

Methodology

Molecular cytogenetic characterization of the *Aegilops biuncialis* karyotype

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ABSTRACT. *Aegilops biuncialis* can be hybridized with wheat (*Triticum* spp) and has been used for wheat breeding and genetic studies. The *A. biuncialis* karyotype ($U^b U^b M^b M^b$) was investigated based on three *A. biuncialis* accessions grown in China. Two pairs of SAT chromosomes were identified as $1U^b$ and $5U^b$, with a karyotype formula of $2n = 4x = 28 = 14m + 10sm + 4st$. Fluorescence *in situ* hybridization (FISH) and C-banding approaches were used to analyze the *A. biuncialis* accession chromosomes at the mitotic stage. Based on the C-banding and FISH patterns, all U^b and M^b chromosomes could be discriminated simultaneously; the three *A. biuncialis* accessions exhibited similar patterns, suggesting a common origin. The U^b genome from *A. biuncialis* resembled the U genome in the diploid species *A. umbellulata*, and it may be related to the tetraploid species containing the U genome. The M^b genome had some differences compared to the M genome in the diploid species

A. comosa, and it may be related to the tetraploid species possessing the M genome. A generalized ideogram was proposed for the *A. biuncialis* genome, which could be useful for standardized and accurate identification of the *A. biuncialis* karyotype and chromosomes.

Key words: *Aegilops biuncialis*; Chromosome; Karyotype; C-banding; Fluorescence *in situ* hybridization

INTRODUCTION

The genus *Aegilops* comprises 11 diploid, 10 tetraploid, and 2 hexaploid species. Based on the C, D, S, U, and M pivotal genomes, *Aegilops* is classified into *Polyeides*, *Cylindropyrum*, *Vertebrata*, *Amblyopyrum*, *Comopyrum*, and *Sitopsis* sections (van Slageren, 1994). *Aegilops* species are the closest relatives of the diverse *Triticum* genus and present a reservoir of useful traits for improving the agronomic traits of wheat. A number of pest- and disease-resistant genes of *Aegilops* have been transferred to wheat (*Triticum aestivum* L.) through wide hybridization and chromosome engineering (Riley et al., 1968; Dhaliwal et al., 2002).

Aegilops biuncialis is generally associated with the *Polyeides* section. The species in this section are annual and have tetraploid genomes ($U^b U^b M^b M^b$, $2n = 4x = 28$). The U^b genome is donated by an *A. umbellulata* diploid progenitor ($2n = 2x = 14$, UU), whereas the modified M^b genome is donated by *A. comosa* ($2n = 2x = 14$, MM) (Resta et al., 1996; Badaeva et al., 2004). Some *A. biuncialis* accessions are highly resistant to barley yellow dwarf disease (Makkouk et al., 1994), and others have superior rust resistance (Dhaliwal et al., 2002). *A. biuncialis* genotypes from dry environments generally have a drought tolerance better than that of wheat. These genotypes may be important for improving drought tolerance in wheat via intergenetic crossing (Molnár et al., 2004).

The early C-banding technique has been successfully used to analyze wheat genomes and chromosomes (Gill and Kimber, 1974). This approach has also been useful in the characterization of genomes and chromosomes of *Aegilops* species. For example, C-banding has been used to construct standard karyotypes of *A. umbellulata*, *A. comosa*, and *A. geniculata* (Friebe et al., 1995, 1996). It has also been used to detect alien chromatins in *T. aestivum*-*A. ovatum*, *T. aestivum*-*A. squarrosa*, *T. aestivum*-*A. caudate*, and *T. aestivum*-*A. geniculata* hybrids (Friebe and Heun, 1989; Friebe et al., 1992a,b, 2000).

Multicolor fluorescence *in situ* hybridization (FISH), based on the labeling of repetitive sequences as probes, has been developed and used extensively for *Triticum* chromosome identification. The repetitive sequences harbored by the plasmids pSc119.2 (Bedbrook et al., 1980; McIntyre et al., 1990), pSc74 (Bedbrook et al., 1980), pAs1 (Rayburn and Gill, 1986), pTa71 (Gerlach and Bedbrook, 1979), and pTa794 (Gerlach and Dyer, 1980) are most frequently used as probes. Owing to their excellent hybridization signal stability and intensity, the probes derived from pSc119.2 and pAs1 plasmids have gained widespread application in chromosome discrimination and alien chromatin identification in wheat-rye and wheat-barley chromosome translocations (Mukai et al., 1993; Nagy et al., 2002; Wang et al., 2004).

