\[ A(k^2) = p_1 \exp(-D_1 t_d k^2) + p_2 \exp(-D_2 t_d k^2) + p_3 \exp(-D_3 t_d k^2). \]

Here \( p_i \) \( D_i \) (i = 1, 2, 3) – populations and SDC (\( D_1 < D_2 < D_3 \)) respectively. Differentiation of DD components was performed on computer by algorithm step-by-step subtraction with «ORIGIN 7.5».

1.2 Objects of research

Population and SDC of the components were identified while approximating by linear functions.
Objects of research were primary roots of 7-day-old plantlets of winter wheat (Triticum aestivum) of Mironovskaja 808 brand, handled in a water culture with luminance 100 W/m² and 12-hour photoperiod. The above-ground part of fasciculate plants was cut off, but 2-3 cm of spear. This material was incubated in double distilled water pending for 3 h. After that the segment of 8 mm lengthwise matching tension zone and the origin of differentiation zone were cut out of each root. Parallel oriented segments of roots, 60-70 in number, were shaped in the pack, which was wrapped up by thin fluoroplastic film and put in hermetic glass ampoule. The sample was disposed in the NMR probe perpendicularly to vector pulse gradient of the magnetic field. Due to the said position we could observe the self-diffusion in direction, perpendicularly to longitudinal axis of root. In each case measurements were completed before defatigation phase, thus and so further alongside with the term «adaptative aging» we’ll use the term «lifetime».

2. Results and discussion
In this report we discuss only the dependences, obtained at $t_d = 350$ ms, the plots of them shown on figure 2. Mean-square displacement, to which the molecule of water is subjected in the direction of the gradient at $t_d = 350$ ms, according to Einstein equation $<r^2>^{1/2} = 2D_t t_d$ is ranged ≈ from 19 to 33 micron, what is comparable with the cell size of root tension zone.

![Figure 2. Dependences of $D_2$ (1), $p_2$ (2) и $D_3$ (3), $p_3$ (4) on a lifetime sample and $t_d = 350$ mc.](image)

According to existing opinions [7-9], molecules of apoplast and simplast vacuole water contribute to component DD with biggest SDC $D_3$. Protons simplast cytoplasm water contributes to the component characterized by $D_2$. As for the component, characterized by $D_1$, the definite opinion is not known at the time being [3]. The parameters $D_1$ and $p_1$ of the slow component do not depend upon lifetime and therefore are not shown in figure 2.

The present results testify practically determined change of translational mobility of water molecules, starting in the cellular space of roots’ segments after they were cut off. This process should be considered as the part of the total cell reaction against the stress caused by the stop of the directed water stream and the replacement the external water medium by the air medium. It is experimentally determined [1], that biggest stimulation of a number of functional systems: breath and oxidative association with phosphorus, ion pump, and also polarization and restoration of barrier properties of plasmatic membrane takes place in the cells of roots in 4 – 6 hours after their cutting off. Since maximums of diffusion characteristics ($D_2$, $D_3$ и $p_2$) are being observed in the same time range (figure 2), there are good reasons to expect close interconnection between translational mobility of water molecules and functional stressful responses of the roots.

The response to water deficiency is the compression of protoplast [10] and, as consequence, the reduction of vacuole volume, which normally takes up to 80 – 90% of the cell volume in the tension zone [11]. As a result, the osmotic pressure in vacuole increases, and the water stream running
through tonoplast and plasmalemma makes its way in direction to the cytoplasm. Therefore population $p_3$ decreases, while $p_2$ and $D_2$ of cytoplasmic water increase due to more mobile water molecules of vacuole. SDC $D_3$ also increases as a result of probable augmentation of the relative portion of apoplastic water molecules.

**Conclusion**

The research has shown that diffusion decay in segments of roots of plantlet winter wheat under hours-long effect of mechanical stress is of irregular shape. The analysis of DD by its approximation by the sum of the three exponents reveals the response to the stress in a form of a distinct extreme dependence of self-diffusion coefficients and populations of intermediate and rapidly decaying component on the lifetime of the sample. For explanation of results thus obtained the model based on the change of the water diffusion direction from vacuole to cytoplasm and inversely through aquaporin water canals of tonoplast has been proposed. It is hypothesized, that periodical change of diffusion direction prevents the critical deaquation and, eo ipso, provides for preservation of the cells water homeostasis being subjected to stresses, by maintenance of coordinated variations of volume and water content of vacuole and cytosolic compartments.

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