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A morpho-physiological approach differentiates bread wheat cultivars of contrasting tolerance under cyclic water stress

Katalin Jäger[a], Attila Fábián[a], Gabriella Eitel[a], László Szabó[b], Csilla Deák[c], Beáta Barnabás[a], István Papp[c,*]

[a] Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik u. 2, 2462 Martonvásár, Hungary
[b] Department of Functional and Structural Materials, Institute of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Pusztaszeri út 59-67, 1025 Budapest, Hungary
[c] Department of Plant Physiology and Plant Biochemistry, Faculty of Horticultural Science, Corvinus University of Budapest, Villányi út 29-43, 1118 Budapest, Hungary

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ABSTRACT

Leaf micromorphological traits and some physiological parameters with potential relevance to drought tolerance mechanisms were investigated in four selected winter wheat varieties. Plants were subjected to two cycles of drought treatment at anthesis. Yield components confirmed contrasting drought-sensitive and -tolerant behavior of the genotypes. Drought tolerance was associated with small flag leaf surfaces and less frequent occurrence of stomata. Substantial variation of leaf cuticular thickness was found among the cultivars. Thin cuticle coincided with drought sensitivity and correlated with a high rate of dark-adapted water loss from leaves. Unlike in *Arabidopsis*, thickening of the cuticular matrix in response to water deprivation did not occur. Water stress induced epicuticular wax crystal depositions preferentially on the abaxial leaf surfaces. According to microscopy and electrolyte leakage measurements from leaf tissues, membrane integrity was lost earlier or to a higher extent in sensitive than in tolerant genotypes. Cellular damage and a decline of relative water content of leaves in sensitive cultivars became distinctive during the second cycle of water deprivation. Our results indicate strong variation of traits with potential contribution to the complex phenotype of drought tolerance in wheat genotypes. The maintained membrane integrity and relative water content values during repeated water limited periods were found to correlate with drought tolerance in the selection of cultivars investigated.

Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important staple crops with potential sensitivity to water stress, especially in the reproductive stage. Drought tolerance of wheat is a complex trait with multifactorial determination, depending on interactions of genotypes and environmental conditions (Araus et al., 2002; Szűcs et al., 2010).

In addition to stomatal transpiration (Sirichandra et al., 2009), the cuticle contributes significantly to water loss, especially under water limited stress conditions (Riederer and Schreiber, 2001). Micromorphological features and properties of this layer may have an impact on water loss and drought tolerance. The cuticle of cereals was found to be relevant in this respect (Rawson and Clarke, 1988), which was also confirmed by recent research efforts (González and Ayerbe, 2010; Wang et al., 2012a,b).

Water limitation and a range of other abiotic stresses may result in oxidative burden to plants (Mittler, 2002). Diverse antioxidant defense responses are mobilized in order to prevent excessive accumulation of potentially harmful reactive oxygen species (ROS) (Gill and Tuteja, 2010). Sustained drought stress may result in abundant ROS production that cannot be balanced by the antioxidant system, leading to deleterious oxidative events including membrane damage and cell death (Cruz de Carvalho, 2008). Membrane injury has been shown to occur during severe water stress in wheat and has been associated with drought sensitivity and poor induction of antioxidant defense responses (Khanna-Chopra and Selote, 2007). Structural damage of organelles has been found to be associated with drought sensitivity in some other crops as well (Zhang et al., 2010).

This study investigates drought-sensitive Cappelle Desprez, GK Élet and tolerant Plainsman V, Mv Emese winter wheat cultivars subjected to two cycles of drought during anthesis and at early

* Corresponding author. Tel.: +36 1 482 6095; fax: +36 1 209 6388.
E-mail address: istvan.papp2@uni-corvinus.hu (I. Papp).

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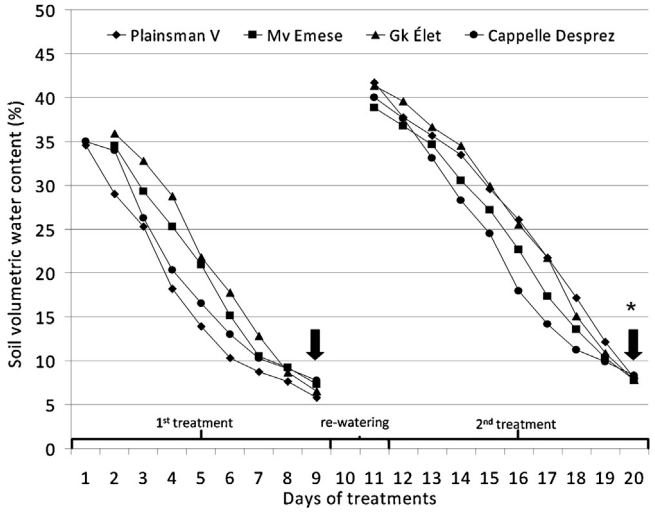

Fig. 1. Volumetric water content of soil during the two drought cycles of the experiment. Arrows indicate sampling dates for relative water content (RWC), relative water loss (RWL) and ion leakage measurements; asterisk marks samplings for light and electron microscopic studies.

stages of kernel development and grain filling. Cycles of water deprivation are thought to approximate natural conditions better than single treatments (Izanloo et al., 2008). Yield parameters as well as micromorphological traits with potential significance to drought hardiness were explored. We aimed to reveal differences of epidermal structures among the genotypes, with an emphasis on stomata and the cuticle. Cellular integrity of mesophyll cells and the rate of electrolyte leakage from flag leaves were also investigated during cyclic drought stress periods.

