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A wheat-*Aegilops umbellulata* addition line improves wheat agronomic traits and processing quality

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Wheat processing quality is mainly correlated with high-molecular-weight glutenin subunits (HMW-GS) of grain endosperm. In bread wheat, the number of HMW-GS alleles are limited. However, wheat relative species possess numerous HMW-GS genes. In our previous study, a pair of novel HMW-GS 1Ux3.5+1Uy1.9 was characterized in *Aegilops umbellulata*. In this work, a novel wheat-*Ae. umbellulata* addition line, GN05, carrying a pair of 1U chromosome was developed and identified via cytogenetic analysis. Protein composition analysis indicated that GN05 carried HMW-GS of *Ae. umbellulata*. Accumulation of glutenin macropolymer (GMP) showed that GN05 had a much higher GMP content than the recurrent parent Chinese Spring. Rheological characteristics were analyzed by mixing test and the dough quality of GN05 was significantly improved compared to Chinese Spring. The results presented here may provide a valuable resource for the improvement of bread wheat quality.

Key Words: *Aegilops umbellulata*, wheat, addition line, agronomy traits, processing quality.

Introduction

The processing quality of wheat is mainly determined by glutenins, which are composed of high-molecular-weight glutenin subunit (HMW-GS) and low-molecular-weight glutenin subunit (LMW-GS) (Payne 1987). They play a critical factor in determining the viscoelastic properties and end-use qualities of dough (Shewry *et al.* 1992). In bread wheat, HMW-GS is located at the *Glu-1* loci on the long arms of chromosomes 1A, 1B, and 1D (Lawrence and Shepherd 1980, Payne 1987). LMW-GS can be separated into B, C, and D subunits based on their electrophoretic mobilities on an SDS-PAGE gel (Zhen *et al.* 2014). Genetic analysis showed that these subunits are encoded by the *Glu-3* loci on the short arms of chromosomes 1A, 1B, and 1D, respectively (Jackson *et al.* 1983). Among the storage proteins in wheat, HMW-GS plays a key role in affecting dough quality (Payne *et al.* 1987).

Although the HMW-GS and LMW-GS are important

determinants of wheat quality, the alleles of HMW-GS and LMW-GS associated with excellent dough quality remain limited (Garg *et al.* 2009). Fortunately, variations of HMW-GS present in wheat relative species may have positive effects on dough quality (Payne 1987). Previous studies have shown that some addition or substitution lines carrying the HMW-GS or LMW-GS of wheat relative species could affect wheat quality (Li *et al.* 2013, Liu *et al.* 2007, Wen *et al.* 2017). For example, a wheat-*Dasyphyrum villosum* translocation line carrying the HMW-GS of *D. villosum* significantly improve grain quality (Wen *et al.* 2017).

Aegilops umbellulata is an important diploid species that provides a U genome to many polyploid species (Friebe *et al.* 1992), so it is expected to serve as a valuable genetic source for wheat improvement. In our previous study, a pair of novel HMW-GS was identified in *Ae. umbellulata* that greatly contributed to dough quality in an *in vitro* test (Hou *et al.* 2017). For further application in breeding, a wheat-*Ae.umbellulata* addition line has been developed and its effect on wheat processing quality been evaluated. This addition line may provide a valuable genetic resource for improving the quality of wheat.

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Materials and Methods

Plant materials

The plant materials used in this study included *Ae. umbellulata* accession Y361 ($2n = 2x = 14$, UU), the common wheat accession Chinese Spring ($2n = 4x = 42$, AABBDD), and the wheat-*Ae.umbellulata* disomic addition line GN05. GN05 was developed and selected from the BC₁F₅ progeny of common wheat Chinese Spring/Y361//Chinese Spring. In briefly, thousands of crosses between CS and Y361 were carried out and three healthy embryos were finally obtained, GN05 is developed by one of the healthy embryos and detected by genomic *in situ* hybridization (GISH), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and plant phenotype. All stocks used in this study are maintained at Guizhou Normal University, Guizhou Province, China. Chinese Spring and GN05 were grown in a well-fertilized field in Guiyang, Guizhou, China, according to a completely randomized design with three replicates.

