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Ability of chromosome 4H to compensate for 4D in response to drought stress in a newly developed and identified wheat–barley 4H(4D) disomic substitution line

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Abstract

A spontaneously developed wheat–barley 4H(4D) disomic substitution line was identified cytogenetically using genomic *in situ* hybridization (GISH), multicolour fluorescent *in situ* hybridization (FISH) and microsatellite markers. The ability of the barley 4H chromosome to compensate for wheat 4D in response to mild drought stress was also investigated. In the barley cv. 'Betzes' and the 4H(4D) substitution line, mild osmotic stress induced intensive stomatal closure, resulting in reduced water loss through transpiration and unchanged relative water content in the leaves. As the CO_2 assimilation rate remained relatively high, the water use efficiency, which is an important factor associated with drought tolerance, increased extensively under mild osmotic stress in these lines. In the case of the parental wheat genotypes, however, mild drought stress induced less intense stomatal closure and a greater decrease in the CO_2 assimilation rate than in barley or in the substitution line, resulting in unaugmented or reduced water use efficiency. The results demonstrate that genes localised on the 4H chromosome of barley were able to increase the water use efficiency of the wheat substitution line, which is suitable for improving wheat drought tolerance through intergeneric crossing.

Key words: *Triticum aestivum* — *Hordeum vulgare* — 4H(4D) substitution — drought stress — CO_2 assimilation — water use efficiency — two-colour FISH

Barley is a potential gene source for wheat, as it has many favourable properties, such as good drought tolerance (Cattivelli et al. 2002), earliness (Murai et al. 1997) and good nutrition quality parameters (Islam and Shepherd 1981). As the first successful hybridization of wheat and barley (Kruse 1973), a set of wheat/barley addition lines (Islam et al. 1978, Islam and Shepherd 1981) and several wheat/barley substitution lines have been developed. The substitution of barley chromosomes 3H, 4H, 6H and 7H for group 3, 4, 6 and 7 homoeologous chromosomes of wheat was reported by Islam and Shepherd (1992, 1993) and the substitution of 2H for group 2 homoeologous chromosomes by Ya-Ping et al. (2003). Several methods have been elaborated for producing alien substitution lines (Gale and Miller 1987), but substitutions have also been reported to arise spontaneously (Zeller and Hsam 1983).

The wheat/barley addition and substitution lines developed earlier were used mainly for the study of homoeologous relationships between wheat and barley and the ability of the alien chromosomes to compensate for phenotypic traits such as plant vigour and fertility. During the investigation of wheat–barley 4H(4A), 4H(4B) and 4H(4D) disomic substitution lines, Islam and Shepherd (1992, 1993) found that only plants of the 4H(4D) substitution had moderate fertility and vigour, making it the most suitable for wheat improvement. However, no information is available on the ability of any barley chromosome to compensate for a wheat chromosome as regards abiotic stress tolerance, especially drought stress tolerance traits.

Using disomic wheat–barley addition lines for chromosomes 2H-7H and the wheat/barley translocation line 2AS.1HS, Handley et al. (1994) demonstrated that only the addition line containing barley chromosome 4H was able to increase the water use efficiency of wheat, indicating that chromosome 4H contains genes controlling water use efficiency (WUE). Investigations on this substitution line, were carried out to obtain more precise information on the ability of barley chromosome 4H to control water use efficiency.

Water use efficiency (WUE) is one of the most important factors associated with drought tolerance (Ehdaie and Waines 1993), and can be determined at whole plant level as biomass production per water used (WUE_T) (Begg and Turner 1976). However, as WUE is primarily controlled by photosynthetic CO_2 assimilation processes in the leaf and by water loss through the stomata (Sinclair et al. 1984) it can be determined at single leaf level (WUE_I) as the ratio of photosynthetic net CO_2 assimilation rate (A) to the stomatal conductance for water vapour (g_s) (Martin and Ruiz-Torres 1992). It was also observed that under mild drought stress WUE_I increased extensively, especially in drought-tolerant plants (Medrano et al. 2002). Under severe drought stress, however, WUE_I decreased in both drought-sensitive and drought-tolerant plants (Martin and Ruiz-Torres 1992).