Both FISH and C-banding methods have been used to analyze karyotype and polygenetic relationships in *A. biuncialis*. Using 27 accessions of diverse origin, Badaeva (2002) and Badaeva et al. (2004) have reported detailed C-banding karyotype and FISH patterns in *A. biuncialis*. However, these authors failed to show the FISH patterns of the investigated

chromosomes graphically. Using three *A. biuncialis* accessions, Schneider et al. (2005) have proposed an FISH pattern for all *A. biuncialis* chromosomes; however, they reported no corresponding C-banding results for *A. biuncialis* chromosomes.

In 2003, we hybridized these three *A. biuncialis* with common wheat and obtained 433 strains. Hence, the main objective of this study was to analyze FISH and C-banding patterns of three drought-tolerant *A. biuncialis* accessions concurrently, with the aim of establishing a generalized *A. biuncialis* ideogram for facilitating the accurate identification of its component genomes and chromatin in the wheat genetic background.

MATERIAL AND METHODS

Plant materials

The *Biun 65*, *Biun 76*, and *Biun 77* *A. biuncialis* accessions (introduced from Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, 12th South Street, Zhongguancun, Haidian District, Beijing, China) were used in this study.

Chromosome preparation

Approximately 2- to 3-cm root tips were excised and pretreated in ice water for 22 to 24 h. They were then fixed in a freshly prepared 3:1 solution of absolute ethanol and glacial acetic acid for 1 to 7 days. The root caps were removed with a razor blade, and the small pieces of meristem were squashed in 45% acetic acid. The slides were frozen in liquid nitrogen, and the cover slips were removed with a razor blade. The slides were subsequently dehydrated through a 70, 90, and 100% ethanol series for 5 min each, dried overnight at room temperature, and stored at 4°C for further use.

C-banding

Slides were treated in 0.2 M HCl at 60°C for 2 min (in a water bath), washed in distilled water, incubated in saturated barium hydroxide solution at room temperature for 7 min, rinsed in distilled water, and then incubated in 2X saline sodium citrate (SSC) at 60°C for 1 h. The slides were placed directly in 1 to 5% Giemsa staining solution of phosphate buffer for up to 30 min. The chromosome staining process was monitored until optimal staining was obtained.

DNA probes

DNA probes were prepared using the plasmids pSc119.2 (Bedbrook et al., 1980) and pAs1 (Rayburn and Gill, 1986). The DNA of the two plasmids was labeled with biotin-16-dUTP (red) and digoxigenin-11-dUTP (green), respectively, via nick translation (Roche Company, Switzerland).

FISH

Denatured hybridization solution (40 µL) containing 2X SSC, 10% dextran sulfate,

50% formamide, 0.1% sodium dodecyl sulfate, 0.9 μg salmon sperm DNA, and 30 ng labeled DNA probe was applied to each denatured slide and incubated at 37°C for 6 h. Stringency washing was carried out in 4X SSC-Tween 20 for 10 min at 42°C, and 20 μL antidigoxigenin-rhodamine (Roche) was applied to each slide. After incubation at 37°C for 1 h, the slides were again washed in 4X SSC-Tween 20 for 20 min at 42°C. The preparations were counterstained with 4,6-diamidino-2-phenylindole and examined under a Zeiss Axioskop epifluorescence microscope (Zeiss, Oberkochen, Germany). The microscope was equipped with filter 10 for fluoresce in isothiocyanate, filter 15 for Texas Red, and a triple-band filter for fluorescence in isothiocyanate, 4,6-diamidino-2-phenylindole, and Texas Red. Images were taken with a Spot-CCD camera (DVC Company, USA).

RESULTS

Karyotype and C-banding analysis

Each of the three accessions of *A. biuncialis* had seven pairs each of U^b and M^b chromosomes and two pairs of satellite (SAT) chromosomes were identified on the $1U^b$ and $5U^b$ chromosomes (Figures 1A, B, and 2). The karyotype was composed of seven chromosome pairs with median centromeres (m), five pairs with submedian centromeres (sm), and two pairs with subterminal centromeres (st). The formula for the chromosome karyotype was $2n = 4x = 28 = 14m + 10sm + 4st$. The arm ratios (L/S), relative arm lengths (L+S) and arm types (m, sm, st) of the chromosomes are listed in Table 1.

