PEG 8000 INDUCED OSMOTIC STRESS CAUSES DIFFERENTIAL EFFLUX OF METAL CATIONS FROM WHEAT LEAVES

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ABSTRASCT

Osmotic stress induced by incubation of young wheat (*Triticum aestivum*, L) plants roots in PEG 8000 solution led to reduction in leaf relative water content and caused increased ion leakage from the leaves. Higher amounts of metal caions tended to leak from the leaves of stressed compared to untreated plants and the overall electrolyte leakage from PEG-treated samples was also greater. It was concluded that the capacity for better water retention could be connected with the retention of potassium ions, which participate in many cellular processes and represent the major osmotic solute in plant cells. Enhanced electrolyte leakage from stressed leaves could be indicative of altered membrane permeability or could be connected with specific protective mechanisms involved in the process of osmotic adjustment at the cellular level.

Key words*: cell membranes, electrolyte leakage, metal cations, polyethylene glycol, wheat.*

Water deficit severely inhibits plant growth and development thus limiting production and performance of cultivated plants. Dehydration causes wilting and decrease in cell turgor and thus contributes to the increasing of ion concentration in the cytosol. When plants are exposed to water deprivation certain inorganic ions and so called compatible solutes tend to accumulate in the cytosol in a process known as osmotic adjustment (Filek et al., 2012; Hare et al., 1998). These osmolytes lower cellular osmotic potential, allow turgor maintenance and protect biomacromolecules against destabilizing effects of oxidative stress.

Study of physiological processes in plants under suboptimal or stress conditions could contribute to our knowledge of metabolic plasticity and could reveal some of the mechanisms involved in overcoming the effects of unfavorable climatic conditions. A common and widely applied alternative to measuring drought stress response in the field is to study the reaction of plants to polyethylene glycol (PEG)-induced osmotic stress under laboratory conditions. The use of PEG solutions for the induction of osmotic stress provides an option for precise control of the degree of dehydration within a wide range of osmotic potentials (Filek et al., 2012).

Plasma membranes are the first receptors of stress and they can protect the cell through modifications that affect perception and rigidity of cellular structures (Farooq and Azam 2006). Changes in membrane organization and composition lead to enhanced electrolyte leakage which could be determined by conductometric measurement (Bajji et al., 2002). Increased ion leakage has long been used as an indicator of stress impact on cell membrane permeability (Prášil and Zámečník, 1998). The implementation of conductometry as a highly sensitive and precise method is justified for assessment of ion leakage from plant tissues since it offers valuable information of membrane damages caused by various environmental factors (Farooq and Azam 2006; Roy et al. 2009). In numerous investigations this data substantially expands the view of the plant overall physiological status.

Wheat (*Triticum aestivum*, L.) is an important and widely distributed crop which is often exposed to the environmental adversities. Investigating plant stress responses is essential for understanding the mechanisms leading to tolerance and adaptation. The aim of the present study was to assess the effect of osmotic stress on ion accumulation and leakage in young wheat plants and their relation to membrane functioning under normal conditions and dehydration.

MATERIALS AND METHODS

Seeds of common Bulgarian wheat cultivar Katya were hydroponically grown under controlled light and temperature conditions with 14 hours photoperiod, 23/17 °C day/night temperature, irradiance of 250 µmol m^{-2} s^{-1} and 70 % relative humidity. Nutrient solution contained 3.4 mM $Ca(NO_3)_2$, 1.5 mM KH_2PO_4, 2.0 mM KNO_3, 0.8 mM $MgSO_4$, 1.3 mM KCl, 90 µM Fe-EDTA and micronutrients. Plants grown on nutrient solution served as untreated controls, while others were subjected to osmotic stress by immersing their roots for 24 hours in 30% PEG 8000 dissolved in nutrient solution. The calculated osmotic potential of the resulting medium was –1.9MPa (Money, 1989).

Relative water content (RWC) in the leaves was measured according to Turner (1981) and was calculated as:

$$RWC, \% = (FW-DW)/(TW-DW) \times 100,$$

where FW is fresh weight, TW is leaf weight at full turgescence, and DW is dry weight.