The present paper reports on the molecular cytogenetic identification and phenotypic characterization of a spontaneously produced wheat–barley 4H(4D) disomic substitution line and its analysis for drought tolerance. The line was characterised cytogenetically using genomic *in situ* hybridization (GISH) with total genomic DNA as a probe (Le et al. 1989), multicolour fluorescent *in situ* hybridization (FISH) with probes having highly repeated DNA sequences (Pedersen and Langridge 1997) and microsatellite markers (Nagy et al. 2002). To demonstrate the effects of barley chromosome 4H on the drought stress response, the substitution line and its parental barley (*Hordeum vulgare* L. cv. 'Betzes') and wheat (*Triticum aestivum* L. cv. 'Chinese Spring' and line Mv9kr1) genotypes were also investigated under drought stress conditions induced by osmotic (PEG 6000) treatment. The relative

water content (RWC) and the gas-exchange parameters of leaves, including stomatal conductance (g_s), CO_2 assimilation rate (A) and WUE_I, were determined so as to characterize the drought tolerance of the plants.

Materials and Methods

Plant materials: A wheat–barley 4H(4D) disomic substitution line was spontaneously produced among backcross progenies of the wheat cv. 'Chinese Spring' × barley cv. 'Betzes' hybrids developed in Martonvásár (Molnár-Láng and Sutka 1994). The hybrids were multiplied in tissue culture, backcrossed twice with wheat line Martonvásári 9 kr1 (Mv9kr1) and self-pollinated as described by Molnár-Láng et al. (2000). The wheat line Mv9kr1 was used for backcrossing, as it possesses agronomically better traits than the cv. 'Chinese Spring' and also carries the crossability gene originating from 'Chinese Spring' (Molnár-Láng et al. 1996).

Identification of the wheat–barley 4H(4D) disomic substitution line by sequential GISH and FISH analysis: A chromosome preparation and the sequential GISH and FISH analysis was made from the wheat–barley 4H(4D) disomic substitution line as described by Nagy et al. (2002). Total barley genomic DNA was Nick translated with Fluorored (tetramethylrhodamine-5-dUTP, Roche Mannheim, Germany) and used as a probe with unlabelled wheat genomic DNA as a competitor at 30 times the quantity of the probe (Molnár-Láng et al. 2000). The slides were counterstained with DAPI (4′,6-diamidino-2-phenylindole, Amersham Vienna, Austria). A Zeiss Axioskop-2 microscope fitted with a Spot CCD camera (Diagnostic Instruments, Sterling Heights, MI, USA) was used to document the hybridization signals. The images were compiled with Image Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). GISH-ed slides were reprobed with the clones pAs1 (Rayburn and Gill 1986) labelled with Fluorored and GAA satellite sequences (Vrána et al. 2000) labelled with Fluorogreen (fluorescein-12-dUTP, Roche). The FISH procedure and the composition of the hybridization solution were the same as those described by Logojan and Molnár-Láng (2000).

Identification of the wheat–barley 4H(4D) disomic substitution line with SSR markers: Microsatellite (SSR) markers Bmag0353 and HVM40, specific for the 4HS and 4HL chromosome arms (Ramsay et al. 2000), respectively, were used to confirm the presence of the 4H chromosome in the 4H(4D) substitution line. The PCR amplifications of the SSR markers and the separation of PCR products on 1.5% agarose gel along with the DirectLoad Wide-Range DNA size marker (Sigma) were performed as described by Nagy et al. (2002).

Phenotypic characterization of the wheat–barley 4H(4D) disomic substitution line and of its wheat and barley progenitors: Plants identified as 4H(4D) substitutions and the parental wheat cv. 'Chinese Spring' and line Mv9kr1 and the barley cv. 'Betzes' genotypes (Molnár-Láng et al. 1996) were characterized morphologically. Fifteen plants of each genotype were grown in the field in Martonvásár under natural conditions in 2004. The rainfall from January until July was 408 mm, which represents satisfactory irrigation in this region. Plant height, tillering, the length of the main spike and fertility were estimated for all the plants. Differences between the treatments and genotypes were determined by means of a one-factor analysis of variance.

