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Wheat-*Aegilops biuncialis* amphiploids have efficient photosynthesis and biomass production during osmotic stress

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ABSTRACT

Osmotic stress responses of water content, photosynthetic parameters and biomass production were investigated in wheat-*Aegilops biuncialis* amphiploids and in wheat genotypes to clarify whether they can use to improve the drought tolerance of bread wheat. A decrease in the osmotic pressure of the medium resulted in considerable water loss, stomatal closure and a decreased CO_2 assimilation rate for the wheat genotypes, while the changes in these parameters were moderate for the amphiploids. Maximal assimilation rate was maintained at high level even under severe osmotic stress in the amphiploids, while it decreased substantially in the wheat genotypes. Nevertheless, the effective quantum yield of PS II was higher and the quantum yield of non-photochemical quenching of PS II and PS I was lower for the amphiploids than for the wheat cultivars. Parallel with this, higher cyclic electron flow was detected in wheat than in the amphiploids. The elevated photosynthetic activity of amphiploids under osmotic stress conditions was manifested in higher biomass production by roots and shoots as compared to wheat genotypes. These results indicate that the drought-tolerant traits of *Ae. biuncialis* can be manifested in the wheat genetic background and these amphiploids are suitable genetic materials for improving drought tolerance of wheat.

Introduction

Many physiological processes of major crops, including photosynthesis, are extremely sensitive to water deficit, which strongly limits biomass production and yield formation (de Souza et al., 2003; Zsófi et al., 2009). During drought stress the water potential (ψ) and relative water content (RWC) of the leaves and the net photosynthetic CO_2 assimilation rate (A) substantially decrease (Bajji et al., 2001; Molnár et al., 2004). Under mild to moderate water deficit the reduction in A results from stomatal closure (stomatal limitation, L_s) (Chaves, 1991; Medrano et al., 2003), which represents the most efficient way to reduce water loss, while the CO_2 diffusion into the leaves is also restricted resulting in a decrease in the intercellular CO_2 concentration (C_i) (Cornic, 2000). During severe water deficit photosynthesis may also be limited by factors other than stomatal closure (called non-stomatal or metabolic limitation, L_m). The non-stomatal limitation of A may be caused by the restricted diffusion of CO_2 from the intercellular spaces to the chloroplasts (Delfine et al., 1999; Loreto et al., 2003) or by metabolic factors such as decrease in the ribulose-1,5-bisphosphate-carboxylase-oxygenase (Rubisco) activity, disturbances in the regeneration of ribulose-1,5-bisphosphate (Medrano et al., 1997; Maroco et al., 2002; Centritto et al., 2003; Chaves et al., 2003) and oxidative damage resulting from the excess excitation energy (Flexas et al., 2006). During water deficit, the increasingly severe limitation of photosynthesis often leads to the plant absorbing more light energy than that can be used by CO_2 fixation (Smirnoff, 1993). Although the excess light can be partially dissipated as heat, it has the potential to cause the over-reduction of the linear electron transport chain, leading

Abbreviations: A, net CO_2 assimilation rate; AL, actinic light; Amphi, Mv9kr1-*Aegilops biuncialis* amphiploids; C_i, intercellular CO_2 concentration; FISH, fluorescent *in situ* hybridization; F_m, maximal fluorescence; $F_m{}'$, maximal fluorescence of illuminated samples; F_0, initial level of fluorescence; F, steady-state fluorescence; F_v, variable fluorescence; F_v/F_m, maximum (optimal) quantum yield of PS II; $\Delta F/F_m{}'$, Y(II), effective quantum yield of PS II; GISH, genomic *in situ* hybridization; g_s, stomatal conductance; L_m, non-stomatal (mesophyll, or metabolic) limitation; L_s, stomatal limitation; NPQ, non-photochemical quenching; Ψ, osmotic potential; PEG, polyethylene glycol; P_0, minimal P700 signal; P_m, maximal P700 level; $P_m{}'$, maximal P700 signal in a given light state; PS I, photosystem I; PS II, photosystem II; Rubisco, ribulose-1,5-bisphosphate-carboxylase-oxygenase; RWC, relative water content; Y, yield; Y(I), photochemical quantum yield of PS I; Y(NA), Y(ND), non-photochemical quantum yields of PS I; Y(NO), quantum yield of non-regulated energy dissipation; Y(NPQ), quantum yield of regulated energy dissipation.

