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PARAMETERS OF CELL MEMBRANE STABILITY AND LEVELS OF OXIDATIVE STRESS IN LEAVES OF WHEAT SEEDLINGS TREATED WITH PEG 6000

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Summary. Wheat (*Triticum aestivum* L.) seedlings of cv. Chinese Spring (CS) and hybrid Line 73 (breeding material based on a wide cross CS x *Aegilops geniculata* Roth) were grown for 7 days on a half strength Hoagland nutrient solution and afterwards transferred to 15 % PEG 6000. Changes in the parameters of cell membrane stability (electrolyte leakage kinetics), level of oxidative stress (MDA and hydrogen peroxide content) and leaf water status assessed by RWC during 5-days (mild) and 8-days (strong) water stress were investigated. The longer stress duration caused stronger dehydration. RWC of the leaves was strongly reduced in both genotypes. The level of water stress in the parental genotype correlated with greater lipid peroxidation (assessed as higher content of MDA) and reduced membrane stability (higher electrolyte leakage from damaged tissues) in leaves. These disturbances in the parental genotype corresponded to higher concentration of hydrogen peroxide of which Line 73 showed lower values. The selected genotype Line 73 disclosed higher tolerance to osmotic stress with PEG 6000 in laboratory conditions as assessed by the studied parameters.

Keywords: electrolyte leakage; H_2O_2; osmotic stress; polyethylene glycol; wheat.

Abbreviations: MDA – malondialdehyde; PEG – polyethyleneglycol; RWC – relative water content.

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INTRODUCTION

Drought is among the most damaging abiotic factors (Smirnoff N, 1993). Besides its direct impact on water status osmotic stress often harmfully affects plant cell membranes. Symptoms of these adverse processes include oxidation of unsaturated fatty acids, protein degradation, and the resultant loss of selective permeability of membranes (Hoekstra and Golovina, 1999). Malondialdehyde (MDA) is a harmful lipid peroxidation product and hydrogen peroxide (H_2O_2) represents a highly toxic active oxygen species that can damage many important cellular components (Kuzniak and Urbanek, 2000). Electrolyte leakage from tissues is often used as a measure of the stress impact on plant membrane stability (Murry et al., 1989). Recent results show that cell walls are also involved in plant stress response (Konno et al., 2008; Piro et al., 2003; Hoson, 1998). In the present study, a laboratory test-system was applied for simulating drought by treating the roots with polyethylene glycol (PEG). PEG is a non-toxic and non-penetrating osmotically active polymer which causes dehydration by lowering water availability to the plant.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) plants from cv. Chinese Spring (CS) and hybrid Line 73 were used for the experiments. Line 73 is isolated from advanced backcross derivatives of an amphiploid between CS and the wild related species *Aegilops geniculata* Roth, known for its resistance to abiotic stress, including drought (Zaharieva et al., 2001). By molecular cytogenetic methods (FISH and GISH) it has been proved that the line carries a pair of alien chromosomes substituted for the wheat 2A chromosome pair (Landjeva et al., 2009). Seeds were superficially washed and then soaked in tap water for 2 h. Seedlings were grown 7 days on half strength Hoagland nutrient solution and afterwards were transferred to 15 % PEG 6000 dissolved in Hoagland. Thus the water potential (Ψ) of the medium is reduced to approximately - 0.75 MPa. Two types of osmotic stress were imposed - 5-days on PEG, referred to as mild and 8-days on PEG – referred to as strong.

Relative water content (RWC)

RWC was estimated according to Turner (1981) and was evaluated from the equation:

$$RWC = (FW–DW)/(TW–DW).100$$

where FW is the fresh weight of the leaves, TW is the weight at full turgor, measured after floating the leaves for 24 h in water in the light at room temperature and DW is the weight estimated after drying the leaves for 4 h at 80°C or until a constant weight is achieved.

Measurements of malondialdehyde and hydrogen peroxide

Accumulation of malondialdehyde (MDA) in leaves was determined according to the method of Cakmak and Horst (1991). For the analyses, 0.3 g leaves were homogenized in 3 ml 0.1% trichloroacetic acid and extract was clarified by centrifugation (10,000 x *g* for 20 min at 4°C). A 0.5 ml aliquot of supernatant was added to 1.5 ml 0.5% (w/v) thiobarbituric acid in 20 % (w/v) trichloroacetic acid. The mixture was kept in a boiling water bath for 30 min and then quickly cooled in an ice bath. Absorbance was measured spectrophotometrically at 532 nm and corrected for non-specific absorption at 600 nm. MDA content was calculated using a molar extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol g^{-1} DW. Hydrogen peroxide was measured spectrophotometrically according to Alexieva et al. (2001). The reaction mixture contained 0.5 ml leaf extract in 0.5% trichloroacetic acid (TCA), 0.5 ml 100 mM K-phosphate buffer (pH 7.4) and 2 ml reagent (0.5M KI w/v in fresh distilled water). The blank probe contained 0.5% TCA in the absence of leaf extract. The reaction was developed for 1 h in darkness and absorbance measured at 352 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2.