Drought stress response: Polyethylene glycol (Mr ≥ 6000) is widely used to stimulate drought stress in hydroculture systems, as it causes similar symptoms in many plant species to those induced by water deficit stress in soil (Nepomuceno et al. 1998, Molnár et al. 2004). However, the experimental conditions can be controlled better in hydroculture than in soil. In this paper PEG 6000 (Sigma) was used in a hydroculture system to induce mild drought stress (Molnár et al. 2004). The seeds of two wheat genotypes (Mv9kr1, 'Chinese Spring'), the barley cv. 'Betzes' and the disomic substitution line Mv9kr1-'Betzes' 4H(4D) were germinated and twenty plants of each genotype (5 plants/1.5 l pot) were grown in half-strength modified Hoagland nutrient solution (Nagy and Galiba 1995) in a plant growth chamber (Conviron, Canada) under the conditions described by Molnár et al. (2004) for 21 days. At day 14, osmotic stress was imposed by applying PEG 6000 (Sigma) at a concentration of 15% (w/v) for 14 days. The osmotic potential of the half-strength modified Hoagland solution with or without 15% PEG was −0.72 MPa and 0.025 Mpa, respectively, as determined using a cryoscopic osmometer (Osmomat O30-D Gonotec GMBH, Berlin, Germany). As a control, samples were taken prior to PEG application and on the 14th day after treatment, as preliminary experiments indicated that no significant modification was found in the parameters measured during the 14 days without treatment (data not shown). This is because only the youngest, fully expanded leaves of different plants were used for the measurements. The relative water content (RWC) of the leaves was determined according to the equation: $RWC = (FW-DW) \times 100/(SW-DW)$, where FW is the fresh weight, SW the water-saturated weight and DW the dry weight after drying for 12 h at 105°C.

Gas-exchange measurements: The CO_2 assimilation rate of intact leaves was measured using an infrared gas analyser (LCA-2, Analytical Development Co. Ltd, Hoddesdon, UK) as described by Molnár et al. (2004). The rates of net CO_2 fixation (A) and stomatal conductance (g_s) were calculated in the light-saturated state of photosynthesis at 350 ppm CO_2 using the equations of von Caemmerer and Farquhar (1981). The WUE_I was calculated as A/g_s (Martin and Ruiz-Torres 1992).

The drought stress response was investigated in two independent series of experiments. In each experiment, the results were the means of six independent measurements per treatment for CO_2 gas exchange and eight measurements per treatment for RWC. Differences between the treatments and genotypes were determined by means of a two-factor analysis of variance.

Results

Identification of the wheat–barley 4H(4D) disomic substitution line by sequential GISH and FISH and SSR markers

Two chromosomes were intensively labelled after GISH using Fluorored-labelled barley genomic DNA in a wheat background to demonstrate the presence of a pair of barley chromosomes (Fig. 1a). The chromosome number was determined with the Feulgen method, and was found to be 42. Meiotic analysis revealed 21 bivalents in metaphase I of meiosis (data not shown). It was thus determined that the line was a substitution, as two barley chromosomes were present but the chromosome number was 42 (Fig. 1a). In order to identify the barley chromosomes, FISH was performed by reprobing the slides as shown in Fig. 1b. All the wheat and barley chromosomes of the 4H(4D) disomic substitution line were identified by FISH using the pAs1 probe and GAA satellite sequences (see Fig. 1b). The 4H chromosomes of barley, identified from the strong hybridization band at the centromeric region, are illustrated in the Fig. 1b insert.

SSR markers Bmag0353 and HVM40, specific for the 4HL and 4HS chromosome arms, also confirmed the presence of the barley 4H chromosome in the substitution line (Fig. 2). A single, intensive PCR product with the expected size could be detected for each marker in both the barley cv. 'Betzes' and the substitution line, but no product was detected in the wheat line

