Cereal Research Communications 44(2), pp. 198–205 (2016)
DOI: 10.1556/0806.43.2015.048
First published online 23 December 2015

Characterization of a New Wheat-*Aegilops biuncialis* 1M^b(1B) Substitution Line with Good Quality-associated HMW Glutenin Subunit

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(Received 13 April 2015; Accepted 27 August 2015;
Communicated by M. Molnár-Láng)

In this study, a new substitution line, 12-5-1, with 42 chromosomes that was derived from BC_3F_2 descendants of the hybridization between *Triticum aestivum* cv. CN19 and *Aegilops biuncialis* was created and reported. The 12-5-1 was immune to both powdery mildew and stripe rust and has stable fertility. Multi-color fluorescence *in situ* hybridization indicated that 12-5-1 was a substitution line 1M^b(1B). The seed storage protein electrophoresis showed that 12-5-1 presented high molecular weight glutenin subunits (2+12) of CN19 and a new subunit designated as M which apparently originated from parent *Ae. biuncialis*, and absent 7+8 subunits. Additionally, the flour quality parameters showed that the protein content, Zeleny sedimentation value, wet gluten content, and grain hardness and mixing time of 12-5-1 were signifiantly higher than those of its parent CN19. Moreover, 5 pairs of the chromosome 1M^b-specifi polymerase chain reaction-based landmark unique gene markers, TNAC1021, TNAC1026, TNAC1041, TNAC1-02 and TNAC1-04, were also obtained. The new substitution line 1M^b(1B) 12-5-1 could be a valuable source for wheat improvement, especially for wheat end product quality and resistance to disease.

Keywords: wheat, *Aegilops biuncialis*, bread-making quality, HMW-GS, multi-colour FISH

Introduction

Aegilops species, one wild relative of wheat, carry many agronomically useful traits, such as biotic and abiotic stress resistance or tolerance, yield components (Schneider et al. 2008), and high ability to improve wheat flour quality (Garg et al. 2009a, b). Therefore, Aegilops has been used widely and successfully as a valuable source for wheat improvement. The literature presented that more than 200 wheat-Aegilops interspecific hybrid,

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addition, and translocation lines have been released, and more than half of hundreds of stress resistance genes have been introgressed to wheat from 15 Aegilops species (Schneider et al. 2008).

Aegilops biuncialis Vis., a tetraploid U^b and M^b genome species, is highly resistant to wheat stem and leaf rusts (Damania and Pecetti 1990), barley yellow dwarf luteovirus (Makkouk et al. 1994), drought stress (Molnár et al. 2004), salt tolerance (Colmer et al. 2006) and osmotic stress (Dulai et al. 2014). And the species also has a high potential for improving the protein content and flour quality of the wheat grain (Zhou et al. 2014). These important genes can be incorporated into common wheat through amphiploids, addition lines, substitution lines or translocation lines (Schneider et al. 2005; Schneider and Molnár-Láng 2012; Farkas et al. 2014).

The High Molecular Weight Glutenin (HMW-GS), encoded by many allelic genes that were designated as x- and y-types are located on the long arms of homeologous group 1 chromosomes, was a major effect on bread-making quality in wheat (Rasheed et al. 2014). Although a lot of studies aimed at finding functionally useful HMW-GSs in wheat, only a very limited number HMW-GSs have shown functional differences. It is therefore very important to screen "good quality HMW-GS" in related wild wheat species for improving bread making quality. Recently, Only the potential good-quality subunits, Glu-1E subunits from *Agropyron elongatum*, Glu-1S subunits from *Aegilops searsii* (Garg et al. 2009a, b) and Glu-1U^b subunits from *Ae. biuncialis* (Zhou et al. 2014) were identified. In this study, we identified and released new wheat-*Ae. biuncialis* 1M^b(1B) substitution line which contain good HMW-GSs from *Ae. biuncialis* for improving wheat bread-making quality, and also screened and obtained the specific markers of the polymerase chain reaction (PCR)-based landmark unique gene (PLUG) to the homeologous group 1 chromosomes of *Ae. biuncialis*. Our observations could be a valuable source for wheat improvement, especially for wheat flour quality and resistance to disease.

Materials and Methods

Plant materials

The wheat-*Ae. biuncialis* substitution line 12-5-1 has been created and released by our research group. It was one of the BC_3F_2 descendants derived from the hybridization between *Triticum aestivum* cv. Chuannong 19(CN19) and *Ae. biuncialis*. *T. aestivum* cv. Chinese Spring (CS) and CN19 were collected and preserved by our lab. *Ae. biuncialis* (PI 550935) were obtained from National Plant Germplasm System (USA) and preserved by our lab.

