GISH reveals different levels of meiotic pairing with wheat for individual *Aegilops biuncialis* chromosomes

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Abstract

The *Triticum aestivum* - *Aegilops biuncialis* (2n=4x=28; $U^bU^bM^bM^b$) disomic addition lines $2M^b$, $3M^b$, $7M^b$ and $3U^b$ were crossed with the wheat cv. Chinese Spring *ph1b* mutant genotype in order to induce homoeologous pairing, with the final goal of introgressing *Ae. biuncialis* chromatin into cultivated wheat. Wheat-*Aegilops* homoeologous chromosome pairing was studied in metaphase I of meiosis in the F_1 hybrid lines. Using U and M genomic probes, genomic *in situ* hybridization (GISH) demonstrated the occurrence of wheat-*Aegilops* homoeologous pairing in the case of chromosomes $2M^b$, $3M^b$ and $3U^b$, but not in the case of $7M^b$. The wheat-*Aegilops* pairing frequency decreased in the following order: $2M^b > 3M^b > 3U^b > 7M^b$, which may reflect differences in the wheat-*Aegilops* homoeologous relationships between the examined *Aegilops* chromosomes. The selection of wheat-*Aegilops* homoeologous recombinations could be successful in later generations.

Additional key words: disomic addition lines, *ph1b* mutant, *Triticum aestivum*.

Introduction

Aegilops species, which are closely related to *Triticum* sp., are potential gene sources for wheat improvement, especially its resistance against pests and diseases (Kuraparthy *et al.* 2007, Schneider *et al.* 2008) and against abiotic stresses (Rekika *et al.* 1997, Zaharieva *et al.* 2001, Landjeva *et al.* 2003). Disomic addition lines could be suitable starting points for the production of interspecific translocations having the useful agronomic characters of *Aegilops sp.* in a cultivated wheat background. As reviewed by Schneider *et al.* (2008), sets of addition lines have been produced with several *Aegilops* species, such as *Ae. umbellulata, Ae. peregrina, Ae. longissima, Ae. ventricosa,* and *Ae. geniculata* in recent decades (Friebe *et al.* 1995, 1996a, 1999).

Aegilops biuncialis Vis. (2n=4x=28; $U^bU^bM^bM^b$) is an annual allotetraploid species with good tolerance of drought stress (Molnár *et al.* 2004), barley yellow dwarf luteovirus (Makkouk *et al.* 1994), yellow rust and brown rust (Damania and Pecetti 1990, Dimov *et al.* 1993), which would be desirable to transfer into cultivated wheat. *Triticum aestivum-Ae. biuncialis* disomic addition lines (Schneider *et al.* 2005) could also be suitable genetic material for the production of wheat-*Ae. biuncialis* interspecific translocations.

The utilisation of interspecific translocations in breeding processes is only successful if the introgressed alien chromosome segment is able to compensate for the loss of wheat chromatin (Friebe *et al.* 1996b). Compensating wheat-alien translocations are likely to be formed from wheat and alien chromosomes belonging to the same homoeologous group due to the very similar gene order along the chromosomes. In wheat, however, homoeologous chromosome pairing, and consequently recombination, is suppressed by the function of the *Ph1* locus, localised on the long arm of chromosome 5B (Riley and Chapman 1958). The Chinese Spring *ph1b* (CS*ph1b*) mutant genotype (Sears 1977), which lacks the *Ph1* locus, has been used successfully for the introgression of alien genetic material into the wheat genome by the induction of homoeologous pairing (Lukaszewski 2000). Crossing wheat-*Aegilops* disomic addition lines with CS*ph1b* results in monosomic wheat-*Ae. biuncialis* addition lines having a single copy of the *Ph1* locus, which may lead to the pairing of wheat and

Received 28 June 2008, *accepted* 15 January 2009.
Abbreviations: DAPI - 4',6-diamidino-2-phenylindole; GISH - genomic *in situ* hybridization.
Acknowledgements: This work was supported by the Hungarian Wheat Ear Consortium and the National Scientific Research Fund (K67808). The authors thank Mrs I. Bucsi and Mrs. J. Havasi for their excellent technical assistance.
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Aegilops chromosomes, the prerequisite for the formation of new wheat-*Ae. biuncialis* homoeologous recombinations. However, some alien chromosomes do not pair with wheat chromosomes even in the absence of the *Ph1* locus. Thus, information on the meiotic pairing ability of individual *Ae. biuncialis* chromosomes with wheat chromosomes may provide a new insight into wheat-*Ae. biuncialis* homoeologous relationships at the chromosomal level, which is essential for the production of the compensating type of introgression lines.

