

Full Length Research Paper

Characterization of a new synthetic wheat – *Aegilops biuncialis* partial amphiploid

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The aim of the experiments was to identify the synthetic wheat – *Aegilops biuncialis* germplasm Line 15-3-2 with 42 chromosomes. Morphologically, the spike of line 15-3-2 is intermediate to those of its wheat and *Aegilops* parents. Line 15-3-2 displays stable fertility and immunity to wheat powdery mildew and stripe rust. The seed storage protein electrophoresis showed that Line 15-3-2 presented almost all gliadin bands and a majority of glutenin subunits of its parents. Fluorescence *in situ* hybridization (FISH) suggested that Line 15-3-2 contained all 14 D-genome chromosomes and chromosome 5U. Therefore, it could be concluded that Line 15-3-2 is a new synthetic wheat – *A. biuncialis* partial amphiploid, and could be used to transfer the disease resistance genes to wheat.

Key words: SDS-PAGE, A-PAGE, fluorescence *in situ* hybridization, wheat – *Aegilops biuncialis* partial amphiploid.

INTRODUCTION

The genus *Aegilops*, which is the closest relative to wheat, comprises 11 diploid, 10 tetraploid, and 2 hexaploid species (Van Slageren, 1994). *Aegilops* species play an important role not only in the evolution of cultivated wheat but also in the processes of improving the genetic variation of common wheat (*Triticum aestivum* L.) (McFadden and Sears, 1946). *Aegilops* species carry many agronomically important traits including disease resistance, insect pests resistance (Gill et al., 1985; 1987; Raupp et al., 1993, 1995), drought tolerance (Molnár et al., 2004), salt tolerance (Colmer et al., 2006) and high protein quality. It was therefore used widely as a valuable source for wheat improvement. Up to now, about 200 wheat-*Aegilops* interspecific hybrids, addition and translocation lines have been developed, and 53 disease and insect resistance genes have been incorporated into the wheat gene pool from 15 *Aegilops* species (Schneider et al., 2008).

Aegilops biuncialis ($2n = 4x = 28, U^bU^bM^bM^b$) is a tetraploid wild relative of wheat belonging to the section *Poly-*

eides of the genus *Aegilops*. Its agronomically interested traits such as drought tolerance (Molnár et al., 2004), salt stress tolerance, barley yellow dwarf luteovirus resistance (Makkouk et al., 1994), yellow rust resistance (Damania and Pecetti, 1990), and brown rust resistance (Dimov et al., 1993) have been reported. There are, however, only a few studies on the utilization of this species to wheat genetic improvement. Logojan and Molnár-Láng (2000) first produced hybrid between *A. biuncialis*, MvGB642 and the winter wheat line Martonvásári 9 kr1 (Mv9kr1), and its progenies. Recently, Schneider et al. (2005) identified 5 wheat – *A. biuncialis* disomic addition lines by molecular cytology from those materials. Up till now, no more detailed information concerning the gene transfer from this germplasm was available. The present study was undertaken to characterize a new synthetic wheat-*A. biuncialis* partial amphiploid.

MATERIALS AND METHODS

Plant materials

Line 15-3-2 derived from the *T. aestivum*-*A. biuncialis* amphiploid was created and released by our research group. We produced F₁ hybrid plants by crossing *T. aestivum* cv. Chuannong 19 (CN19), an