Cytological observations

Somatic chromosome counts and meiosis analysis were done as described previously (Zhou et al. 2012). Root tips and the chromosome spreads of materials were prepared according to Han et al. (2006). Probes oligo-pSc119.2-1 (6-FAM-5'-CCGTTTTGTGGAC-

TATTACTCA CCGCTTTGGGGTCCCATAGCTAT-3') and oligo-pTa535-1 (Tamra-5'-AAAAACTTGACG CACGTCACGTACAAATTGGACAAACTCTTTCGGAGTAT-CAGGGTTTC-3') were prepared and fluorescence *in situ* hybridization (FISH) were done as described previously (Tang et al. 2014). Slides were examined on an Olympus BX-51 fluorescence microscope. Photographs were taken with a cooled CCD camera system (DP70 on an Olympus BX-51 fluorescence microscope).

Seed storage protein electrophoresis and quality testing

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate endosperm glutenin proteins as described previously (Zhou et al. 2007). Quality testing was carried out according to Zhang et al. (2005). Grain samples were conditioned to 14% moisture (AACC method 26-95) and milled in a Quadrumat Junior mill (Duisburg, Germany; AACC method 26-21A), grain protein content, grain hardness and Zeleny sedimentation value were measured with a near infrared (NIR) analyzer (1241 Grain Analyzer, FOSS Inc., Denmark; AACC method 39-10A), wet gluten was tested by Perten Glutomatic System (AACC method 38-12.02), rheological properties were tested by Brabender Farinograph (AACC method 54-21).

PLUG marker analysis

Total genomic DNA from CS, CN19, *Ae. biuncialis*, and 12-5-1 were extracted as described previously (Zhou et al. 2012). A total of 48 pair primers of PLUG markers, including 22 that were group 1 primers in Ishikawa et al. (2009) and 26 (TNAC1-01, TNAC1-02, TNAC1-26) that were designed by us, were used in this study. PCR, products digestion and separation were carried out according to Zhou et al. (2014).

Results

Agronomic traits and cytological observation of 12-5-1

Line 12-5-1 was obtained from BC_3F_2 descendants of the hybrids between wheat CN19 and *Ae. biuncialis*. The adult plant height of 12-5-1 is around 85 cm, and the number of spikes per plant varied from 4 to 7. These agronomic traits are very close to those of its wheat parent CN19. In addition, line 12-5-1 has stable fertility and immunity to wheat powdery mildew and stripe rust under both inoculated and natural conditions in all growth stages. Moreover, the somatic chromosome number per cell of 12-5-1 was 42. Twenty-one bivalents and very low probability of univalents and multivalents were observed at metaphase I (MI) in meiotic pollen mother cells (PMCs). These above results demonstrated that the 12-5-1 was a stable genetic line and it would be used in wheat breeding program act as a resource for a continuous supply of desirable genes from *Ae. biuncialis*.

FISH analysis of 12-5-1

Oligonucleotide probes end-labelled with 6-carboxyfluorescein (6-FAM) or 6-carboxytetramethylrhodamine (Tamra) are usually used in multi-colour FISH analysis to distinguish wheat A-, B-, and D-genome chromosomes and wild relative species of wheat (Tang et al. 2014). Probes oligo-pSc119.2-1 and oligo-pTa535-1 were used to identify chromosomes in line 12-5-1. As illustrated in Fig. 1, twenty-one chromosome pairs were detected. Except for two 1B chromosomes, the other wheat chromosomes were distinguished according to the standard FISH signal patterns of wheat (Tang et al. 2014). In addition, Oligo-pSc119.2-1 (green) generated strong signals on two distal arms of a pair chromosome, which should be chromosome 1M^b compared with the 1U^b chromosomes described previously (Zhou et al. 2014). Moreover, GISH with M-genomic probes on the line 12-5-1 showed that one pair of chromosome gave signals (data not given). Based on the above data, we can conclude that 12-5-1 is a 1M^b (1B) substitution line.