The introgression of chromatin from alien species such as barley, rye, *Ae. geniculata* and *Ae. biuncialis* into the wheat genome can be detected by means of genomic *in situ* hybridization (GISH) using one or more differentially labelled genomic DNA probes (Le *et al.* 1989, Schwarzacher *et al.* 1989, Friebe *et al.* 2000, Molnár-Láng *et al.* 2000, Benavente *et al.* 2001, Molnár *et al.* 2005). GISH is also used extensively for the analysis of meiotic pairing between wheat and alien chromosomes, such as barley (Molnár-Láng *et al.* 2005), rye (Miller *et al.* 1994) and *Ae. geniculata* (Benavente *et al.* 2001).

In this study, GISH was applied for the analysis of wheat-*Ae. biuncialis* chromosome pairing in meiotic metaphase I in F_1 hybrids produced by crossing the $2M^b$, $3M^b$, $7M^b$ and $3U^b$ wheat-*Ae. biuncialis* disomic addition lines with the Chinese Spring *ph1b* mutant genotype.

Materials and methods

Plants: The $2M^b$, $3M^b$, $7M^b$ and $3U^b$ *Triticum aestivum* (Mv9kr1) – *Aegilops biuncialis* disomic addition lines produced in Martonvásár (Schneider *et al.* 2005) were crossed with the wheat cv. Chinese Spring *ph1b*/*ph1b* mutant (CS*ph1b*) (Sears 1977). The addition lines were used as female parent in all the crosses. The F_1 hybrids were grown in the nursery and their meiotic behaviour was analysed in pollen mother cells (PMCs) at metaphase I (MI) of meiosis. Anthers of the F_1 hybrids containing PMCs at MI were fixed in 1:3 (v/v) acetic acid/ethanol and stored at -20 °C for a maximum of 2 months. The anthers were squashed in 45 % acetic acid and the slides were stored at -20 °C until *in situ* hybridization.

DNA probes and labelling: Genomic DNA isolation was carried out as described by Sharp *et al.* (1988). The total genomic DNA of *Ae. comosa* Sm. in Sibth. & Sm. (2n=2x=14; MM) and *Ae. umbellulata* Zhuk. (2n=2x=14; UU) was labelled with biotin (biotin-5-dUTP, *Roche*, Mannheim, Germany) or digoxigenin (digoxigenin-12-dUTP, *Roche*) by random priming and used as M and U genome probes, respectively. Occasionally, *Ae. tauschii* (2n=2x=14; DD) genomic DNA labelled with biotin or digoxigenin was also used parallel with the M or U genomic probes. Unlabelled durum wheat (*Triticum turgidum ssp. durum* L., 2n=4x=28; AABB) genomic DNA was sheared by autoclaving and used as a block.

Genomic *in situ* hybridization (GISH): The pretreatment and stringency washing of the slides were carried out as described by Schneider *et al.* (2005). The hybridization mixture (0.025 cm^3 per slide), containing 50 % formamide, 2× SSC, 10 % dextran sulphate, 70 ng of the U or M genome probes and 2.1 μg competitor DNA, was denatured at 80 °C for 10 min and stored on ice for 5 min. The chromosome DNA was denatured in the presence of the hybridization mixture at 80 °C for 2 min and allowed to hybridize overnight at 42 °C. For the detection of the hybridization signals, 10 μg cm^{-3} each of streptavidin-FITC (*Roche*) and anti-digoxigenin-Rhodamin (*Roche*) were used. Finally, the slides were counterstained with 2 mg cm^{-3} 4',6-diami-dino-2-phenylindole (DAPI; *Amersham*, Freiburg, Germany).

Images were acquired through a *Zeiss Axioskop-2* fluorescence microscope using a *Plan Neofluar* oil objective (*Zeiss*, Oberkochen, Germany) equipped with filter sets appropriate for DAPI, FITC and Rhodamin (*Zeiss* filter set 24) with a *Spot* CCD camera (*Diagnostic Instruments*, Sterling Heights, MI, USA). The images were compiled with *Image Pro Plus* software (*Media Cybernetics*, Silver Spring, MD, USA).