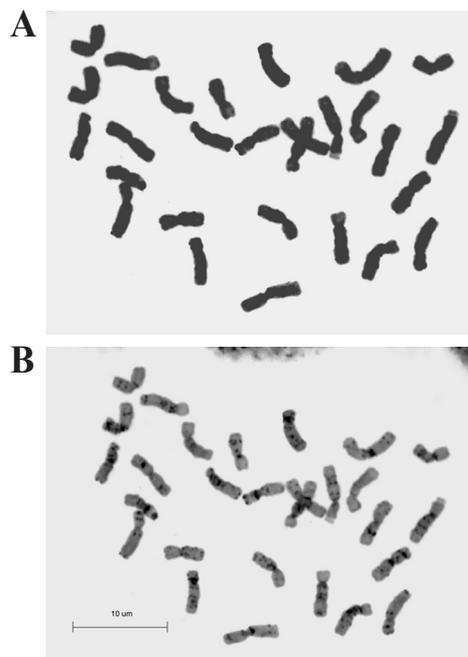


Figure 1. Mitotic metaphase chromosomes of *Aegilops biuncialis* accession *Bium* 65. **A.** Phase contrast. **B.** C-banding.

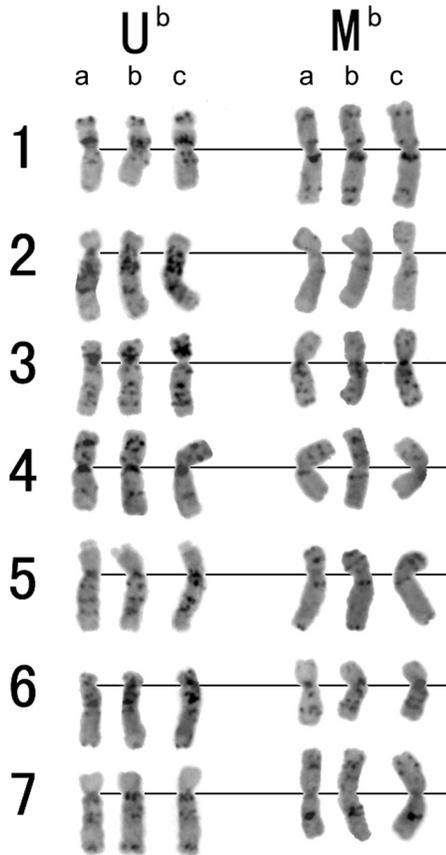


Figure 2. C-banding patterns of chromosomes in the 3 *Aegilops biuncialis* accessions. **a.** *Biun 65.* **b.** *Biun 76.* **c.** *Biun 77.*

Table 1. Arm ratio, relative arm length and type of *Aegilops biuncialis* chromosomes.

| Chromosome | Arm ratio (L/S) | Relative arm length | Type of chromosomes |
|-----------------|-----------------|---------------------|---------------------|
| 1U ^b | 1.23 | 6.26 | m |
| 2U ^b | 3.90 | 7.26 | st |
| 3U ^b | 2.61 | 6.70 | sm |
| 4U ^b | 1.45 | 7.46 | m |
| 5U ^b | 1.74 | 8.06 | sm |
| 6U ^b | 5.51 | 6.58 | st |
| 7U ^b | 2.88 | 7.34 | sm |
| 1M ^b | 1.24 | 8.99 | m |
| 2M ^b | 2.11 | 7.05 | sm |
| 3M ^b | 1.44 | 6.45 | m |
| 4M ^b | 1.09 | 6.64 | m |
| 5M ^b | 2.23 | 7.46 | sm |
| 6M ^b | 1.45 | 5.67 | m |
| 7M ^b | 1.00 | 8.09 | m |

L/S = long arm length and short arm length; m = median centromere; st = subterminal centromere; sm = submedian centromere.

All *A. biuncialis* chromosomes had identifiable C-bands (see Figure 1B). Similar C-banding patterns were observed among the three *A. biuncialis* accessions (see Figure 2). A generalized C-banding ideogram was established based on measurements from 10 chromosomes of each *A. biuncialis* accession (Figure 3).