Cytogenetic analysis

Genomic *in situ* hybridization (GISH) analysis was conducted to detect the alien chromosome in GN05. Seeds were germinated on moistened filter paper in Petri dishes then germinated in a constant temperature incubator at 23°C. When the roots were 2–3 cm long, roots were placed in ice water (0–4°C) for 24 h, then fixed in Carnoy's fixative fluid (ethanol:acetic acid = 3:1) at 4°C for 2 days. The root tips were removed and digested in 1% pectinase and 2% cellulase at 37°C for 1 h, then stained with 1% (w/v) aceto-carmine solution for 2 h and squashed in 45% (v/v) acetic acid. Young spikes were stripped and fixed in 6:3:1 ethanol:chloroform:acetic acid mixture for 2 days. The anthers were squashed on a slide in 1% aceto-carmine solution. The GISH procedure was performed as described in Wang *et al.* (2016). The images captured were viewed and photographed with a Photometrics SenSys CCD camera (BX61, Olympus, Japan).

Gluten extraction and SDS-PAGE

The glutenin of Y361, GN05, and Chinese Spring were extracted from mature seeds according to our previous study (Du *et al.* 2018) and then separated in a 10% SDS-PAGE gel (Ma *et al.* 2013).

Agronomic traits and dough quality analysis

Agronomic traits include flowering time, plant height, number of spikes per plant, number of seeds per spike, thousand seed weight, and grain protein content. The data of agronomic traits were collected according to Liu *et al.* (2017) and Zou *et al.* (2017). Dough quality was evaluated using 10 g of flour with a Mixograph instrument (National Mfg. Co., Lincoln, NE) according to AACC method 54-40A (AACC 2000). At least three replicates were performed of all of the tests and statistical analysis followed that described by Du *et al.* (2018).

Extraction and analysis of glutenin macropolymer (GMP)

To analyze the dynamic accumulation trend of GMP, seeds in the middle spikelets were collected at 3, 5, 10, 15, 20, 25, and 28 days after anthesis. GMP concentration was measured according to Zhang *et al.* (2013).

Results and Discussion

Chromosome constitution and HMW-GS composition

SDS-PAGE analysis indicated that HMW-GS 1Ux3.5+1Uy1.9 and LMW-GS of Y361 are present in GN05 (Fig. 1), and a pair of exogenous chromosomes was detected in GN05 (Fig. 2A, 2B). In *Aegilops umbellulata*, HMW-GS and LMW-GS genes are located on the long arm and short arm of chromosome 1U, respectively (Rawat *et al.* 2011, Wang *et al.* 2018). In this work, HMW-GS pair 1Ux3.5+1Uy1.9 used as a molecular marker, finally, the additional chromosome was identified as 1U and GN05 is a wheat-*Ae. umbellulata* 1U disomic addition line.

Hybridization between wheat and wheat relative species for the development of introgression lines is an important strategy frequently used in wheat improvement programs.

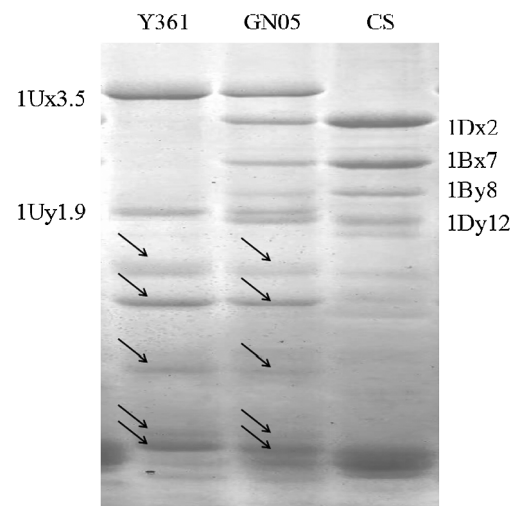


Fig. 1. SDS-PAGE analysis the HMW-GS and LMW-GS expression in Chinese Spring (CS) and 1U addition line GN05. The specific LMW-GS encoded by 1U chromosome marked by arrows. Y361 is the *Ae. umbellulata* contributed 1U chromosomes of the addition line GN05.

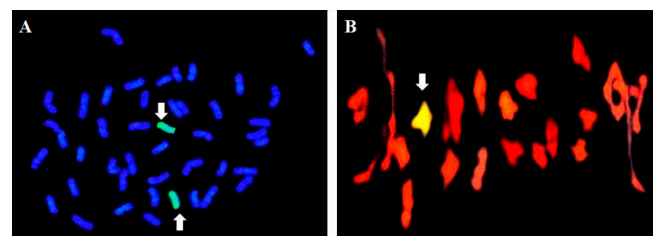


Fig. 2. Genomic *in situ* hybridization performed using the (A) genomic DNA in root tip and (B) pollen mother cell. Arrows marked the 1U chromosome.