Differences between the frequency of the individual wheat-*Aegilops* chromosome associations were determined by means of *t*-tests.

Results

Production of F_1 hybrids between wheat-*Ae. biuncialis* addition lines and CS*ph1b*: In the first step F_1 hybrids (AABBDD + U^b or M^b; 2n = 6x = 42+1) were developed by crossing Mv9kr1-*Ae. biuncialis* $2M^b$, $3M^b$, $7M^b$ or $3U^b$ disomic addition lines with the *ph1b* mutant of Chinese Spring (Table 1). For each addition line 17 - 20 plants were pollinated with the CS*ph1b* genotype. Around 50 % of the flowers became fertilised and produced seeds. The lowest fertility was observed for the $2M^b$ addition line (42.64 %) and the highest for the $3U^b$ addition line (69.03 %).

Visualization of *Ae. biuncialis* chromosomes using genomic in situ hybridization: Pairing between wheat and *Aegilops* chromosomes was detected by means of genomic *in situ* hybridization in metaphase I of meiosis in the F_1 hybrid plants. By using genomic DNA from *Ae. umbellulata* (UU, 2n=2x=14) and *Ae. comosa* (MM, 2n=2x=14) as U and M genome-specific probes, the *Ae. biuncialis* chromosomes were clearly distinguishable on the basis of the green fluorescent signal (red if digoxigenin was used for labelling). Crosshybridization

Table 1. Production of F_1 hybrids by crossing wheat and *Ae. biuncialis* disomic addition lines ($2M^b$, $3M^b$, $3U^b$, $7M^b$) with the Chinese Spring *ph1b* mutant (CS*ph*) genotype.

	Number of pollinated plants	Number of pollinated flowers	Number of seeds obtained	Fertility [%]
$2M^b \times$ CS*ph*	20	598	255	42.64
$3M^b \times$ CS*ph*	20	586	349	59.55
$7M^b \times$ CS*ph*	19	466	230	49.35
$3U^b \times$ CS*ph*	17	436	301	69.03

signals of the probe were occasionally observed in the centromeric and peri-centromeric regions of the wheat chromosomes (Fig. 1*A,D*), but this did not affect the ability to discriminate between wheat and *Aegilops* chromosomes. Regardless of which *Ae. biuncialis* chromosome they contained, the most frequently occurring meiotic configuration in the F_1 hybrid lines was 21 wheat bivalents plus one *Aegilops* univalent (Fig. 1*A*). In the F_1 hybrid derived from a cross with the $3M^b$ addition line 20 wheat plus one wheat-*Aegilops* bivalent were observed (Fig. 1*C*). In a few cases pairing was observed between wheat and *Aegilops* chromosomes (Fig. 1*B-F*), involving both bivalents (Fig. 1*E,*F) and trivalents (Fig. 1*G-I*). The most frequent form of wheat-*Aegilops* trivalent was V-shaped, with the *Aegilops* chromosome in the distal position (Fig. 1*G*), but Y-shaped and "frying pan"-shaped forms were also