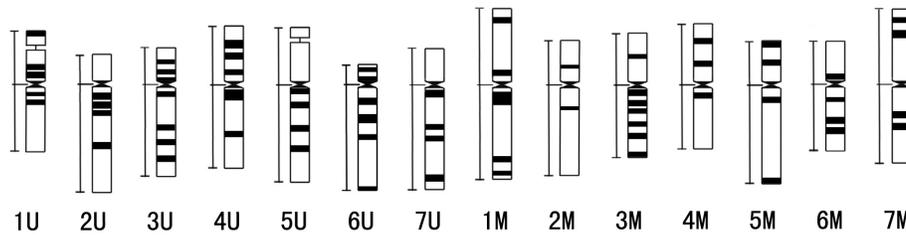


Figure 3. Generalized ideogram of the 3 *Aegilops biuncialis* accessions. The chromosome arm length and arm ratio data were based on the measurements of 10 chromosomes of each *A. biuncialis* accession.

Chromosome 1U^b [arm ratio (L/S), 1.2; relative arm length (L+S), 6.3; type of chromosome, m] was an SAT chromosome with secondary constriction in the distal region of the short arm. C-bands were present on both sides of the centromere, and at the telomere of secondary constriction. Chromosome 2U^b (L/S, 3.9; L+S, 7.3; type of chromosome, st) had C-bands on the proximal and interstitial regions of the long arm. However, chromosome 2U^b had no C-bands on either the telomeres or the short arm. Chromosome 3U^b (L/S, 2.6; L+S, 6.7; type of chromosome, sm) had C-bands on both sides of the centromere. Some dense C-bands were noted on the short arm of chromosome 3U^b. However, C-bands were completely absent on both telomeres. Chromosome 4U^b (L/S, 1.5; L+S, 7.5; type of chromosome, m) had C-bands on both sides of the centromere. Interestingly, three C-bands were present on the short arm and two were present on the long arm. However, no C-band was observed on the telomeres. Chromosome 5U^b (L/S, 1.7; L+S, 8.1; type of chromosome, sm) was a second SAT chromosome. Three C-bands were evenly distributed on the long arm, but no C-bands were present on the telomeres and short arm. Chromosome 5U^b was easily distinguishable from SAT chromosome 1U^b by its short satellite and different chromosome length. Chromosome 6U^b (L/S, 5.5; L+S, 6.6; type of chromosome, st) was easily distinguishable by its large arm ratio. C-bands were present on both sides of the centromere, and some C-bands were observed on the long arm, one of which was on the telomere. Chromosome 7U^b (L/S, 2.9; L+S, 7.3; type of chromosome, sm) had no C-bands on the short arm. Several C-bands were observed on the long arm, one of which was on the telomere.

Chromosome 1M^b (L/S, 1.2; L+S, 9.0; type of chromosome, m) was the longest chromosome. Two C-bands were present on the short arm and three were present on the long arm, one of which was denser in the proximal region of the centromere. Chromosome 2M^b (L/S, 2.1; L+S, 7.1; type of chromosome, sm) had two weak C-bands, with one of each in the interstitial regions of the short and long arms. Chromosome 3M^b (L/S, 1.4; L+S, 6.5; type of chromosome, m) had C-bands on both sides of the centromere. More C-bands occurred on the long arm than on the short arm; only one C-band was observed on the short arm, and six C-bands were observed on the long arm. Chromosome 4M^b (L/S, 1.1; L+S, 6.6; type of chromosome, m) had two C-bands on the short arm. One of the C-bands close to the centromere was on the

long arm. Chromosome 5M^b (L/S, 2.2; L+S, 7.5; type of chromosome, sm) had 2 C-bands in the proximal regions of the short and long arms. Two additional C-bands were present on both telomeres. Chromosome 6M^b (L/S, 1.5; L+S, 5.7; type of chromosome, m) was the shortest chromosome, with C-bands on the centromere and long arm. Chromosome 7M^b (L/S, 1.0; L+S, 8.1; type of chromosome, m) was a metacentric chromosome with two C-bands close to the telomere on the short arm and two notably dense C-bands at the middle of the long arm.

FISH analysis

The three *A. biuncialis* accessions exhibited similar FISH patterns. The sites of pSc119.2 were observed on all of the chromosomes except 6U^b, 2M^b, and 3M^b. Similarly, pAs1 sites were found on all of chromosomes except 2U^b, 3U^b, 4U^b, 3M^b, and 4M^b. With the exception of 3M^b, all the chromosomes had one or both of the pSc119.2 and pAs1 hybridization sites. These characteristics allowed the distinction of all chromosomes (Figure 4). A generalized FISH ideogram was established for *A. biuncialis* (Figure 5). The presence and absence of pSc119.2 (red bands) or pAs1 (green bands) hybridization sites and their locations on the *A. biuncialis* chromosomes are described below.