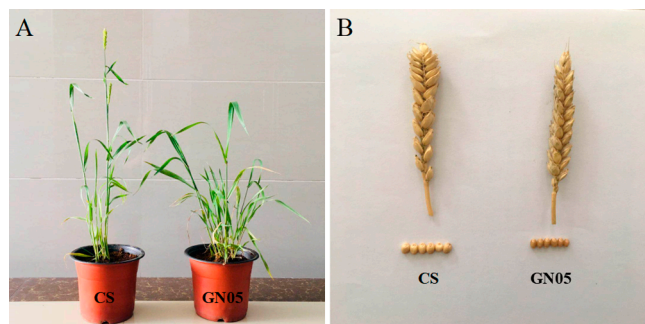


Fig. 3. Phenotype of GN05 compared with Chinese spring. (A) The plant phenotype; (B) The spike and grain phenotype.

Several aneuploidy and translocation lines derived from wheat and its closely related species have been developed (Garg *et al.* 2009, Wang *et al.* 2016, Wen *et al.* 2017, Zhao *et al.* 2010). Molecular markers and fluorescent tags were traditionally used for the identification of exogenous chromosome fragments (Zhao *et al.* 2010). In this work, the HMW-GS pair 1Ux3.5+1Uy1.9 was used as a key marker to identify the additional chromosome because this pair was only expressed in chromosome 1U (Hou *et al.* 2017).

Agronomic traits of GN05

Phenotype and agronomic comparisons between Chinese Spring and GN05 have been shown in **Fig. 3** and **Table 1**. GN05 showed shorter plant height and more spikes per plant than Chinese Spring, contributing to lodging resistance and yields increasing. However, the average duration of anthesis of GN05 is 198.7 days, much longer than Chinese Spring (172.2 days), limiting the rotation of wheat and other crops. The analysis of protein content revealed that total protein concentration of GN05 is 13.2%, significantly higher compared with Chinese Spring (10.7%). There were no significant differences among the number of seeds per spike and thousand seed weight (**Table 1**).

Spikes per acre, numbers of seeds per spike, and thousand seed weight are three factors affecting wheat yield (Yang *et al.* 2016). Possession of multiple tillers is an important trait that could increase wheat yield. *Ae. umbellulata* possesses the trait of multiple tillers and this characteristic has been transferred to the addition line (**Fig. 3**, **Table 1**). The reduced plant height of modern wheat cultivars is an important breeding objective because shorter plants have an improved rate of floret survival, increased grain number per

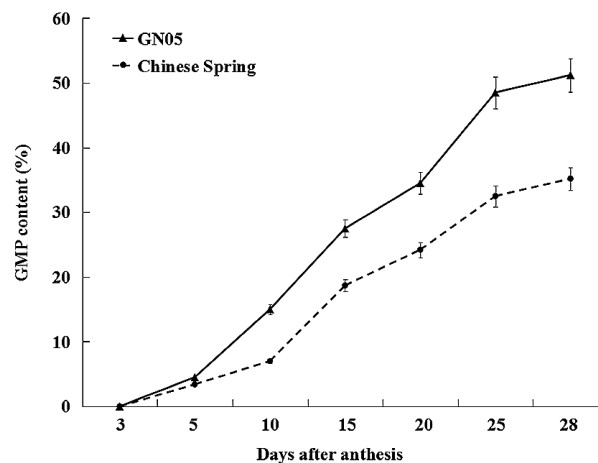


Fig. 4. The dynamic accumulation of glutenin macropolymers of Chinese Spring and GN05.

spike, and reduction of lodging when plants are grown under high fertilizer regimes (Mo *et al.* 2018). The height of GN05 was 50.6 cm, shorter than Chinese Spring, suggesting that GN05 possesses a high yield potential. Previous studies indicated that a high protein content of wheat is required for bread making, in addition, flour protein content had a significant impact on Chinese steamed bread quality (Würschum *et al.* 2016, Zhu *et al.* 2001). As indicated in **Table 1**, protein content of GN05 significantly higher than Chinese Spring that suggesting GN05 possessed a better dough strength.