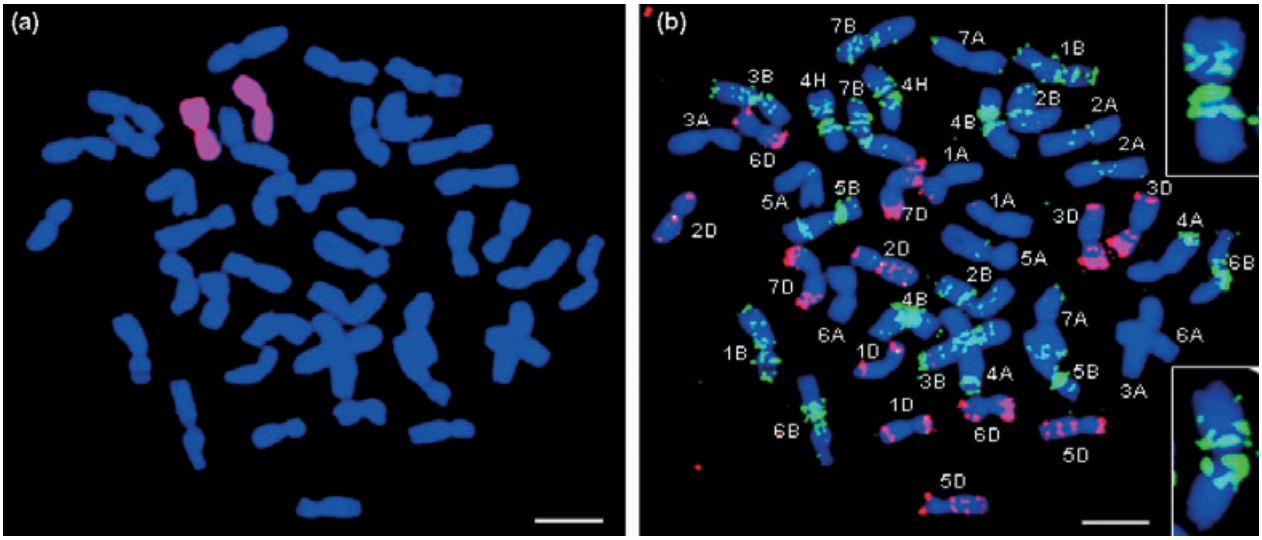

Fig. 1: Genomic *in situ* hybridization (GISH) (a) and multicolour fluorescence *in situ* hybridization (FISH) (b) on the metaphase chromosome spread of the 4H(4D) disomic wheat–barley substitution line. Red signals on the GISH image mark the barley chromosomes. On the FISH image, all the chromosomes both wheat and barley (inserts) could be identified using the probes GAA (green) and pAs1 (red). The chromosomes were counterstained with DAPI (blue) on both the GISH and FISH images

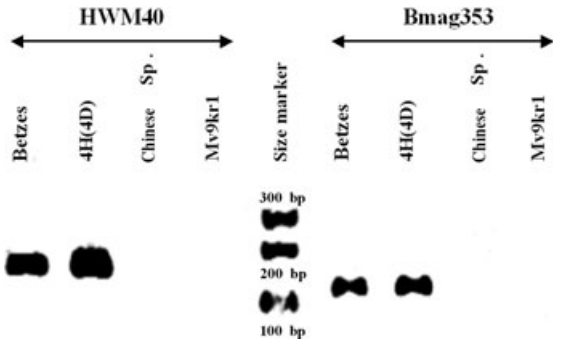

Fig. 2: Electrophoretic pattern of the Bmag353 and HVM40 SSR markers tested on the 4H(4D) wheat–barley substitution line and on its wheat (line Mv9kr1 and the cv. 'Chinese Spring') and the barley (cv. 'Betzes') progenitors

Mv9kr1 and the cv. 'Chinese Spring', confirming that the barley chromosome was 4H in the substitution line.

Phenotypic characterization of the wheat–barley 4H(4D) disomic substitution line and its wheat and barley parental genotypes

The effect of substituting barley chromosome 4H for 4D was manifested in spike morphology (Fig. 3) as well as in plant morphology and fertility (Table 1). The spikes of the substitution line resembled those of the wheat parents, but were shorter (Table 1, Fig. 3). The plants of the 4H(4D) substitution line were shorter than those of the parental genotypes the barley cv. 'Betzes' and the wheat cv. 'Chinese Spring', but were similar to the wheat line Mv9kr1 (Table 1). The substitution of barley chromosome 4H for wheat chromosome 4D resulted in a substantially higher number of tillers than in the parental wheat genotypes. However, the disomic substitution 4H(4D) plants led to moderate fertility as indicated by the number of grains/spikelet (Table 1).

Fig. 3: Spike morphology of the barley cv. 'Betzes', the 4H(4D) wheat–barley disomic substitution line, the wheat cv. 'Chinese Spring' and the wheat line Mv9kr1. CS, 'Chinese Spring'

Table 1: Means ± s.e. from fifteen plants for plant height, number of tillers, spike length and fertility of the 4H(4D) substitution line and of its wheat (cv. 'Chinese Spring', line Mv9krl) and barley cv. 'Betzes' progenitors

	Plant height (cm)	Number of tillers	Spike length (cm)	Fertility (grains/spikelet)
CS[1]	110.8 ± 2.7*	7.9 ± 1.5	7.6 ± 0.8	2.3 ± 0.4
Mv9kr1	81.5 ± 4.9	8.2 ± 1.9	9.4 ± 1.0	2.1 ± 0.4
4H(4D)	76.9 ± 2.6	12.9 ± 1.5*	5.6 ± 0.4*	1.6 ± 0.4
Betzes	104.4 ± 6.5*	15.6 ± 2.8*	10.3 ± 1.2	0.8 ± 0.1*

*: Significantly different from Mv9 kr1 at P = 0.05.
[1]CS = Chinese Spring.