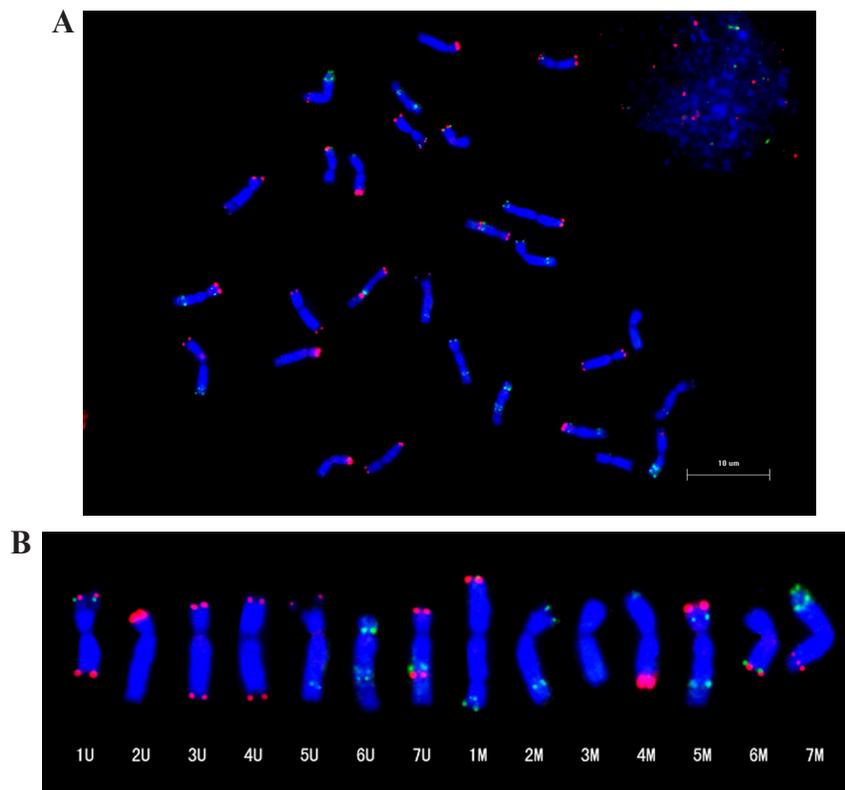


Figure 4. Fluorescence *in situ* hybridization (FISH) depicting the locations of pSc119.2 (red) and pAs1 (green) hybridization sites on individual somatic chromosomes of the *Aegilops biuncialis* accession *Biun 76*. **A.** Mitotic metaphase chromosomes. **B.** FISH patterns.

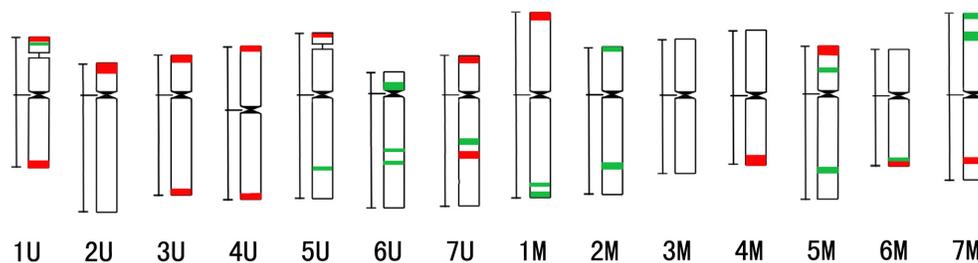


Figure 5. Graphical presentation of chromosomal fluorescence *in situ* hybridization patterns of the 3 *Aegilops biuncialis* accessions using pSc119.2- (red bands) and pAs1- (green bands) derived DNA probes.

