Can the drought tolerance traits of *Ae. biuncialis* manifest even in the wheat genetic background?

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**ABSTRACT**  The physiological responses to water stress induced by PEG were investigated in *Aegilops biuncialis* (Vis.) genotypes which differ in the annual rainfall of their habitat (1050, 550 and 225 mm/year) and in *Triticum aestivum* (L.) wheat genotypes differing in drought tolerance, in order to find *Ae. biuncialis* accessions suitable for improving wheat drought tolerance through intergeneric crossing. A decrease in the osmotic pressure of the nutrient solution from -0.027 MPa to -1.8 MPa resulted in intense water loss, a low extent of stomatal closure and a decrease in the intercellular CO₂ concentration (C) in *Aegilops* genotypes originating from dry habitats, while in wheat genotypes high osmotic stress induced increased stomatal closure, resulting in a low level of water loss and high C. Nevertheless, under saturating light at normal atmospheric CO₂ level, the rate of CO₂ assimilation was higher for the *Aegilops* accessions under strong osmotic stress than for the wheats. Moreover, in the wheat genotypes, CO₂ assimilation exhibited less or no O₂ sensitivity. These physiological responses were manifested in changes in the growth rate and biomass production, since *Aegilops* (*Ae550, Ae225*) genotypes retain a higher growth rate (especially in the roots), biomass production and yield formation after drought stress than wheat. On the basis of the results it seems that *Aegilops* genotypes originating from a dry habitat have better drought tolerance than wheat, making them good candidates for improving the drought tolerance of wheat through intergeneric crossing.

**KEY WORDS**  wheat-*Aegilops biuncialis* amphiploids, drought tolerance, CO₂ fixation, stomatal conductance

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*Aegilops* sp., which are closely related to *Triticum* sp. (Van Slageren 1994), are widely used as genetic resources for wheat improvement, especially against pests and diseases (Kerber and Dyck 1990; McIntosh 1991; Ceoloni et al. 1992). On the other hand, only limited information is available on chromosome mediated gene transfer from *Aegilops* sp. to wheat in order to improve abiotic stress tolerance (Farooq and Azam 2001). *Aegilops biuncialis* Vis., (*2n = 4x = 28, U⁰U⁰M⁰M⁰*) grows in Mediterranean and Western Asiatic regions (Van Slageren 1994), in a habitat where the annual rainfall ranges from 225–1250 mm. In a previous experiment *Ae. biuncialis* accessions originating from a dry habitat showed better drought tolerance than wheat genotypes under a PEG-induced drought stress (Molnár et al. 2004). This phenomenon was characterized by an intense water loss, a low extent of stomatal closure, a decreased intercellular CO₂ concentration (C), and high rate of CO₂ assimilation (A) under strong osmotic stress. These physiological responses of *Ae. biuncialis* accessions were manifested in a higher growth rate, biomass production and yield formation after drought stress than wheat.

The first stable step during the transfer of these useful agronomic traits by interspecific hybridization is the production of wheat-*Ae. biuncialis* amphiploids (*2n=10x=70, AABB-DDU⁰U⁰M⁰M⁰*). The fertility of the amphiploids are partially restored since all of the wheat and *Aegilops* homologous chromosome pairs are present. Moreover, amphiploids are good object to study the effect of an alien genome on abiotic stress tolerance in wheat genetic background.

The aim of the present study was to investigate the effect of U⁰ and M⁰ genome chromosomes on responses to drought stress in two wheat-*Aegilops biuncialis* amphiploid genotypes in which the *Aegilops* parents originated from dry habitats. The experiments were performed in order to clarify that *Ae. biuncialis* accessions suitable for improving wheat drought tolerance through intergeneric crossing.

**Materials and Methods**

**Plant materials**

A comparison was made of the responses to drought stress in the amphiploids Mv9kr1-*Ae. biuncialis* _MGI_ 470 (Amphi470) and Mv9kr1-*Ae. biuncialis* _MGI_ 1112 (Amphi1112) and in wheat (*Triticum aestivum* L.) genotypes with good drought tolerance. The two wheat-*Ae. biuncialis* amphiploids were

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produced in Martonvásár by the crossing of wheat genotype Mv9kr1 with two Aegilops biuncialis Vis. accessions (MvGB470 and MvGB1112), originating from habitats having annual rainfall of 300 and 400 mm (Logojan and Molnár-Láng 2000). The winter wheat Mv9kr1 contains a crossability gene (Molnár-Láng et al. 1996) and wheat cultivar Plainsmann V. is drought-tolerant control.