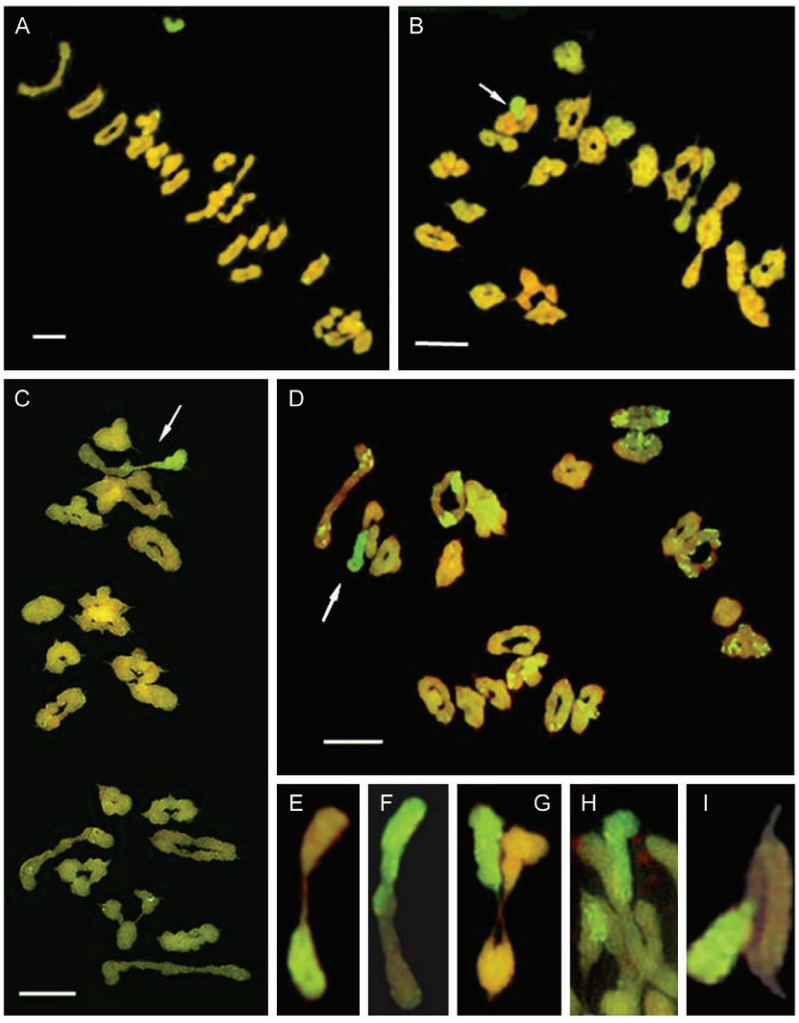

Fig. 1. GISH discrimination of *Aegilops* chromosomes labelled using biotinilated U or M genomic probes (*green*) and unlabelled wheat chromosomes (*brown*) on meiotic metaphase I chromosome spreads of PMCs from F_1 hybrids of wheat and *Ae. biuncialis* additions × CS*ph1b*. *A* - F_1 hybrid involving the $7M^b$ chromosome ($21^{II} + 1^{I}$), *B* - F_1 hybrid involving the $3U^b$ chromosome ($20^{II} + 1^{III}$), *C* - F_1 hybrid involving the $3M^b$ chromosome (21^{II}), *D* - F_1 hybrid involving the $2M^b$ chromosome ($20^{II} + 1^{III}$). Examples of five types of wheat-*Aegilops* MI associations: rod bivalent without a chiasma (*E*), rod bivalent with a chiasma (*F*), V-shape trivalent (*G*), Y-shape trivalent (*H*), frying pan trivalent (*I*). *Arrows* indicate wheat-*Ae. biuncialis* chromosome associations. *Bar* = 10 μm.

Table 2. Frequency of wheat-*Aegilops* chromosome pairing configurations in metaphase I of meiosis in F_1 hybrids originating from crosses between wheat-*Ae. biuncialis* addition lines ($2M^b$, $3M^b$, $7M^b$, $3U^b$) and the *ph1b* mutant of Chinese Spring (CS*ph*). MI associations [1] - in 3 trivalents the *Aegilops* chromosome was in the central position,[2] - in 2 trivalents the *Aegilops* chromosome was in the central position, [3] - in 1 trivalent the *Aegilops* chromosome was in the central position.

	Cell No.	Wheat-*Ae. biuncialis* pairing configurations				MI association	
		rod II	ring II	III	mean	total	mean
$2M^b \times$ CS*ph*	57	0	0	14	0.245	17[1]	0.298
$3M^b \times$ CS*ph*	113	6	0	7	0.115	15[2]	0.132
$7M^b \times$ CS*ph*	87	0	0	0	0	0	0
$3U^b \times$ CS*ph*	139	0	1	3	0.028	5[3]	0.036

Table 3. Results of *t*-tests describing differences in the means of wheat-*Aegilops* chromosome pairing between the F_1 hybrids (*,** - significant difference between the two F_1 hybrids at the $P = 0.05$ and $P = 0.01$ significance levels, respectively).

	t-value	*P*-value
$2M^b \times$ CS*ph* - $3M^b \times$ CS*ph*	2.010	0.023000*
$2M^b \times$ CS*ph* - $7M^b \times$ CS*ph*	4.269	0.000038**
$2M^b \times$ CS*ph* - $3U^b \times$ CS*ph*	3.659	0.000259**
$3M^b \times$ CS*ph* - $7M^b \times$ CS*ph*	3.815	0.000111**
$3M^b \times$ CS*ph* - $3U^b \times$ CS*ph*	2.587	0.005200**
$7M^b \times$ CS*ph* - $3U^b \times$ CS*ph*	2.022	0.022000*

detected. In most cases the wheat and *Aegilops* chromosomes were connected through a thin chromatin bridge (Fig. 1*D*,*E*,*G*) and were thus considered as achiasmatic chromosome associations, which could be distinguished from the chiasmatic associations, where a pronounced chromatin loop could be seen at the linkage site (Fig. 1*C*,*F*).