Chromosome 1U was an SAT chromosome with two pSc119.2 sites on both telomeres and a pAs1 site on the secondary constriction. Chromosome 2U had one broad pSc119.2 site on the telomere of the short arm but no pAs1 sites on the whole chromosome. Chromosome 3U had two pSc119.2 sites on both telomeres. Chromosome 4U also had two pSc119.2 sites on both telomeres. The difference between 3U and 4U was in the arm ratio and relative arm length of the chromosomes. Chromosome 5U was a second SAT *A. biuncialis* chromosome. One pSc119.2 site occurred on the telomere of the secondary constriction and one pAs1 site was present on the long arm. No band was observed on the telomere of the long arm. Chromosome 6U had 3 pAs1 sites; one on the centromere and two in the middle of the long arm. Chromosome 7U had 2 pSc119.2 sites; one on the telomere of the short arm and another in the middle of the long arm. One pAs1 site was present in the middle of the long arm on chromosome 7U.

Chromosome 1M had one pSc119.2 site on the telomere of the short arm. It also had two pAs1 sites on the long arm - one on the telomere and another near the telomere. Chromosome 2M had two pAs1 sites; one on the telomere of the short arm and another on the long arm. Chromosome 3M had no sites on its entire length. Chromosomes 4M and 5M had one broad pSc119.2 site on the telomere of the long arm and short arm, respectively. Chromosome 5M also had two pAs1 sites; one in the middle of the short arm and another near the telomere of the long arm. Chromosome 6M had one pSc119.2 and one pAs1 site on the telomere of the long arm. Chromosome 7M had one pSc119.2 site on the long arm and two pAs1 sites on the short arm, one of which was on the telomere.

DISCUSSION

Detailed C-banding and FISH analyses of three *A. biuncialis* accessions were carried out and showed that C-bands were present in all *A. biuncialis* chromosomes. Each chromosome of *A. biuncialis* displayed unique C-banding and FISH patterns that could be used for chromosome identification. Neither large structural rearrangements nor polymorphisms were observed in the C-banding and FISH patterns of the investigated accessions, indicating both close relationships and similar origins among the three *A. biuncialis* accessions.

The C-banding patterns of the three *A. biuncialis* accessions were generally similar to those of the *A. biuncialis* accessions reported by Badaeva et al. (2004), which belonged to type II chromosomes. For the individual component genomes, U^b had intense centromere bands. This finding was consistent with that for the *A. umbellulata* karyotype reported by Friebe et al.

(1995). The M^b genome was somewhat different from the *A. comosa* subsp *comosa* karyotype ($2n = 2x = 14 MM$) and notably different from the *A. comosa* subsp *heldreichii* karyotype ($2n = 2x = 14 M^h M^h$) (Friebe et al., 1996).

Although no hybridization sites were observed in the 3M^b chromosome, the FISH patterns for the U^b and M^b chromosomes of the three *A. biuncialis* accessions were also identified in terms of the pSc119.2 and pAs1 probes. The hybridization pattern of the U^b genome was the same as those of four *A. umbellulata* accession diploids (Schneider et al., 2005) and similar to those recorded by Schneider et al. (2005) for the accessions we examined. The 5U^b chromosome pattern differed. The 5U^b chromosome in this study had one pAs1 site on the long arm, and no band was observed on the telomere of the long arm, whereas that of Schneider et al. (2005) had one pSc119.2 site on the telomere of the long arm and no band on the long arm. The hybridization pattern of the M^b genome was variable for the *A. comosa* (MM) genome accession diploids on the 1M^b, 3M^b, 4M^b, and 6M^b chromosomes but entirely different from the *A. biuncialis* genome (U^b U^b M^b M^b) accession tetraploids on the 1M^b, 3M^b, and 6M^b chromosomes. In particular, the 1M^b chromosome had only one pSc119.2 band on the telomere of the long arm and one pAs1 band on the telomere of the short arm. This result agreed with that of Badaeva et al. (2004), but disagreed with that of Schneider et al. (2005).

Our analyses confirmed that the U^b genome in *A. biuncialis* originated from *A. umbellulata* (UU) diploid species, and that the M^b genome of *A. biuncialis* may be a modified version of the M genome in *A. comosa*. The analysis further supported the hypothesis that most polyploid *Aegilops* species may have originated from limited hybridization events and share one or more common component genomes (Badaeva, 2002; Badaeva et al., 2004).

In conclusion, the results of this study have provided further details on the characteristics of *A. biuncialis* chromosomes. The detailed karyotype information of *A. biuncialis* may be useful for standardized and accurate identification of *A. biuncialis* chromosomes either among different *A. biuncialis* accessions or in wheat-*A. biuncialis* hybrids.

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Conflict of interest

The authors declare no conflict of interest.

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