ELECTROLYTE LEAKAGE AND K^+ IONS CONTENT IN THE LEAVES OF WHEAT PLANTS SUBJECTED TO POLYETHYLENE GLYCOL TREATMENT

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ABSTRASCT

The effect of PEG-induced osmotic stress on relative water content and electrolyte leakage from the leaves of two contrasting wheat (*Triticum aestivum*, L) cultivars was compared in the study. Drought tolerant cv. Katya retained higher water content in the leaves after imposition of osmotic stress in comparison with cv. Prelom. Higher amount of potassium ions tended to leak from the leaves of stressed cv. Prelom plants and the overall electrolyte leakage from the leaves of this cultivar was also greater in comparison with cv. Katya. It was concluded that capacity for better water retention in the tolerant cv. Katya could be connected with the retention of potassium ions which participate in many cellular processes and represent the major osmotic solute in plant cells. Lower electrolyte leakage from stressed Katya leaves in comparison to Prelom was indicative of less damaged cellular membrane permeability which could be connected with superior protective mechanisms.

***Key words**: electrolyte leakage, polyethylene glycol, potassium ions, wheat.*

Wheat is a major and widely distributed crop which is often exposed to climatic adversities. Among the abiotic factors affecting yield and quality of production drought is one of the most frequent. Owing to the complex nature of drought tolerance, the problems related to plants ability to withstand water deprivation require investigation at many different levels. 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 environmental conditions. Induction of stress in laboratory conditions has certain advantages over field experiments as controlled growing conditions offer better reproducibility of the results and the possibility for separate evaluation of a particular limiting factor thus avoiding the interference of stressors. The use of polyethylene glycol (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).

Cellular membranes are among the first targets of water stress and plant dehydration. Commonly, changes in membrane organization and composition lead to enhanced electrolyte leakage which could be determined by conductometric measurement. Ion leakage from plant tissues is widely used as a measure of cell membrane stability in the assessment of the impact of various stress factors. It is measured by common techniques for analysis of conductivity of the solutions in which tissues are immersed (Bajji et al. 2002). Thus the conductivity of distilled water in which the samples are submerged naturally increases owing to the flow of ions from the intercellular spaces driven by concentration gradient in the process of diffusion. When the normal membrane permeability is disturbed an increased leakage occurs. 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 (Filek et al. 2012). In numerous investigations this data substantially expands the view of the plant overall physiological status. Empirical dependences exist which connect conductivity of the outer solution to the concentration of main ion species (mostly K^+) flowing out of plant tissues (Palta and Li, 1980). Potassium is the most abundant ion in the plant cell which is related to its multiple functions such as osmoticum, role in maintenance of membrane potentials, regulation of stomatal and nastic movements etc. The objective of the present study is to evaluate electrolyte leakage from the leaves of control and osmotically stressed wheat plants and to relate it to with the amount of potassium ions efflux in the outer solution.

MATERIALS AND METHODS

Seeds of two common Bulgarian wheat (*Triticum aestivum*, L.) cultivars, Katya and Prelom, 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. Osmotic stress was induced by application of 30% PEG 8000 dissolved in nutrient solution for 24 hours. The calculated osmotic potential of the media is – 1.9MPa (Money, 1989). Plants were grown on nutrient solution served as untreated control.

Relative water content (RWC) in the leaves was measured according to Turner, 1980 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 a conductometer Elwro 5721 (Poland). Ten leaf pieces 2 cm in length were immersed in 20 ml of distilled water. Conductivity of the solutions was first measured after 24 hours of incubation at 20ºC in the dark (κ) and once again after subsequent boiling of samples (κ_{max}, total ion content). Results were expressed as the relation κ/κ_{max}. High values of the relative conductivity are indicative of greater amount of ions flown into the outer solution which signifies damaged membrane permeability.

Potassium ion content in water extracts from leaves was measured by ICP spectrometer Jobin Yvon Ultima (France) before and after boiling of the samples.

RESULTS AND DISCUSSION

Relative water content in the leaves of the two wheat cultivars declined significantly after the imposition of osmotic stress with PEG 8000 (Fig. 1). This could be ascribed to the effect of dehydration which PEG caused as a strong osmoticum. It reduced the osmotic potential of the media and consequently hampered root water suction ability as well as water transport toward leaves, while stomatal transpiration continued. Greater RWC values were found in drought tolerant cv. Katya which probably actuated better water managing mechanisms under osmotic stress.