Chromosome pairing behaviour of F_1 hybrids between wheat–*Ae. biuncialis* additions and CS*ph1b*: The various *Aegilops* chromosomes ($2M^b$, $3M^b$, $7M^b$, $3U^b$) paired with wheat chromosomes at different frequencies (Table 2). The results of the *t*-test revealed that $2M^b$ paired with wheat chromosomes more frequently than the other *Aegilops* chromosomes (Table 3). The number of $3M^b$- and particularly $3U^b$-wheat chromosome associations was significantly lower (Tables 2, 3), while no pairing with wheat chromosomes could be observed for chromosome $7M^b$. The pairing frequency of *Ae. biuncialis* chromosomes with wheat chromosomes could thus be ranked as follows: $2M^b > 3M^b > 3U^b > 7M^b$.

Table 4. Chiasmatic (chiasma+) and achiasmatic (chiasma–) wheat-*Aegilops* chromosome associations in metaphase I of meiosis in F_1 hybrids originating from crosses between wheat-*Ae. biuncialis* addition lines ($2M^b$, $3M^b$, $7M^b$, $3U^b$) and the *ph1b* mutant of Chinese Spring (CS*ph*).

	Total	Chiasma+	Chiasma–
2M × CS*ph*	17	0	17 (100%)
3M × CS*ph*	15	5 (33.3 %)	10 (66.6%)
7M × CS*ph*	0	0	0
3U × CS*ph*	5	0	5 (100%)

The chiasmas found on paired chromosomes in metaphase I of meiosis represent the sites where recombination (crossing over) occurs. When producing homoeologous wheat-*Aegilops* recombinations, it could be useful to know the extent to which they contain chiasmas. It can be seen from the data in Table 4 that the majority of the wheat-*Aegilops* chromosome associations were achiasmatic. Chiasmas were only observed in approximately a third of the $3M^b$-wheat chromosome associations.

Discussion

F_1 hybrids were developed by crossing wheat-*Ae. biuncialis* disomic addition lines ($2M^b$, $3M^b$, $7M^b$, $3U^b$) with the *ph1b* mutant of Chinese Spring (CS*ph1b*). Genomic *in situ* hybridisation was employed to determine the frequency of pairing between the individual *Aegilops* and wheat chromosomes in the meiosis of the F_1 hybrids, as this is a precondition for the development of homoeologous recombinations. The crosshybridization signals observed in some wheat chromosomes could indicate that similar repetitive DNA sequences are localized at the centromeric and peri-centromeric regions of wheat and *Aegilops* chromosomes. GAA satellite sequences, which are one of the major components of the heterochromatic regions (Pedersen and Langridge 1997), were localized mainly in the centromeric and/or pericentromeric regions of the U-genome chromosomes of *Ae. umbellulata* (Molnár *et al*. 2005) and also of the B-genome chromosomes of bread wheat (Vrána *et al.* 2000). Despite the crosshybridization, the GISH analysis made it clear that wheat-*Aegilops* chromosome associations were formed in the case of chromosomes $2M^b$, $3M^b$ and $3U^b$. When studying the meiosis of wheat (Mv9kr1)-*Ae. biuncialis* F_1 hybrids ($ABDU^bM^b$, 35), Logojan and Molnár-Láng (2000) reported a lower frequency of pairing between the wheat and *Ae. biuncialis* chromosomes than was observed in the present work. This could indicate that the *ph1b* mutation is capable of promoting the pairing of homoeologous chromosomes even when present in the heterozygous form, as in the F_1 hybrid lines used here.