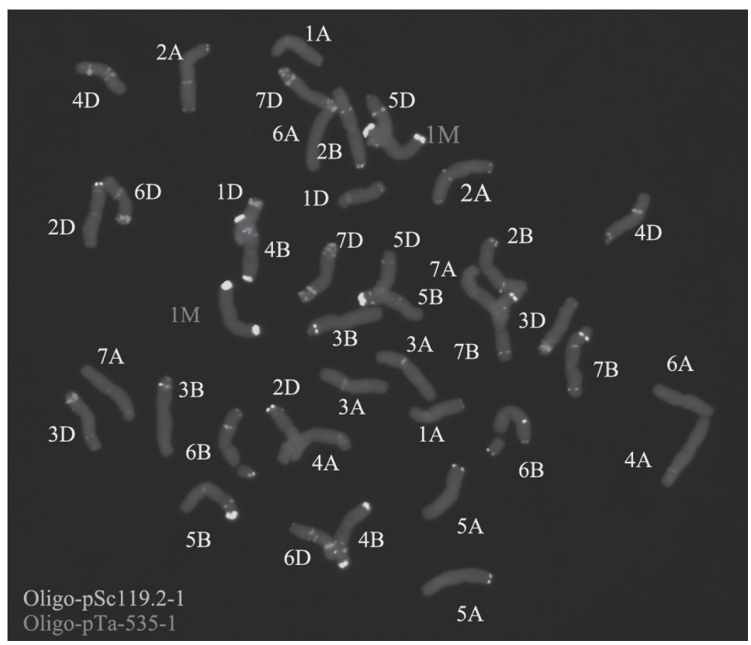

Figure 1. FISH analyses root tip metaphase chromosomes of 12-5-1 using Oligo-pSc119.2-1 (green), Oligo-pTa535-1 (red) as probes. Chromosomes were counterstained with DAPI (blue)

The chromosome 1M^b-specific PLUG markers

Five pairs of the chromosome 1M^b-specific PLUG markers, TNAC1021, TNAC1021, TNAC1041, TNAC1-02, and TNAC1-04, were obtained from 48 pairs of the screening markers (Fig. 2). As shown in Fig. 2, only one 1M^b -specific band was observed from each

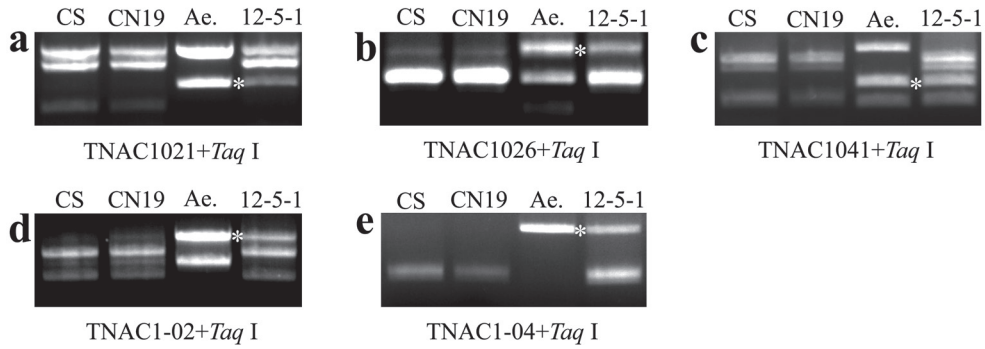

Figure 2. Agarose gel electrophoresis pattern of the PLUG-PCR markers. The chromosome-specific bands were indicated by asterisk. CS, Chinese Spring; Ae., *Ae. biuncialis*. The primers, TNAC1021, TNAC1026, and TNAC1041 were the same as described previously (Ishikawa et al. 2009); TNAC1-02 (sense 5'-CCTTTCTA CTCAGATTTTGATC-3', antisense 5'-CTTTGGCAGGAAATATTGTTCC-3'), TNAC1-04 (sense 5'-GACA-TCCACGCGAAGCCATC-3', antisense 5'-GACACTAATCAT GAGTGGTTG-3')

primer + *Taq* I (Fig. 2a–e). These results can be used to screen wheat-1M^b introgression lines in the near future.

HMW-glutenin and quality testing of 12-5-1

The seed glutenin composition of *Ae. biuncialis*, 12-5-1, CS, and CN19 was analyzed by SDS-PAGE. As illustrated in Fig. 3, both CN19 and CS contain the HMW-GSs of 7 + 8, and 2 + 12, encoded by Glu-B1 and Glu-D1, respectively. Line 12-5-1 has 2 + 12 subunits, a new subunit designated as M which apparently originated from parent *Ae. biuncialis*, and absent 7 + 8 subunits. Compare to the subunits glutenin proteins 1Ux and 1Uy described previously (Zhou et al. 2014), we can know the new subunit belongs to chromosome 1M^b. These results strongly indicated that 12-5-1 was a 1M^b(1B) substitution line.