Hydroculture system

Germinated seedlings were grown in half-strength modified Hoagland nutrient solution (Nagy and Galiba 1995) in a plant growth chamber (Conviron, Canada) as described by (Molnár et al. 2004). Osmotic stress was imposed after three weeks by applying PEG 6000 (Sigma) in 7-day cycles at increasing concentrations of 12, 15, 18 and 21% (w/v), resulting in osmotic potentials of −0.45 MPa, −0.72 MPa, −1.14 MPa and −1.8 MPa, respectively. Samples were taken prior to PEG application (control), on the 7th day after the application of various PEG concentrations, and after 2 and 7 days of regeneration without PEG.

Determination of leaf water potential, water content and growth parameters

The water content of the leaves was expressed as relative water content (RWC) according to the following equation:

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RWC = \frac{(FW - DW)}{(SW - DW)} \times 100
\]

where FW is the fresh weight, SW the water-saturated weight and DW the dry weight after drying for 12 hours at 105°C.

Gas exchange measurements

The CO₂ assimilation of intact leaves was measured in a standard gas mixture of 340 ppm CO₂ and 21% O₂ in N₂ using an infrared gas analyser (LCA-2, Analytical Development Co. Ltd., Hoddesdon, UK). The rates of net CO₂ fixation (Anow), stomatal conductance (gs) and stomatal conductance (gsc) were calculated in the light-saturated state of photosynthesis using the equations of von Caemmerer and Farquhar (1981). In order to discriminate the limitation of photosynthesis driven from stomatal closure and from the metabolic injury A was also determined at 1000 ppm CO₂ from a standard gas mixture (1000 ppm CO₂ and 21% O₂ in N₂) at saturating light intensity.

Statistical analysis

The results are the means ± LSDα of 6 measurements per treatment for CO₂ gas exchange and 8 measurements per treatment for RWC parameters. The measurements were performed on different plants. Differences between the treatments and genotypes were determined by means of two-factor analysis of variance (ANOVA) at the P<0.01 or P<0.05 level and three-factor ANOVA in the case of CO₂ gas exchange measurements at high CO₂ concentration.

Results and Discussion

Osmotic stress caused a more rapid reduction in RWC in the wheat genotypes, where the greatest decline in water loss was recorded for Plainsmann V., than in the amphiploids (Fig. 1A). During the regeneration the amphiploids and the wheat line Plainsmann V. regained their normal water contents by
Drought tolerance of wheat-Ae. biuncialis amphiploids

The leaves is limited, resulting in a decrease in the leaf intercellular CO$_2$ concentration ($C$) and the CO$_2$ fixation (Cornic 2000). At this stage, the metabolic processes of photosynthesis are not impaired as the maximum rate of CO$_2$ fixation ($A_{max}$) can be restored to the control (non-stressed) level by the increase in the ambient CO$_2$ concentration (Lawlor and Cornic 2002). At severe stress, the relative importance of metabolic to stomatal limitation increase, i.e. elevated CO$_2$ concentration is not able to restore $A_{max}$ to the non-stressed level.

No significant differences were found between the amphiploids and Mv9kr1 genotypes for the $A_{max}$ values before PEG treatment (Fig. 2). In the case of amphiploids, the $A_{max}$ was able to return to the control values after exposure to mild stress (-0.7 MPa). At severe stress (-1.8 MPa), the elevated CO$_2$ concentration could restore the $A_{max}$ only in the Amphi470 genotype suggesting that there was no metabolic inhibition during the stress treatment in this genotype. In the case of wheats $A_{max}$ could not reach the control value even at mild stress indicating a strong metabolic impairment in these plants.

All of these results evidenced that the good drought tolerance traits of Ae. biuncialis can be manifested even in the wheat genetic background. The $U^b$ and $M^b$ genome chromosomes, where these traits are localized can be selected by the backcrossing with wheat in the future.

**Abbreviations used**

$A$, net CO$_2$ assimilation rate; $g_s$, stomatal conductance; RWC, relative water content

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Molnár et al.

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