Table 2: Means ± s.e. of relative water content (RWC) of leaves, stomatal conductance (g_s), net CO_2 assimilation rate (A) and instantaneous water use efficiency (A/g_s) (for details see text)

	RWC (%)		g_s (mmolm^{-2}s^{-1})		A (μmol m^{-2}s^{-1})		A/g_s (μmol mol^{-1})	
	C[1]	PEG	C	PEG	C	PEG	C	PEG
CS	94.5 ± 1.0 *	87.7 ± 1.8 #	757 ± 33.7	425 ± 28.4 *,#	14.2 ± 0.5	6.0 ± 0.3 #	18.6 ± 0.5	14.1 ± 0.8 *,#
Mv9kr1	91.8 ± 1.3	87.6 ± 0.9 #	731 ± 16.8	350 ± 26.6 #	13.4 ± 0.6	6.4 ± 0.5 #	18.4 ± 1.1	18.7 ± 2.6
4H(4D)	92.6 ± 0.8	90.3 ± 1.3 *,#	534 ± 28.1 *	128 ± 12.5 *,#	12.4 ± 0.5	9.1 ± 0.2 *,#	23.1 ± 1.1 *	71.3 ± 6.8 *,#
Betzes	95.4 ± 1.1 *	93.9 ± 4.4 *	749 ± 28.8 -	260 ± 33.9 *,#	12.4 ± 0.4	11.5 ± 0.6 *	16.2 ± 0.7 *	44.2 ± 5.9 *,#

*: Significantly different from Mv9 kr1 within the same treatment at P = 0.05.
#: Significantly different from the control at P = 0.05.
[1]C = control, PEG = 15% PEG.
[2]4H(4D) = wheat-barley 4H(4D) disomic substitution line. CS = Chinese Spring.

Effect of PEG-induced osmotic stress on the water content and photosynthetic parameters

In order to determine the effect of substituting barley chromosome 4H for wheat 4D on the physiological responses to drought stress, a comparison of the water content and photosynthetic parameters was made between the wheat–barley 4H(4D) substitution line and the wheat and barley parental genotypes. Mild drought stress was induced by PEG treatment in a hydroculture system. When PEG 6000 was added to the nutrient solution the osmotic pressure of the nutrient solution decreased from −0.025 to −0.72 MPa. This mild osmotic stress only resulted in a slight reduction in the relative water content (RWC) of the leaves (Table 2) in all the genotypes compared with the control. A small but statistically significant difference in RWC between the control and stressed plants was only found in the case of wheat line Mv9kr1 and the cv. 'Chinese Spring'.

As the stomata play an important role in the regulation of both transpirational water loss and photosynthetic CO_2 assimilation rate, gas-exchange measurements were made during the osmotic stress treatment in order to determine changes in the stomatal conductance (g_s), which is proportional to the closure of the stomatal aperture, in the photosynthetic CO_2 assimilation rate (A) and in the water use efficiency (WUE_I) determined at single leaf level as A/g_s.

Before osmotic treatment, a significant difference in stomatal conductance (g_s) could be detected between the 4H(4D) substitution line and its parental genotypes (Table 2). When the osmotic pressure of the nutrient solution decreased to −0.72 MPa during the PEG treatment, g_s decreased in all the genotypes. During mild drought stress, the stomatal conductance (g_s) decreased more extensively in the barley cv. 'Betzes' and in the substitution line than in the wheat genotypes, indicating intensive stomatal closure in these genotypes. The decrease in g_s was not >50% in wheat, indicating that the stomata of wheat remained more widely open than those of plants having chromosome 4H. Besides the modification of g_s, the net CO_2 assimilation rate (A) at 350 ppm CO_2 concentration also decreased during PEG-induced osmotic stress. Before osmotic treatment, no significant difference between the genotypes could be detected in the net CO_2 assimilation rate (Table 2). In the case of mild osmotic stress the greatest decrease in A was observed for the wheat plants, but there was no significant modification in the CO_2 assimilation rate in the barley. In the substitution line, a small decrease in A was observed, but the CO_2 assimilation rate remained higher than in wheat.

Before osmotic stress, WUE_I, calculated as A/g_s at leaf level, was a little lower in barley than in the other genotypes (Table 2). The osmotic stress triggered an increase in WUE_I for genotypes having barley chromosome 4H [cv. 'Betzes' and the 4H(4D) substitution line'], while no significant changes were observed in the parental wheat genotypes (cv. 'Chinese Spring' and line Mv9kr1), indicating greater water utilization efficiency in genotypes possessing barley chromosome 4H.

Discussion

A spontaneously produced wheat–barley 4H(4D) substitution line was identified using sequential GISH and FISH, and the ability of barley 4H chromosome to compensate for the wheat 4D chromosome was studied in the photosynthetic responses to mild drought stress.