Differences in the numbers of various wheat-*Aegilops* chromosome associations suggest that the individual *Aegilops* chromosomes do not have the same likelihood of pairing with the corresponding homoeologous chromosomes of wheat. Of the *Aegilops* chromosomes tested, $2M^b$ paired with wheat chromosomes at the highest frequency, followed by $3M^b$. The pairing frequency was much lower for $3U^b$, while no wheat-*Aegilops* chromosome associations were observed in the case of chromosome $7M^b$. In studies on CS*ph1b* × rye F_1 hybrids, Cuadrado *et al.* (1997) also found differences in the frequency with which the individual rye chromosomes paired with wheat chromosomes. These authors reported that similar sequences of coding and non-coding regions were decisive for the meiotic pairing of homoeologous chromosomes. If this collinearity has declined as the result of (intra- and intergenomic) structural rearrangements in the course of evolution, there is a reduced chance of pairing between the homoeologous chromosomes (Sybenga 1999, Maestra and Naranjo 2000). On the basis of pairing frequencies it is logical to conclude that chromosomes $2M^b$ and $3M^b$ are in a closer homoeologous relationship with the chromosomes of homoeologous groups 2 and 3 of wheat than the $3U^b$ and $7M^b$ chromosomes with the corresponding wheat chromosomes. In this connection, RFLP analyses carried out by Zhang *et al.* (1998) revealed that the U genome of *Ae. umbellulata* and the genomes of wheat differed by at least 11 chromosome rearrangements (reciprocal translocations and inversions). In a study on meiotic pairing in *Triticum turgidum* and *Ae. ovata* (syn. *Ae. geniculata*) amphiploids (2n=8x=56, $AABBM^oM^oU^oU^o$) in the presence of a *ph1c* deletion (which is in a position similar to the *ph1b* deletion), Benavente *et al.* (2001) detected five M^o-U^o and four M^o-wheat translocations in twelve plants, but no U^o-wheat translocations. The present investigations on homoeologous group 3 confirm these findings, as the $3M^b$ chromosomes paired considerably more frequently with wheat chromosomes than the $3U^b$ chromosomes. In some F_1 hybrid lines derived from a cross with the $3M^b$ addition line 21 bivalents (41 wheat plus one *Aegilops* chromosome) were observed. As cytologically tested disomic addition lines have exhibited high genetic stability in recent years, these meiosis data probably reflect the unbalanced genomic constitution of the *ph1b* crossing partner. This idea is also supported by the comprehensive karyotypic analysis of the *ph1b* mutants, where Sánchez-Morán *et al.* (2001) demonstrated 11 A, 14 B and 17 D chromosomes in some *ph1b* plants by mcGISH. Despite the fact that the *ph1b* mutant plant in the present work also had the euploid chromosome number (42, as evidenced by the Feulgen method), a change in the genomic constitution cannot be excluded.

The chiasmas observed in metaphase I are indicative of recombinations between two chromosomes in meiosis (Benavente *et al.* 1996), although there are also indications of achiasmatic recombinations (Irick 1994). The fact that chiasmas were only observed in the $3M^b$-wheat chromosome associations leads to the conclusion that there is a greater likelihood of homoeologous recombinations with wheat in the case of the $3M^b$ chromosome than for the other *Aegilops* chromosomes tested.

On the basis of the meiotic chromosome pairing observed in the present work it can be stated that, among the *Ae. biuncialis* chromosomes tested, $2M^b$ and $3M^b$ exhibited the closest homoeologous relationship with the corresponding wheat chromosomes.

Several agronomically important genes are localized on the chromosomes of homoeologous groups 2, 3 and 7 in wheat and their wild relatives. For example, resistance genes to various rusts (Yr8, Sr34; and Lr35, Sr39) are localized on chromosome 2M of *Ae. comosa* and chromosome 2S of *Ae. speltoides,* respectively (McIntosh *et al*. 1982, Gold *et al*. 1999). QTLs for shoot Mn and Cu contents (Bálint *et al*. 2007), for resistance to *Fusarium* head blight in wheat (Waldron *et al*. 1999) and for resistance to leaf rust (Lr24) in *Agropyron elongatum* have also been identified on the homoeologous group 3 (Friebe *et al*. 1996a,b). Important QTLs for physiological traits involved in drought tolerance (such as osmotic adjustment and water use efficiency) were also identified on homoeologous group 7 in wheat and barley (Cattivelli *et al.* 2002). On the basis of this information the wheat-*Aegilops* recombinations in homoeologous groups 2, 3, and 7 could have great genetic potential for wheat improvement. According to the results of the present meiotic pairing analysis, it may later be possible to select new wheat-*Ae. biuncialis* translocation lines.

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