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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 CO_2 concentration (C_i) 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_i. Nevertheless, under saturating light at normal atmospheric CO_2 level, the rate of CO_2 assimilation was higher for the *Aegilops* accessions under strong osmotic stress than for the wheats. Moreover, in the wheat genotypes, CO_2 assimilation exhibited less or no O_2 sensitivity. These physiological responses were manifested in changes in the growth rate and biomass production, since *Aegilops* (*Ae*550, *Ae*225) 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.
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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, $U^bU^bM^bM^b$) 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 CO_2 concentration (C_i), and high rate of CO_2 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-DDU^{b}U^{b}M^{b}M^b). 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 genom on abiotic stress tolerance in wheat genetic background.

The aim of the present study was to investigate the effect of U^b and M^b 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 aphiploids Mv9kr1-*Ae. biuncialis* MvGB 470 (Amphi470) and Mv9kr1-*Ae. biuncialis* MvGB 1112 (Amphi1112) and in wheat (*Triticum aestivum* L.) genotypes with good drought tolerance. The two wheat-*Ae. biuncialis* amphiploids were
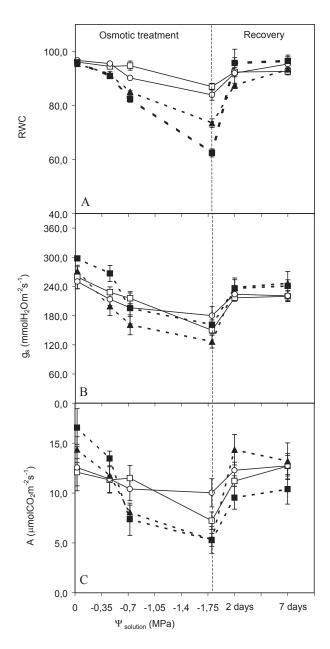
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Figure 1. Effect of increasing osmotic stress followed by 7 d regeneration on (A) relative water content (RWC), (B) stomatal conductance (g$_s$) and (C) net CO$_2$ assimilation rate (A) in two wheat (dotted line) and two wheat-*Ae. biuncialis* amphiploid (continuous lines) genotypes. (▲) Mv9kr1, (■) Plainsmann V., (○) Amphi470, (□) Amphi1112. Data are the means ± s.e. of data of six plants per treatment.

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:

RWC = (FW – DW) * 100/(SW – DW)

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 CO$_2$ assimilation of intact leaves was measured in a standard gas mixture of 340 ppm CO$_2$ and 21% O$_2$ in N$_2$ using an infrared gas analyser (LCA-2, Analytical Development Co. Ltd., Hoddesdon, UK). The rates of net CO$_2$ fixation (A), and stomatal conductance (g$_s$) 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 drived from stomatal closure and from the metabolic injury A was also determined at 1000 ppm CO$_2$ from a standard gas mixture (1000 ppm CO$_2$ and 21% O$_2$ in N$_2$) at saturating light intensity.

Statistical analysis

The results are the means ± LSD$_{5\%}$ of 6 measurements per treatment for CO$_2$ 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 CO$_2$ gas exchange measurements at high CO$_2$ 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 Planinsmann V., than in the amphiploids (Fig. 1A). During the regeneration the amphiploids and the wheat line Plainsmann V. regained their normal water contents by
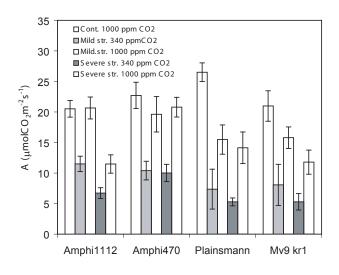
Figure 2. Effect of high CO_2 concentration (1000 ppm) on the rate of net CO_2 assimilation during mild (-0.7 MPa) and severe (-1.8 MPa) osmotic stress in in two wheat (Plainsmann V., Mv9kr1) and two wheat-*Ae. biuncialis* amphiploid (Amphi1112, Amphi470) genotypes. Data are the means ± s.e. of data of six plants per treatment.

the end of the second day. Parallel with the decrease in water content, the stomatal conductance (g_s), which is proportional to the closure of stomatal aperture, decreased in all the genotypes (Fig. 1B). There was no difference in the stomatal closure between the genotypes under the stress and regeneration periods. The results showed that under the same stomatal closure amphiploids were able to maintain their water status more succesfully than wheat genotypes. This phenomenon suggests that an efficient osmoregulation mechanism could be present in the amphiploid genotypes.

Besides the modification of RWC and g_s the net CO_2 assimilation (A) also decreased during PEG-induced osmotic stress. Before osmotic treatment no significant difference could be detected between the Mv9kr1 wheat and the amphiploid genotypes in the net CO_2 assimilation rates. In the case of mild osmotic stress (-0.7 MPa), a substantial decrease in net CO_2 assimilation rate could only be observed in the wheat genotypes (Fig. 1C). When the osmotic stress was severe (-1.8 MPa), the photosynthetic CO_2 fixation was strongly inhibited in the wheat genotypes and remained high in the amphiploid genotypes, especially in Amphi470. In Amphi470 less than 20% decrease in the photosynthetic activity was observed at the end of stress treatment, while this value were more than 60% in the wheat genotypes. These results suggest that the limitation of photosynthesis in the wheats could be attributed to other factors than in the amphiploid genotypes.

At the beginning of drought stress the limitation of net CO_2 assimilation rate (A) is mainly due to stomatal closure, which is the most efficient way to reduce water loss (Cornic 2000). When the stomata are closed, the diffusion of CO_2 into the leaves is limited, resulting in a decrease in the leaf inter-

cellular CO_2 concentration (C_i) 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

Acknowledgements

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