As the hybridization patterns of wheat and barley differ significantly when using the pAs1 and GAA probes, all the wheat and barley chromosomes, even in the substitution line, could be identified in a single hybridization step (Pedersen et al. 1996, Pedersen and Langridge 1997). On the basis of the GAA karyotype of barley, the barley chromosomes labelled by GISH in the substitution line were identified as 4H. The presence of the 4H chromosomes was confirmed by SSR markers specific for barley chromosome 4H (Fig. 2). The 4D chromosome has a characteristic pAs1 hybridization pattern, making it easy to identify. There is a small hybridization site on the short arm close to the centromere and there are larger sites at the centromere and at intercalary, subterminal and terminal positions on the long arm (Rayburn and Gill 1986). This chromosome was not found in any cell of this line, so the loss of the 4D chromosomes was unambiguously demonstrated.

The substitution of barley 4H for wheat chromosome 4D resulted in changes in plant and spike morphology and in an increase in tillering ability. The increased tillering ability of the substitution line may contribute to high yield formation under well-watered conditions, but would not necessarily be advantageous under drought conditions. In the 'Chinese Spring'-'Betzes' 4H(4D) substitution line Islam and Shepherd (1993) observed much better fertility (1.51 ± 0.17 grains/spikelet) than for 4H(4A) (0.14 ± 0.05) or 4H(4B) (0.30 ± 0.08). In the present study, the 4H(4D) substitution line had similar

fertility (1.6 ± 0.39) to the above, indicating that the 4H barley chromosome is more homoeologous to 4D than to other chromosomes in wheat homoeologous group 4.

WUE is primarily controlled by stomatal conductance and CO_2 assimilation processes (Sinclair et al. 1984). The ratio of $^{13}C/^{12}C$ isotopes in the plant tissues was determined primarily by these two processes (Condon et al. 2002), making it possible to use ^{13}C discrimination analysis to characterise the water use efficiency in plants (Farquhar and Richards 1984, Lambrides et al. 2004). However, measuring ^{13}C discrimination provides no information on the magnitude of either CO_2 assimilation or transpiration or on whether variation in ^{13}C discrimination is being driven by variation in stomatal conductance or photosynthetic capacity (Condon et al. 2002). As indicated by Earl (2002), to identify sources of genetic material for improving WUE in wheat breeding programmes, detailed investigation is desirable on the specific physiological traits involved, such as the determination of stomatal conductance and CO_2 assimilation rate.

The present investigations demonstrated that mild osmotic stress (−0.72 MPa) induced intensive stomatal closure, indicated by a 65–75% decrease in g_s, in the barley cv. 'Betzes' and in the 4H(4D) substitution line. High stomatal closure reduced water loss through transpiration, as manifested in the unchanged RWC of the leaves. On the other hand, the CO_2 assimilation rate remained relatively high during mild drought stress in plants having the 4H chromosome. It thus seemed that mild osmotic stress had a greater effect on stomatal closure, and thus on the reduction in water loss, than on the decrease in CO_2 assimilation rate in these genotypes. The high CO_2 assimilation rate and the low water loss through the stomata resulted in an increase in WUE_I. As suggested by Martin and Ruiz-Torres (1992) increase of WUE under mild drought stress, as observed in the wheat–barley 4H(4D) substitution line, could indicates a relative drought tolerance. Similar results were found by Medrano et al. (2002) and Socias et al. (1997) using *Trifolium subterraneum* and Ritchie et al. (1990) with winter wheat. These results suggest that the barley cv. 'Betzes' and the 4H(4D) substitution line operate regulation and protection mechanisms that prevent a decrease in leaf water content and damage to CO_2 assimilation processes under water limitation conditions. This is confirmed by the fact that the RWC content did not change during mild drought stress. These results support the observation of Handley et al. (1994) that barley chromosome 4 plays an important role in the control of water use efficiency in barley (Handley et al. 1994).

In the case of wheat genotypes, however, mild drought stress induced less intense stomatal closure than in barley and the substitution line, resulting in higher water loss through transpiration. This was reflected in the slight decrease in RWC. In spite of the relative opening of the stomata, the CO_2 assimilation rate (A) is more inhibited in wheat than in barley or in the substitution line. In the case of wheat, it seems that mild osmotic stress had a greater effect on photosynthetic CO_2 fixation than on transpiration, resulting in unaugmented or decreased WUE_I. The intense decrease in A indicates that the wheat genotypes were not able to optimise their photosynthetic processes even under mild drought stress, which was manifested as poorer water use efficiency than in barley and the substitution line. As the response of the substitution line to drought stress resembled the response of barley rather than that of wheat, it can be assumed that there are genes localised on chromosome 4H that have a major role in the regulation of photosynthetic processes under water limitation conditions.