Dough quality analysis

As indicated in **Fig. 4**, the rate of GMP accumulation in GN05 is much higher than that of Chinese Spring, and GN05 showed a higher amount of GMP. Rheological properties showed that the parameters of middle peak time, middle peak height, right of peak slope, width at peak, and width at 8 min of GN05 were all considerably higher than those of Chinese Spring (**Fig. 5**). These results revealed that GN05 possessed much higher dough strength than its recurrent parent Chinese Spring.

Although there is a complex mixture of wheat storage proteins, HMW-GS was identified to have a key role in wheat processing quality (Payne 1987). The composition of HMW-GS affected GMP content and further significantly correlated with the quality parameters (Don *et al.* 2003). Our present work has revealed that HMW-GS 1Ux3.5 of

Table 1. Agronomic traits of GN05 and Chinese Spring

	Agronomic traits					
	Flowering time (day)	Plant height (cm)	Number of spike per plant	Number of seeds per spike	Thousand seed weight (g)	Grain protein content (%)
Chinese Spring	172.2 ± 2.12a	152.3 ± 0.22b	9.2 ± 3.44a	31.5 ± 0.12a	31.1 ± 1.17a	10.7 ± 0.32a
GN05	198.7 ± 1.03b	101.7 ± 1.37a	16.5 ± 2.23b	36.7 ± 0.53a	32.5 ± 2.52a	13.2 ± 1.17b

Each trait was investigated five individual plants. Means followed by different letters within the same column were significantly different from each other ($p < 0.05$).

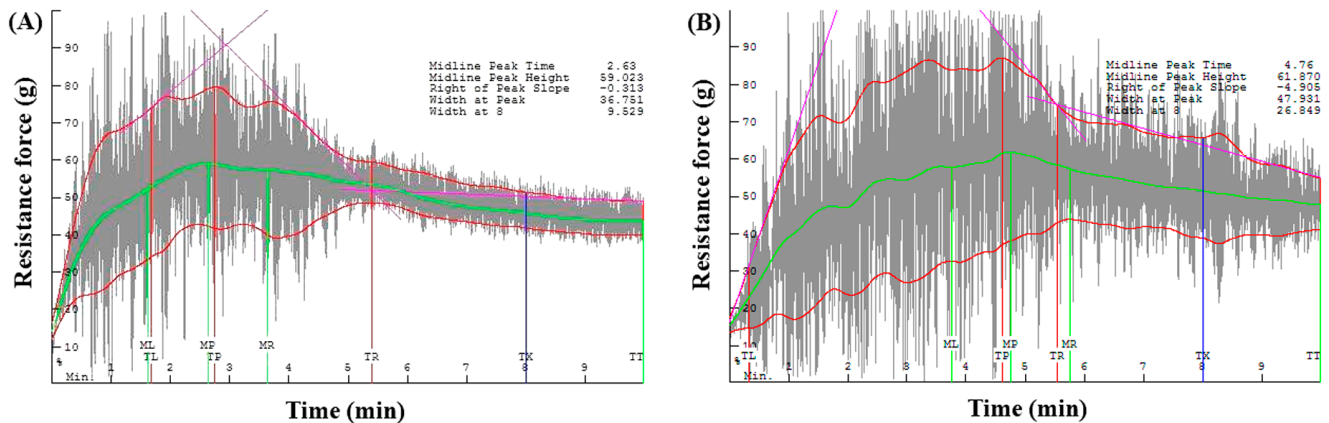


Fig. 5. Mixograph analysis of (A) Chinese Spring and (B) GN05. ML: left margin of peak; TL: left margin of peak time; MP: middle peak; TP: time of middle peak; MR: right margin of peak; TR: right margin of peak time; TX: width of 8 min; TT: time terminal. The curve fitting parameters has been surrounded by two red lines.

Ae. umbellulata possesses a unique structure that contributed to excellent dough quality (Hou *et al.* 2017). The rheological properties of dough indicated that the addition line showed better quality than the recurrent parent Chinese Spring (Fig. 5).

Overall, GN05 is a wheat-*Ae. umbellulata* 1U addition line that possesses much better agronomic traits and dough quality than Chinese Spring. However, its long period of anthesis limited the rotation of wheat and other crops. The flowering time of wheat should be restricted between 170–180 days that is suitable for cultivation mode in Guizhou Province of China. In a future study, an introgression line containing HMW-GS of *Ae. umbellulata* and possessing a short period of anthesis will be developed and applied as a germplasm resource for the further improvement of wheat.

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