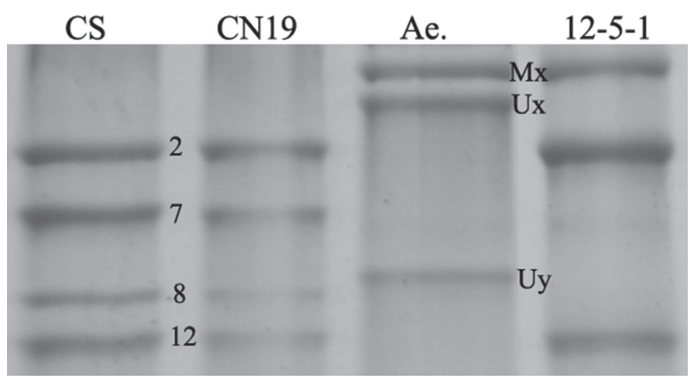

Figure 3. Seed storage protein profiles of 12-5-1. CS, Chinese Spring; Ae., *Ae. biuncialis*

Table 1. The average value of grain and flour characteristics of 12-5-1 in two years

Species	Content of protein (%)	Zeleny sedimentation value (cm^3)	Wet gluten content (%)	Grain hardness	Dough development time (min)	Dough stability time (min)	Content of water (%)	Water absorption (%)
12-5-1	19.5**	59.9*	39.4**	83.3*	2.6**	3.0	10.5	52.8
CN19	15.2	51.6	33.3	65.8	1.7	2.5	10.6	52.2

t-test was used for checking significant difference. *indicated $P<0.05$, **indicated $P<0.01$.

Moreover, the grain flour quality parameters (protein content, Zeleny sedimentation value, wet gluten content, grain hardness, mixing time, mixing tolerance, water content, and water absorption) were tested. The data (Table 1) indicated that the protein content, Zeleny sedimentation value, wet gluten content, grain hardness and mixing time of 12-5-1 were significantly higher than those of its parent CN19. These results showed that 1M^b has a potential to improve wheat end-product quality.

Discussion

Chromosome addition or substitution lines allow us to study the genetic effects of individual alien chromosomes in comment wheat. It is reported that the wheat-*Ae. biuncialis* disomic addition lines (DAL2U^b, DAL2M^b, DAL3U^b, DAL3M^b, DAL5U^b, DAL6M^b, and DAL7U^b) were created, and all of these addition lines had reduced fertility (Schneider et al. 2005; Schneider and Molnár-Láng 2012). And a wheat-*Ae. biuncialis* disomic addition line DAL1U^b which had stable genetic was created and leased (Zhou et al. 2014). In this study, a new wheat-*Ae. biuncialis* 1M^b(1B) substitution line 12-5-1 was created and identified through molecular cytogenetic methods. And the agronomic traits of this substitution line were very close to those of wheat parent CN19. In addition, this substitution line also has stable fertility and heredity compared with that of the parent, suggesting that not only the wheat CN19 has compatibility with the donor chromosome 1M^b, but also chromosome 1M^b can compensate excellently for the genetic loss of chromosome 1B. Therefore, the substitution line 1M^b (1B) is an ideal secondary gene resource for wheat breeding directly.

Reliable alien chromosome-specific molecular markers not only are very useful for detecting alien chromatin in wheat background, but also enable us to screen and identify translocations and recombinants from large progenies (Gong et al. 2014). Up-to-now, very few chromosome-specific molecular markers have been developed in Aegilops species (Gong et al. 2014). Moreover, only 2 simple sequence repeat (SSR) markers GWM44 and GDM61 (Schneider and Molnár-Láng 2012) and some gene-based conserved orthologous set (COS) markers (Molnár et al. 2013) were obtained in *Ae. biuncialis*. In this study, we have obtained five pairs of PLUG markers that were specific to the 1M^b chromosomes (Fig. 2). These markers were good for screening wheat-1M^b introgression lines in further research.

It is well known that protein content and protein quality play important role in flour quality. However, increases in protein content do not always accompany increases in

dough strength (Garg et al. 2009a, b). It has been reported that although the DAL1U from *Ae. umbellulata* contained a large size of x-type HMW-GSs and the protein content was significantly higher than its parent, the dough strength was not obviously different from that of parent, indicating that the factors such as β-spiral structures and conservation of repeat units, interactions between different glutenins and gliadins from different sources may be more key contributors to dough strength (Garg et al. 2009a, b). In this study, the protein content, Zeleny sedimentation value, wet gluten content, and grain hardness of 12-5-1 were significantly higher than those of its parent (Table 1). These results mentioned above indicated the gluten proteins from *Ae. biuncialis* were positive effect on wheat dough strength. And line 12-5-1 would be an excellent gene resource for wheat quality breeding.

Acknowledgements

This research was supported by the Applied Fundamental Research Fund of Sichuan Province (No. 2014JY0006), the National Natural Science Foundation of China (No. 31271420, No. 31330017, No. 31371682 and No. 31201203), and the National Transgenic Major Project (No. 2014ZX0801003B-002). We are grateful to Prof. Dr. Zongxiang Tang and Prof. Dr. Shulan Fu (State Key Laboratory of Plant Breeding and Genetics, Sichuan Agricultural University, Sichuan, China) for help and guidance of FISH analysis.

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