Materials and methods

Plant material and stress conditions

Wheat (*Triticum aestivum* L.) plants were grown in soil–sand–peat mixture (3:1:1, v/v/v) after 7 weeks of vernalization at a temperature of 4 °C, in phytotron chambers (Conviron, Winnipeg, Canada) using the spring climatic program T1 (Tischner et al., 1997). The first cycle of total water withholding started 3 days prior to anthesis (GS58; Zadoks et al., 1974) and lasted for 9 days in Mv Emese and GK Élet, and 10 days in Plainsman V and Cappelle Desprez. After the first treatment, plants were re-watered (150 mL) for two days and subjected to the second run of water deprivation for 10 days. The volumetric water content of the soil dropped below 10% (Fig. 1) at the end of both treatments. Along with the treated plants, a control group was grown with normal water supply. Twenty plants of each genotype and treatment were grown to full maturity at a final max/min temperature of 32/18 °C and yield components were determined. All experiments were repeated at least two times during 2010 and 2011.

Determination of soil and flag leaf water content

Soil volumetric water content (VWC) was measured using an HH2 moisture meter (Delta-T Devices Ltd., Cambridge, UK) at field capacity and during treatments. Relative water content (RWC) of leaves was determined on whole flag leaves of three plants per genotype and treatment at the end of both drying episodes using fresh weight (FW) at excision, saturated weight (SW) after 24 h rehydration on distilled water at 4 °C in the dark, and dry weight (DW) after oven drying for 48 h at 80 °C. The leaf RWC (%) was calculated using the following equation: $\mathrm{RWC}(\%) = \frac{\mathrm{FW}-\mathrm{DW}}{\mathrm{SW}-\mathrm{DW}} \times 100$

Dark adapted water loss from detached leaves

Water loss (WL) was determined from whole flag leaves of five well watered plants per genotype at 5 days after anthesis (DAA) in Mv Emese, GK Élet and at 6 DAA in Plainsman V and Cappelle Desprez cultivars. All steps of the experiment preceding dry weight determination were conducted under dark conditions. Excised leaf blades were brought to 100% relative water content by placing leaves on the surface of moistened filter paper overnight. Fresh weight (FW) was measured at full saturation and after drying for 20, 40, 60, 80, 100, 120, 180, 240, 300, 360, 420 min at 30 °C. Dry weight of flag leaves was determined after drying for 24 h at 80 °C. The leaf WL ($\mathrm{gh}^{-1}\ \mathrm{g}^{-1}$ DW) was calculated using the following equation (Ristic and Jenks, 2002):

Leaf water loss $= \frac{[(\mathrm{FWTx}-\mathrm{FWTx}+1)\times 60]}{[\mathrm{DW}\times(\mathrm{Tx}+1-\mathrm{Tx})]}$, where FWTx is flag leaf fresh weight at time Tx, FWTx + 1 is flag leaf fresh weight at time Tx + 1, DW is flag leaf dry weight, Tx is time (min) when FWTx was determined, and Tx + 1 is time (min) when FWTx + 1 was determined. Data obtained from five plants were averaged and used for statistical analysis. Determination of ion leakage

Measurement of conductivity was carried out according to Murray et al. (1989). Six cm long segments were cut from the middle part of flag leaves ($n = 12$ per genotype and treatment). Each piece was placed in a test tube filled with 10 mL of deionizer water and agitated overnight at room temperature under dark conditions. Next day the actual conductivity of the solution was measured using a Mikro KKT Conductometer (Budapest, Hungary). This was followed by treatment of the samples at 121 °C, 103.4 kPa for 40 min to disrupt all membranes in the cells. Conductivity was measured again and the values obtained were assumed to represent complete (100%) electrolyte leakage.

Yield components

Main spikes of control and drought-stressed plants ($n = 20$ per treatment) for each genotype were harvested at full maturity. Total floret number per spike, seed number per spike, yield per spike and total plant weight were determined. Fertility and harvest index were calculated from these data.

Light microscopy

Flag leaves of main tillers were collected from 6 plants per genotype and treatment at the end of the second exposure to water stress. According to our preliminary studies, trichome density widely differed with location both on adaxial and abaxial leaf surfaces. In order to eliminate data distortion, 3–3 stereomicroscopic images were taken from basal, middle and top portions of each leaf before the clearing procedure. After area measurements and stereomicroscopic determination of trichome density ($\mathrm{n\,mm}^{-2}$), leaves were cleared overnight in a solution containing 95% ethanol (41%), chloroform (21%), lactic acid (17%), phenol (21%) and chloral hydrate (3.63 M), washed, and stored in 50% ethanol. Cleared epidermal peels of each flag leaf were manually dissected from the middle part of the specimen as a thin transparent film using thin forceps, flattened on the surface of distilled water, mounted on microscope slides in 50% glycerol and examined with an Olympus B51 microscope (Olympus, Tokyo, Japan). Ten micrographs were taken of each of the peels dissected from both abaxial and adaxial side of the leaves at 200× magnification. The computer-aided determination of epidermal parameters was performed using Cell^{P} image analysis software (Olympus, Tokyo, Japan). Pavement cell density (PF [$\mathrm{n\,mm}^{-2}$]), silica cell density, stomatal frequency (SF [$\mathrm{n\,mm}^{-2}$]), and guard cell length (μm) were measured. The