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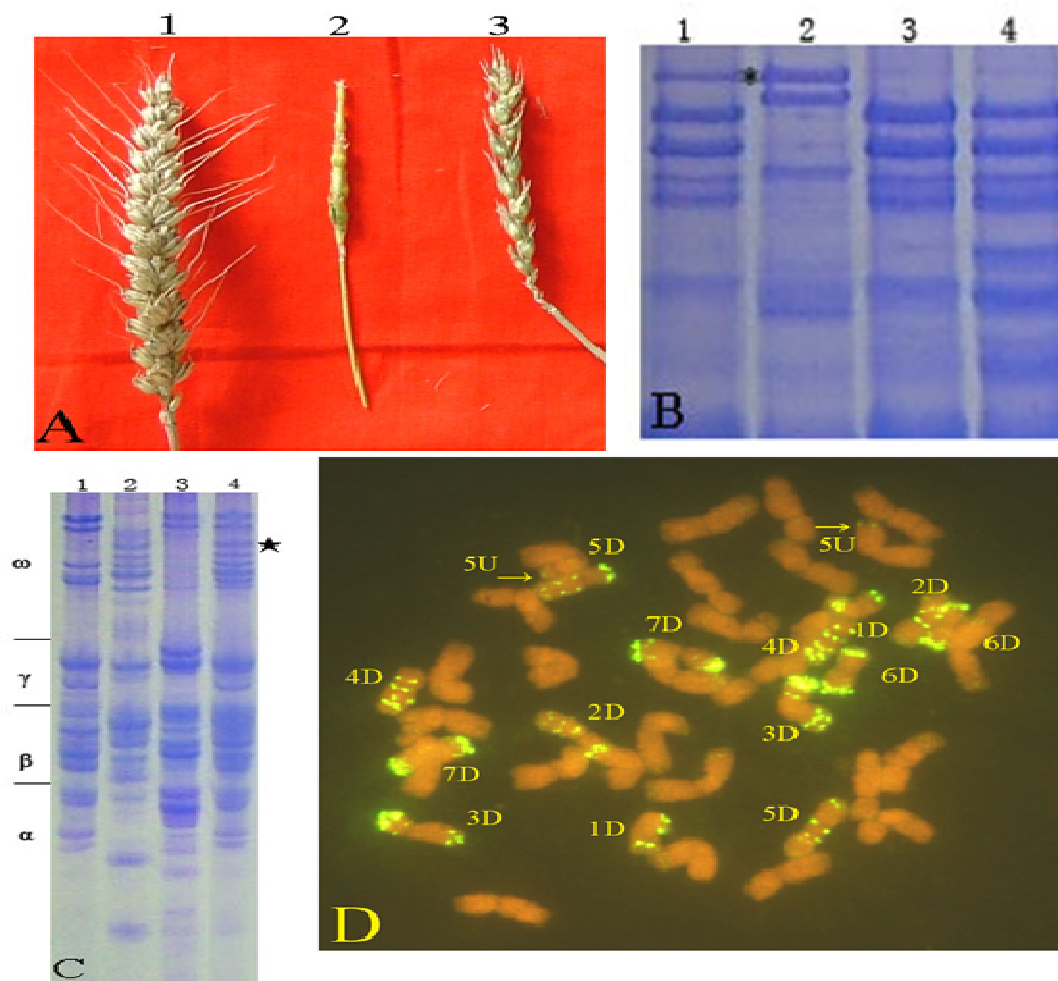


Figure 1. (A) Spike shape of Line 15-3-2. Number 1, 2 and 3 indicated CN19, *A. biuncialis* and Line 15-3-2, respectively. (B) Glutenin subunits of Line 15-3-2 (lane 1), *A. biuncialis* (lane 2), CN19 (lane 3) and Chinese Spring (lane 4) were separated by SDS-PAGE. Numbers indicated the HMW-glutenin subunits. Asterisks (*) indicates the glutenin subunit coming from *A. biuncialis*. (C) Gliadin storage proteins Chinese Spring (lane 1), *A. biuncialis* (lane 2), CN19 (lane 3) and Line 15-3-2 (lane 4) were separated by A-PAGE. Pentacle (*) indicated the gliadin bands coming from *A. biuncialis*. (D) Fluorescence in situ hybridization to meiotic chromosomes of Line 15-3-2 using the probe pAs1 that was labeled with digoxigenin and detected with FITC. Arrowheads indicated hybridization signals of chromosome 5U.

elite cultivar with *A. biuncialis* in 2003. The resulted F_1 hybrids (ABDUM, $2n = 5x = 35$) contained 35 chromosomes and were treated with colchicine at the tillering stage to produce amphiploid plants (AABBDDUUMM, $2n = 10x = 70$), which were backcrossed with wheat and then selfed. At last, we obtained about 110 lines derived from the *T. aestivum*-*A. biuncialis* amphiploid. Line 15-3-2 was one of these descendants.

Somatic chromosome counts and meiosis study

For counting somatic chromosomes, seedling root tips were kept in water at 0°C for 24 h, and fixed in ethanol-acetic acid (3:1) for at least 3 days. They were then stained, using the conventional Feulgen method.