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to oxidative damage (Smirnoff, 1993; Flexas et al., 2006). Under these circumstances the down-regulation of photosynthesis by regulated thermal dissipation (Demming-Adams et al., 2006) and/or photorespiration (Haupt-Herting and Fock, 2002) represent an efficient defence mechanism in C_3 plants. It has also been suggested that the cyclic electron transport, Y(CEF), may have a role in maintaining an adequate pH gradient across the thylakoid membrane (ΔpH) during drought, which is also required for NPQ (Golding and Johnson, 2003).

Drought stress ultimately results in dehydration of the cell which disrupts the osmotic equilibrium. The excess of osmotic changes, the osmotic stress, leads to a cascade of events, including the expression of LEA/dehydrin-type genes, synthesis of molecular chaperones in order to protect the partner protein from degradation (see Mahajan and Tuteja, 2005). The osmotic stress also leads to synthetize sugars and other osmolytes, through the process known as osmotic adjustment, in order to decrease cellular osmotic potential, thereby helping the diffusion of water into the leaf resulting in increase in leaf turgor (Hoekstra et al., 2001). All of these protective mechanisms against the osmotic stress have been considered to play a significant role in the tolerance against drought stress and drought tolerant plants, such as wheat, should also have tolerance to osmotic stress (Ramanjulu and Bartels, 2002).

Hydroponic systems with increased water potential have widely been used to study osmotic stress responses of plants (Munns et al., 2010). The advantage of hydroponics is the applied osmotic stress is more controlled and their effects on the experimental plants are more homogeneous than in soil-based systems (Shavrukov et al., 2012). In this respect, the application of high molecular weight polyethylene glycol (PEG) is more advantageous than the use of other small molecules such as mannitol because of it is less likely to be absorbed by plants (Miller, 1987; Hohl and Schopfer, 1991). The osmotic stress generated by PEG and the water deficit stress in soil usually results in similar symptoms in many plant species, such as wheat and related species. These symptoms have been characterized by a decrease in the relative water content (RWC), leaf water potential, photosynthetic CO_2 fixation, biomass production and yield formation (Zhang and Kirkham, 1995; Molnár et al., 2004), confirming the stimulation of drought stress by PEG-induced osmotic treatments.

Interspecific hybridization of wheat with wild relatives is a widely used method to improve the agronomic characters of wheat, including nutritional parameters or tolerance to biotic and abiotic stresses (Colmer et al., 2006; Schneider et al., 2008; Pradhan et al., 2012). The species in the genus *Aegilops*, which is closely related to *Triticum* (van Slageren, 1994), are widely used as genetic resources in the breeding of bread and pasta wheat (Zaharieva et al., 2001), especially to improve tolerance against pests and diseases (Kerber and Dick, 1990; McIntosh, 1991; Ceoloni et al., 1992). The allotetraploid *Aegilops biuncialis* Vis. ($2n = 4x = 28$, $U^bU^bM^bM^b$) is native in the Mediterranean and Western Asiatic regions characterized by hot, dry vegetation periods, where the annual rainfall ranges from 225 to 1250 mm (van Slageren, 1994). Accessions of *Ae. biuncialis* originating from drought-prone habitats have developed various acclimation or adaptation strategies against drought (Molnár et al., 2004). These are characterized by less intense stomatal closure and a high rate of net CO_2 assimilation under severe osmotic stress, manifested as higher growth rate, biomass production and yield formation in *Ae. biuncialis* accessions in comparison with wheat genotypes (Molnár et al., 2004).