These results confirm previous observations that chromosome 4H plays an important role in the control of water use efficiency in barley (Handley et al. 1994) and that WUE_I can be inherited not just within, but also between species (Hubick and Farquhar 1988, Hall et al. 1990). Direct evidence was found that genes located on chromosome 4H of barley play an important role in the optimization of photosynthetic processes under mild drought and can be manifested in a wheat genetic background. The substitution of barley 4H for wheat 4D had a positive effect on the drought stress responses in wheat. Consequently, the 4H barley chromosome is suitable for improving wheat drought tolerance through intergeneric crossing.

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References

Begg, J. E., and N. C. Turner, 1976: Crop water deficits. Adv. Agric. **28,** 161—217.

von Caemmerer, S., and G. D. Farquhar, 1981: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153,** 376—387.

Cattivelli, L., P. Baldi, C. Crosatti, N. Di Fonzo, P. Faccioli, M. Grossi, A. M. Mastrangelo, N. Pecchioni, and A. M. Stanca, 2002: Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*. Plant Mol. Biol. **48,** 649—665.

Condon, A. G., R. A. Richards, G. J. Rebetzke, and G. D. Farquhar, 2002: Improving intrinsic water-use efficiency and crop yield. Crop Sci. **42,** 122—131.

Earl, H. J., 2002: Stomatal and non-stomatal restrictions to carbon assimilation in soybean (*Glycine max*) lines differing in water use efficiency. Environ. Exp. Bot. **48,** 237—246.

Ehdaie, B., and J. G. Waines, 1993: Variation in water-use efficiency and its components in wheat. I. Well-watered pot experiment. Crop Sci. **33,** 294—299.

Farquhar, G. D., and R. A. Richards, 1984: Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol. **11,** 539—552.

Gale, M. D., and T. E. Miller, 1987: The introduction of alien genetic variation into wheat. In: Lupton, F. G. H. (ed). Wheat Breeding: Its Scientific Basis. 173—210. Chapman and Hall, New York, USA.

Hall, A. E., R. G. Mutters, K. T. Hubick, and G. D. Farquhar, 1990: Genotypic differences in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci. **30,** 300—305.

Handley, L. L., E. Nevo, J. A. Raven, R. Martínez-Carrasco, C. M. Scrimgeour, H. Pakniyat, and B. P. Forster, 1994: Chromosome 4 controls potential water use efficiency ($\delta^{13}C$) in barley. J. Exp. Bot. **45,** 1661—1663.

Hubick, K., and G. Farquhar, 1988: Heritability and genotype × environment interactions of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogaea* L.). Aust. J. Plant Phys. **15,** 799—813.

Islam, A. K. M. R., and K. W. Shepherd, 1981: Production of disomic wheat–barley chromosome addition lines using *Hordeum bulbosum* crosses. Gen. Res. **37,** 215—219.

Islam, A. K. M. R., and K. W. Shepherd, 1992: Substituting ability of individual barley chromosomes for wheat chromosomes. 1. Substitutions involving barley chromosomes 1, 3 and 6. Plant Breeding **109,** 141—150.

Islam, A. K. M. R., and K. W. Shepherd, 1993: Substitution of barley chromosome 4 for group 4 homoeologous of wheat. In: Z. S. Li, and Z. Y. Xin (eds), Proceedings of the Eighth International Wheat Genetics Symposium, 141—144.

Islam, A. K. M. R., K. W. Shepherd, and D. H. B. Sparrow, 1978: Production and characterization of wheat–barley addition lines. In Proceedings of the 5th International Wheat Genetics Symposium, New Delhi, 365–371. Indian Society of Genetics and Plant Breeding, IARI, New Delhi.

Kruse, A., 1973: Hordeum–Triticum hybrids. Hereditas **73,** 157—161.

Lambrides, C. J., S. C. Chapman, and R. Shorter, 2004: Genetic variation for carbon isotope discrimination in sunflower: association with transpiration efficiency and evidence for cytoplasmic inheritance. Crop Sci. **44,** 1642—1653.

Le, H. T., K. C. Armstrong, and B. Miki, 1989: Detection of rye DNA in wheat–rye hybrids and wheat translocation stocks using total genomic DNA as a probe. Plant Mol. Biol. Rep. **7,** 150—158.