For studying meiosis, anthers with pollen mother cells (PMCs) at metaphase I (MI) were fixed in Carnoy's 6:3:1 (ethanol/acetic-acid/chloroform) fixative and meiosis was studied, using the conven-

tional acetocarmine procedure.

Seed storage protein electrophoresis

Acid polyacrylamide gel electrophoresis (A-PAGE) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), carried out according to the procedure of Yang et al. (2001), were used to separate endosperm gliadin and glutenin proteins.

Fluorescence in situ hybridization

The probe pAs1, containing a 1-kb DNA fragment isolated from *A. tauschii* in the plasmid pUC8 (Rayburn and Gill, 1986), was generously provided by Dr. B. Friebe of Wheat Germplasm Resource Center, Department of Plant Pathology, Kansas State University, USA. The plasmid insert was labeled with digoxigenin-11-dUTP by

nick translation based on the protocols of the manufacturer (Roche Diagnostics Indianapolis, IN). Slides were finally mounted in Vectashield antifade solution (Vector Laboratories, Burlingame, CA) with the 0.25 µg/ml propidium iodide (PI). The hybridization and detection of digoxigenin was carried out with fluorescein-conjugated anti-digoxigenin. Fab fragment (Roche Diagnostics) was carried out according to Jiang et al. (1996).

RESULTS AND DISCUSSION

Line 15-3-2 was 42 chromosomes based on the chromosomal counting of cell of the root tips. Line 15-3-2 had full pollen fertility, and the meiotic observation of pollen mother cells displayed that it had few univalent and showed cytological stability. After inoculated at seedling and field tested at adult plants, line 15-3-2 showed immune to both wheat powdery mildew and stripe rust races (data not shown). Moreover, its spike shape was intermediate to those of wheat and *A. biuncialis* (Figure 1A).

The seed glutenin composition of Line 15-3-2, *A. biuncialis*, Chuannong 19 (CN19) and Chinese Spring (CS) were analyzed by SDS-PAGE. As illustrated in Figure 1B, both CN19 and CS contained the high-molecular-weight-glutenin subunits (HMW-GS) of 7+8, and 2+12, encoded by Glu-B1 and Glu-D1, respectively. *A. biuncialis* contained two HMW-GS subunits higher than HMW-GS 2 and one subunit between HMW-GS 7 and 8. Line 15-3-2 exhibited all HMW-GS Subunits (7 + 8 and 2 + 12) of CN19 and the highest subunit which were apparently originated from parent *A. biuncialis*. Although the available studies have indicated that *Aegilops* species is a rich source for novel variants of HMW glutenin subunits (Yan et al., 2002; Wan et al., 2005), the successful cases of transferring the new HMW glutenin subunits into wheat from *Aegilops* species remained rare. Here, we successfully transfer novel HMW-GS of *A. biuncialis* into wheat. On the other hand, the seed gliadin A-PAGE patterns of Line 15-3-2 and its parents are shown in Figure 1C. It can be clearly observed that Line 15-3-2 almost carried all bands of its parents CN19 and *A. biuncialis* in all of 4 gliadin mobility zones (ω , β , γ and α), especially in ω -zone. It was well known that the endosperm storage proteins, as useful genetic markers, usually show additively in hybrids. In our case, Line 15-3-2 presented almost all gliadin bands and a majority of glutenin subunits of its parents, suggesting that Line 15-3-2 may be partial amphiploid.

Because of the repetitive DNA probe pAs1 was not only hybridized well with D-genome chromosomes of wheat but also presented different fluorescent bands on specific D-genome chromosomes which can be useful to distinguish and identify the specific chromosomes (Rayburn and Gill, 1986; Badaeva et al., 2002), the probe pAs1 was used to detect the presence of D-chromosomes in Line 15-3-2. As illustrated in Figure 1D, all the 14 D-genome chromosomes showed strong hybridization signals in Line 15-3-2. Moreover, chromosomes 5U can

be marked according to the pAs1 hybridization sites always presented on the distal of short arms (Schneider et al., 2005).

Therefore, the Line 15-3-2 displays the characteristics of the synthetic wheat – *A. biuncialis* partial amphiploid germplasm. Considering that the Line 15-3-2 contained new HMW-GS from *Aegilops* and novel resistance to powdery mildew and stripe rust, it can be used as a valuable source for wheat improvement of quality and disease resistance.

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