Electrolyte leakage

For the determination of Injury index 15 leaf pieces (2 cm in length) were cut from stressed and control plants and immersed in 15 ml distilled water at room temperature. Conductivity of the solutions was measured periodically during a 24-h-period as previously described (Kocheva et al., 2005). Results are expressed as the relation κ/κ_{max} where κ is conductivity measured at each time point of samples and κ_{max} is the total electrolyte content determined after killing the tissues by boiling. A two-phase kinetics was evidenced. Best fit of experimental data was accomplished by an exponential associate function (NLSF procedure, Origin 5.0) with three variable parameters (a, b and c). An additional parameter T was defined as a combination of the other three and was called 'period of the prompt phase'

$$T = \frac{\ln(ab/c)}{a}$$

Statistical analysis

Three independent experiments were conducted and all parameters were measured in at least 3 replications each time. Data are presented as mean values ±SD.

RESULTS AND DISCUSSION

It was established that RWC correlates well with the degree of stress and could be used as an accurate measure of stress degree (Turner 1981). In our experiments mild osmotic stress had a slighter effect on plant water relations in comparison with prolonged PEG treatment. Mild stress caused a decrease in RWC to about 42 % in the leaves of parental genotype and 51 % in the hybrid line (Fig. 1C). Increasing the duration of PEG treatment resulted in a stronger decrease in RWC corresponding to a greater water loss from the leaves reaching values as low as 22 % in parent, but 36 % in Line 73. In this respect, a more stable water balance was conserved in the hybrid line as compared with the parental genotype especially under conditions of prolonged stress. Although strong stress caused similar changes in H_2O_2 content, under mild stress Line 73 maintained lower values of this reactive oxygen species as compared with the parent (Fig. 1A). Peroxide accumulation in cells has its impact on the levels of lipid peroxidation

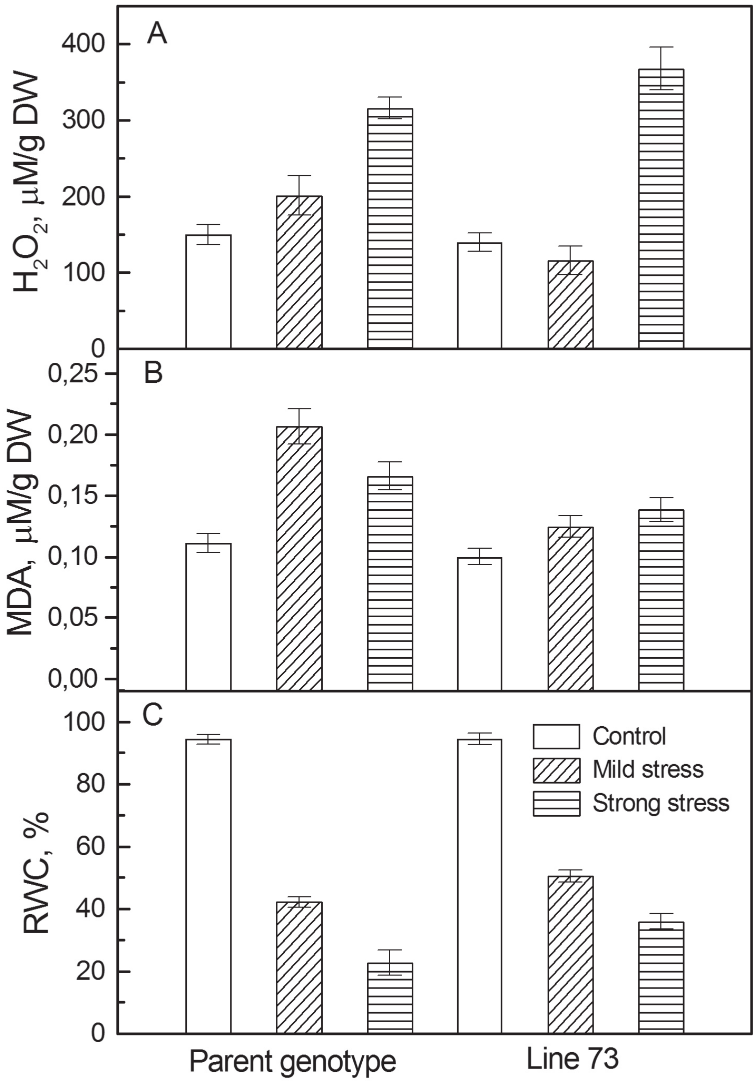

Fig. 1. Effect of PEG treatment on relative water content (RWC), hydrogen peroxide and malondialdehyde (MDA) concentrations in the leaves of two wheat cultivars. Treatment with 15% PEG was performed for 5 days (mild stress) or 8 days (strong stress).

products such as MDA (Noctor and Foyer, 1998). Both genotypes showed increase in MDA content but in the hybrid line lower values were detected (Fig. 1B). Prolonged treatment with 15% PEG had stronger effect on treated plants and disclosed more differences between the genotypes than mild stress. However, the treatment had similar effect on the two genotypes which indicates similarities in the stress response as a whole. The electrolyte leakage kinetics for the two genotypes (Fig. 2, Table 2) showed reduced time of the prompt phase T which describes the ion efflux from the apoplast. As shown previously (Kocheva et al., 2005), this parameter has proven to be useful in assessing the stress degree apart from the commonly used index for cell membrane stability (Farooq and Azam, 2006). Moreover, its variations