The first step in the chromosome mediated gene transfer of drought tolerance traits from *Ae. biuncialis* into wheat is the production of F_1 hybrids, which are sterile due to their haploid genome composition ($n = 5x = 35$, $ABDU^bM^b$). The wheat-*Ae. biuncialis* amphiploids contain the parental genomes in two copy number ($2n = 10x = 70$, $AABBDDU^bU^bM^bM^b$), thus they are partially fertile and the effects of alien genes in the wheat genetic background could be reliably analyzed in subsequent generations. However, some chromosomes could be eliminated during the multiplication of the seeds and sometimes the chromosome number is stabilized at 56 as it was observed in other wheat-alien amphiploids (Sepsi et al., 2008). Furthermore, desirable alien genes are not necessarily expressed in the amphiploids, due to the rapid genetic or epigenetic changes leading to gene silencing (Shaked et al., 2001; Heslop-Harrison, 2003). Consequently, both the karyotypic composition and the drought tolerance of wheat-*Ae. biuncialis* amphiploids are necessary to determine before their use for chromosome-mediated gene transfer.

The aim of the present study was to clarify whether the osmotic stress tolerance of the *Ae. biuncialis* accessions originating from dry habitats, published previously by Molnár et al. (2004), are able to manifest in the wheat genetic background. For this purpose the photosynthetic and physiological responses of wheat-*Ae. biuncialis* amphiploids, developed by crossing the wheat genotype Mv9kr1 with *Ae. biuncialis* (MvGB470 or MvGB1112) accessions, to PEG-induced osmotic stress were compared to those of wheat parent Mv9kr1 and to a known droght-tolerant wheat genotype 'Plainsman V', as controls to clear whether wheat-*Ae. biuncialis* amphiploids are potentially suitable gene sources for improving the drought tolerance of bread wheat.

Materials and methods

Plant materials

Triticum aestivum L. – *Aegilops biuncialis* Vis. amphiploids (Mv9kr1-*Ae. biuncialis* MvGB470, *Amphi*470; Mv9kr1-*Ae. biuncialis* MvGB1112, *Amphi*1112; with genome constitution: $2n = 10x = 70$, $AABBDDU^bU^bM^bM^b$), the parental wheat genotype Mv9kr1 (Molnár-Láng et al., 1996) and wheat cultivar 'Plainsman V', considered as a drought-tolerant control (Jäger, 2010), were used in the experiments. The amphiploids were produced as described in Logojan and Molnár-Láng (2000) and seeds were multiplied through six generations. Fluorsecence *in situ* hybridization (FISH and mcGISH) were performed to check the chromosomal constitution of the multiplied amphiploid genotypes. The photosynthetic response to osmotic stress induced by polyethylene glycol (PEG) in hydroculture system of wheat-*Ae. biuncialis* amphiploids were compared to those of wheat parental line Mv9kr1 and to a known drought-tolerant wheat cultivar 'Plainsman V'.

Fluorsecence in situ hybridization (FISH and mcGISH)

In situ hybridization experiments including the production of mitotic chromosome preparations, probe labelling and hybridization procedures were performed as described by Molnár et al. (2009). Briefly, the total genomic DNA of *Ae. umbellulata* (UU) and *Ae. comosa* (MM), the diploid progenitors of *Ae. biuncialis*, was labelled with biotin (biotin-16-dUTP, Roche) and digoxigenin (digoxigenin-11-dUTP, Roche) by random priming and used as U and M genomic probes, respectively. Unlabelled genomic DNA from durum wheat (*Triticum turgidum ssp. durum* L., $2n = 4x = 28$; AABB) was sheared by autoclaving and used as a block.

The repetitive DNA probes, used for the FISH experiments, pSc119.2 and Afa-family sequences were labelled with biotin-16-dUTP (Roche) and digoxigenin-11-dUTP (Roche), respectively, using PCR (Nagaki et al., 1995; Contento et al., 2005). The clone pTa71 was labelled with 50% Biotin-16-dUTP and 50% Digoxigenin-11-dUTP. Digoxigenin and biotin were detected using anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidin-FITC (Roche), respectively.