Electrolyte leakage from leaves was measured with conductometer Elwro 5721 (Poland). Ten leaf pieces 2 cm in length were immersed in 20 ml of distilled water. Conductivity of the solutions was measured at multiple time points during 24 hours of incubation at 20ºC in the dark (κ) and after subsequent boiling of samples (κ_{max}, total ion content). Ion leakage kinetics was expressed as the relation of κ/κ_{max} for each time point of the incubation period. Fitting of experimental data was performed by the Exponential Associate function of Origin 5.0 software which implements a non-linear regression algorithm with multiple iteration procedures (Kocheva et al., 2014). It was established that time changes in ion concentration in the incubation medium could be given by the following equation:

$$\kappa/\kappa_{max} = C_o(t) \approx A_1(1-e^{-t/t1}) + A_2(1-e^{-t/t2}) + C_o^o$$

Metal ions content in water extracts from leaves was measured by ICP spectrometer Jobin Yvon Ultima (France) twice: (1) at the end of the 24-hours incubation and (2) after subsequent boiling of the samples. Total content of a specific ion type was considered the sum of the two measured concentrations (1+2). In results, leaked ions of a certain type were presented as percentage of the overall concentration of the respective ion species in leaves of untreated and PEG-stressed plants: (1)/(1+2) × 100.

RESULTS AND DISCUSSION

To quantify the degree of dehydration leaf water status was estimated by measurement of relative water content (RWC), which expressed the capacity of conserving cellular hydration under physiological water starvation. Imposition of osmotic stress with PEG 8000 for 24 hours caused a drastic decrease of RWC in the leaves of young wheat plants (Table 1). This simulated dehydration could be ascribed to the ability of PEG to reduce the osmotic potential of the solution and thus to hamper roots suction capacity and water transport toward leaves, without impeding stomatal transpiration.

Variant	*RWC, %*
Control	96.8 ± 0.8
PEG stress	48.8 ± 3.4

Table 1. Relative water content (RWC) in leaves of wheat plants grown on nutrient solution (Control) and subjected to 24-hour treatment with PEG 8000.

PEG-induced osmotic stress caused a two-fold increase in leaf ion leakage in comparison to untreated plants as suggested by higher values of calculated relative conductivity (κ/κ_{max}) (Fig. 1).

It is generally accepted that electrolyte leakage from plant tissues could be a reliable indicator of the degree of cell membrane damage caused by various types stress (Bajjii et al., 2002; Kocheva et al., 2014). It could be speculated that the applied osmotic stress with PEG induced changes in membrane permeability of leaf cells which could reflect either damage or the existence of an adaptive mechanism involving ion expulsion in order to protect the dehydrated cytosol from toxic ion concentrations.

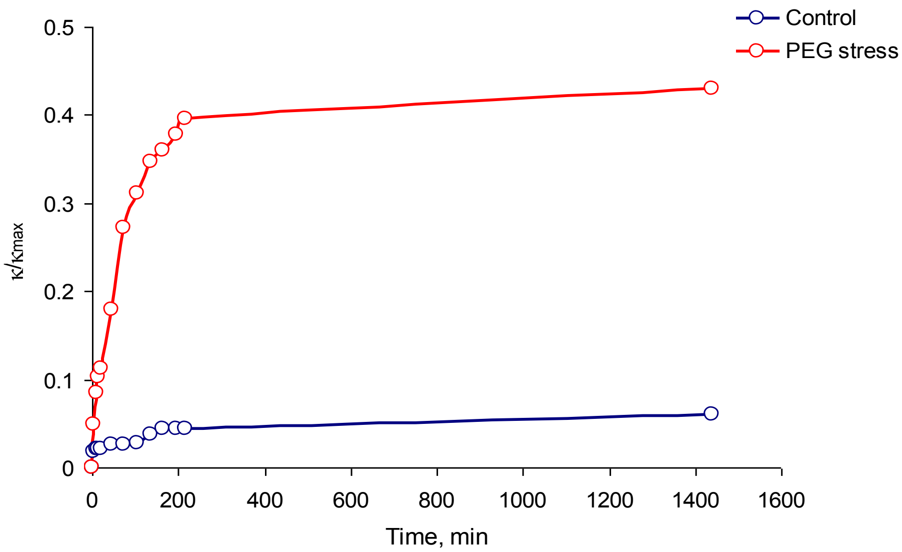

Fig. 1. Kinetics of electrolyte leakage from leaves of wheat plants under normal (Control) and PEG-stress (Stress) conditions.