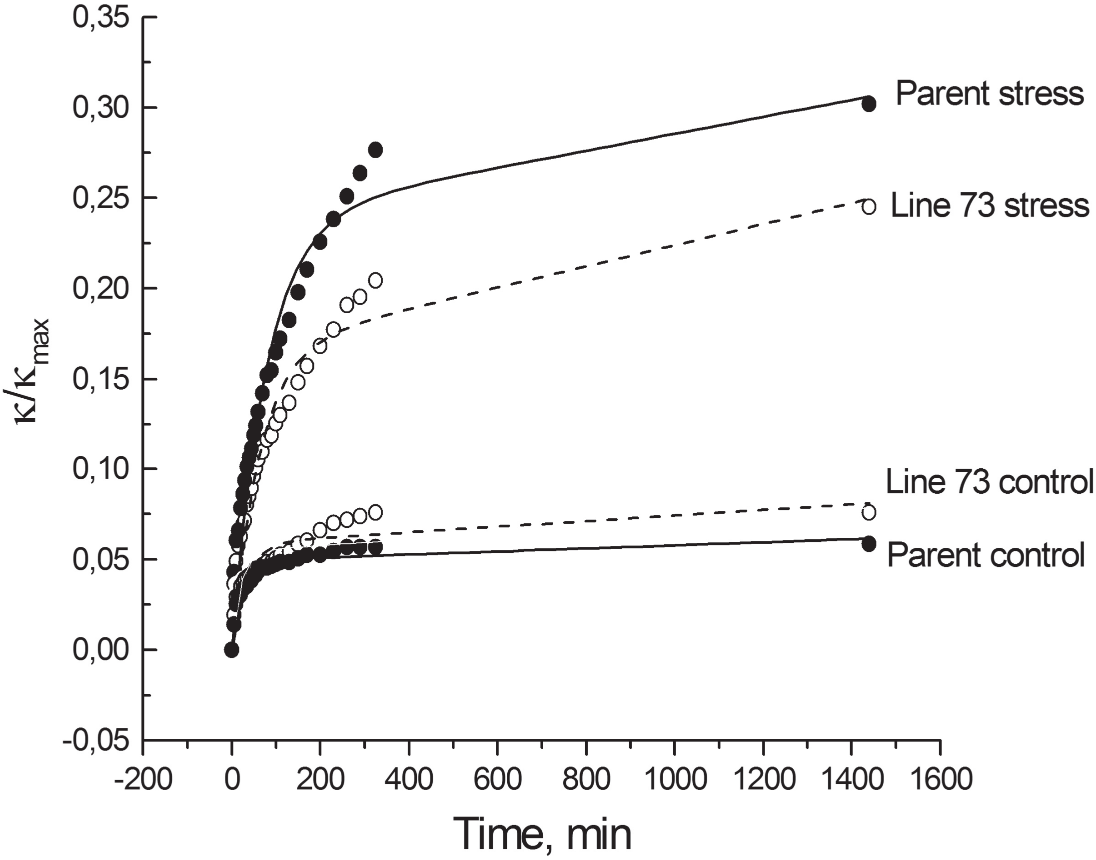

Fig. 2. Kinetics of electrolyte leakage from leaf segments of wheat plants from two cultivars treated with 15 % PEG for 8 days. Normalized curves of conductivity (κ/κ_{max}) versus time of incubation are shown.

correlated well with the maintenance of higher RWC in the leaves of Line 73 in comparison with the parental genotype. The faster ion release from the apoplast (as indicated by T) could be regarded as a result of greater damage in cell membrane permeability. Lower values of T could evidence loss of cell membrane stability or higher membrane injury. This idea is supported by the lower content of lipid peroxidation product (MDA) in stressed leaves of Line 73. In addition, H_2O_2 values in Line 73 could be due to a more effective

Table 1. Estimated period T of the prompt phase of ion leakage kinetics measured in the leaves of two wheat genotypes subjected to prolonged (8-days) osmotic stress with PEG.

Variants	T, min
Parent control	107.95 ± 8.0
Parent stress	16.13 ± 2.3
Line 73 control	106 ± 7.4
Line 73 stress	17.32 ± 3.1

antioxidant defense system of the hybrid line (Fu and Huang, 2001). The presence of alien chromosomes substituted for wheat chromosomes in the hybrid line could have affected the physiological response of wheat plants with positive impact on the water stress tolerance. These experiments demonstrate some differences in plant water relations and cell membrane stability of the studied genotypes as well as various levels of oxidative stress development. It could be concluded that the hybrid line (Line 73) acquired higher RWC than the parent after PEG treatment thus indicating higher capability of maintaining the water status under stress conditions and possibly advanced dehydration tolerance. The latter could be attributed to an effective change in the genotype of the hybrid line.

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