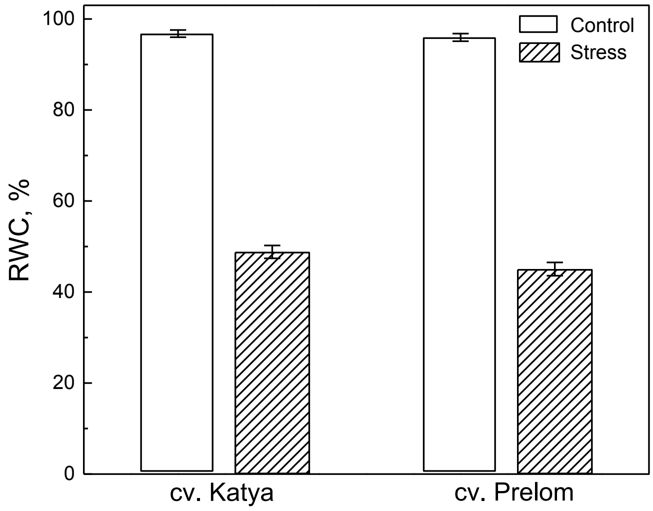

Fig. 1. Relative water content in the leaves of two wheat cultivars under normal and osmotic stress conditions.

Enhanced electrolyte leakage from plant tissues is widely used as a reliable indicator for the degree of cell membrane damage caused by various types stress (Bajjii et al. 2002; Kocheva and Georgiev, 2005). PEG-induced osmotic stress reflected in increased relative conductivity of both cultivars as compared to respective untreated controls (Fig. 2). It was more pronounced in cv. Prelom where a three-fold increase in the value κ/κ_{max} was registered after stress. In stressed cv. Katya relative conductivity was twice increased as compared to untreated control. This justifies our supposition that the applied osmotic stress with PEG induced greater damages in leaf membrane permeability in cv. Prelom plants.

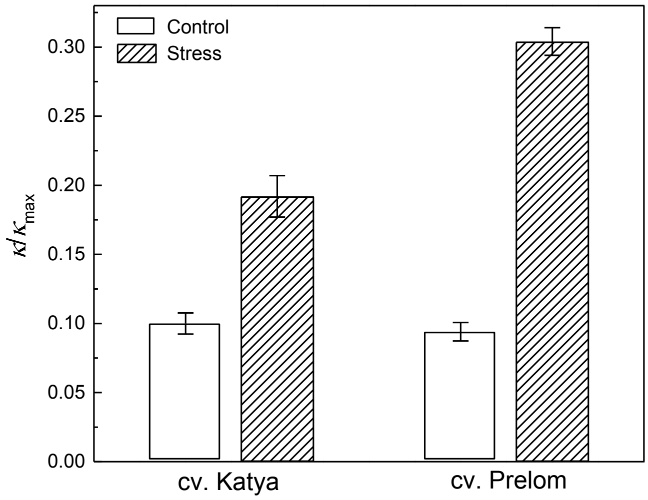

Fig. 2. Relative conductivity of solutions with leaf samples from two wheat cultivars under normal and PEG-stress conditions.

Conductivity measurements in wheat cultivars subjected to osmotic stress by PEG offered information about the total amount of ions which were driven by the concentration gradient and in the processes of osmosis through the plasmalemma passed from the intercellular spaces into the outer solutions in which the leaves were immersed. The amount of potassium ions which leaked into the outer solution was measured spectrometriclly by icp. Untreated samples had lower potassium content while highest levels were found in the leaves of stressed Prelom plants (Table 1). As in the case of increase relative conductivity, this observation was indicative of greater K^+ efflux through cell membranes and hence more damaged permeability and impaired membrane functioning in the leaves of cv. Prelom.

Variant	*K^+, g/L*
Katya control	0.141
Katya stress	0.223
Prelom control	0.199
Prelom stress	0.276

Table 1. Amount of potassium ions leaked from the leaves of two wheat cultivars under normal and PEG-stress conditions.

Presented results indicate lowered water content and increased ion efflux (particularly K^+) from the leaves of wheat plants of cv. Prelom in comparison with drought tolerant Katya. One probable explanation of these observations could be sought in the action of cellular mechanisms for membrane protection from damage, in combination with more effective water management focused on reduction of transpiration losses and maintenance of water balance in the leaves of cv. Katya.

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