Conductivity measurements on wheat plants subjected to osmotic stress by PEG offered information about the total amount of ions that were driven by the concentration gradient in the processes of osmosis and leaked through the plasmalemma into the distilled water in which the leaves were immersed. The discrete amounts of potassium (K^+), sodium (Na^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) ions which leaked into the outer solution were measured by ICP spectrometry. It was found that untreated samples had lower ion content while higher levels of all four measured ions were found in the leaves of stressed plants (Fig. 2). As in the case of increased relative conductivity, this observation was indicative of greater ion efflux through cell membranes and hence more damaged permeability and impaired membrane functioning in PEG-treated leaves.

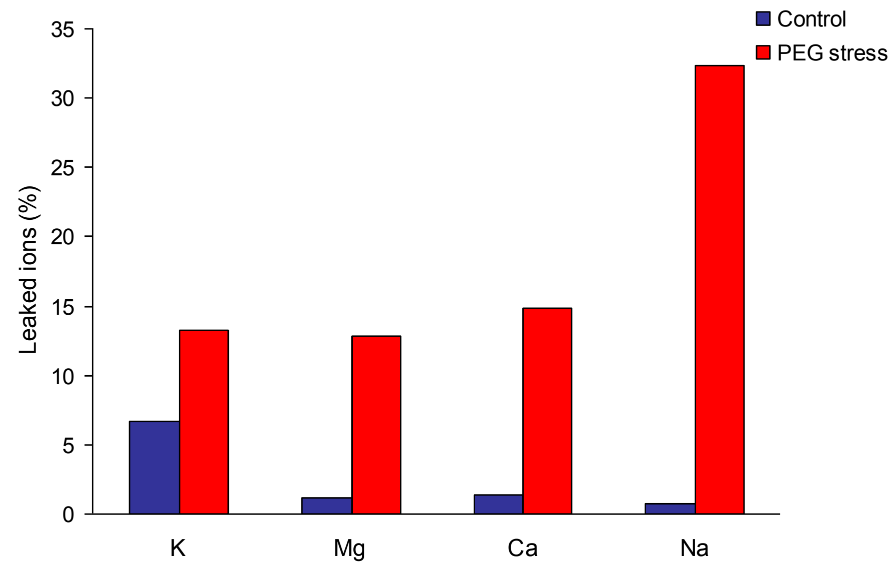

Fig. 2. Percentage of K^+, Mg^{2+}, Ca^{2+} and Na^+ leaked from leaves of untreated (Control) and PEG-stressed wheat plants related to the overall content of the respective ion.

In control plants, among the studied ion species K^+ demonstrated the highest percentage of leakage, assessed on the basis of its total content in the leaves (Fig. 2). This is not surprising since potassium is the most abundant cation in plant cells with important biological functions (Britto and Kronzucker, 2008). In stressed plants, highest percentage of leakage was observed regarding Na^{2+}. It has been hypothesized that Na^+ ions may have a positive impact on water stress response since plants specifically increase their absorption of this cation under drought conditions. Filek et al. (2012) suggested that sodium ions were involved in the resistance to PEG-induced dehydration by directly or indirectly influencing the accumulation of other compounds involved in osmotic adaptation.

It is generally accepted that Ca^{2+} can stabilize membranes or affect the ability of biomembranes to selectively absorb certain ions, while Mg^{2+} are important in their role as cofactors for many cellular enzymes (Karley et al., 2000). Plants also use these inorganic ions for osmoregulation under drought stress (Hare et al., 1998).

Presented here results indicated that lowered water content was accompanied with increased ion efflux from the leaves of PEG-stressed wheat plants in comparison with untreated ones. A probable explanation of these observations could be sought in the action of cellular mechanisms for membrane protection against damage, in combination with effective water management focused on reduction of transpiration losses and maintenance of water balance in the leaves of osmotically stressed wheat plants.

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