Logojan, A. A., and M. Molnár-Láng, 2000: Production of *Triticum aestivum–Aegilops biuncialis* chromosome additions. Cereal Res. Comm. **28,** 221—228.

Martin, B., and N. A. Ruiz-Torres, 1992: Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). Plant Physiol. **100,** 733—739.

Medrano, H., J. M. Escalona, J. Bota, J. Gulias, and J. Flexas, 2002: Regulation of photosynthesis of C-3 plants in response to progressive drought: stomatal conductance as a reference parameter. Ann. Bot. **89,** 895—905.

Molnár, I., L. Gáspár, É. Sárvári, S. Dulai, B. Hoffmann, M. Molnár-Láng, and G. Galiba, 2004: Physiological and morphological responses to water stress in *Aegilops biuncialis* and *Triticum aestivum* genotypes with differing tolerance to drought. Funct. Plant Biol. **31,** 1149—1159.

Molnár-Láng, M., and J. Sutka, 1994: The effect of temperature on seed set and embryo development in reciprocal crosses of wheat and barley. Euphytica **78,** 53—58.

Molnár-Láng, M., G. Linc, and J. Sutka, 1996: Transfer of the recessive crossability allele kr_1 from 'Chinese Spring' into the winter wheat variety Martonvásár 9. Euphytica **90,** 301—305.

Molnár-Láng, M., G. Linc, B. R. Friebe, and J. Sutka, 2000: Detection of wheat–barley translocations by genomic *in situ* hybridization in derivatives of hybrids multiplied *in vitro*. Euphytica **112,** 117—123.

Murai, K., T. Koba, and T. Shimada, 1997: Effects of barley chromosome on heading characters in wheat–barley chromosome addition lines. Euphytica **96,** 281—287.

Nagy, Z., and G. Galiba, 1995: Drought and salt tolerance are not necessarily linked: a study on wheat varieties differing in drought resistance under consecutive water and salinity stresses. J. Plant Phys. **145,** 168—174.

Nagy, E. D., M. Molnár-Láng, G. Linc, and L. Láng, 2002: Identification of wheat–barley translocations by sequential GISH and two-colour FISH in combination with the use of genetically mapped SSR markers. Genome **45,** 1238—1247.

Nepomuceno, A. L., D. M. Oosterhuis, and J. M. Stewart, 1998: Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. Environ. Exp. Bot. **40,** 29—41.

Pedersen, C., and P. Langridge, 1997: Identification of the entire chromosome complement of bread wheat by two-colour FISH. Genome **40,** 589—593.

Pedersen, C., S. K. Rasmussen, and I. Linde-Laursen, 1996: Genome and chromosome identification in cultivated barley and related species of the Triticeae (Poaceae) by *in situ* hybridization with the GAA-satellite sequence. Genome **39,** 93—104.

Ramsay, L., M. Macaulaya, S. degli Ivanissevichb, K. MacLeana, L. Cardlea, J. Fullera, K. J. Edwardsc, S. Tuvessond, M. Morganteb, A. Massarie, E. Maestrie, N. Marmirolie, T. Sjakstef, M. Ganalg, W. Powella, and R. Waugha, 2000: A simple sequence repeat-based linkage map of barley. Genetics **156,** 1997—2005.

Rayburn, A. L., and B. S. Gill, 1986: Isolation of a D genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol. Biol. Rep. **4,** 102—109.

Ritchie, S. W., H. T. Nguyen, and A. S. Holaday, 1990: Leaf water-content and gas-exchange parameters of 2 wheat genotypes differing in drought resistance. Crop Sci. **30,** 105—111.

Sinclair, T. R., C. B. Tanner, and J. M. Benett, 1984: Water use efficiency in crop production. BioScience **34,** 36—40.

Socias, X., M. J. Correia, M. Chaves, and H. Medrano, 1997: The role of abscisic acid and water relations in drought responses of subterranean clover. J. Exp. Bot. **48,** 1281—1288.

Vrána, J., M. Kubaláková, H. Simková, J. Cíhalíková, M. A. Lysák, and J. Dolezel, 2000: Flow-sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). Genetics **156,** 2033—2041.

Ya-Ping, Y., C. Xiao, X. Si-He, A. K. M. R. Islam, and X. Zhi-Yong, 2003: Identification of wheat–barley 2H alien substitution lines. Acta Bot. Sin. **45,** 1096—1102.

Zeller, F. J., and S. L. K. Hsam, 1983: Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: S. Sakamoto, (ed.), Proceedings of the Sixth International Wheat Genetics Symposium, 161—173. Plant Germ-Plasm Institute, Kyoto University, Kyoto.