Introduction

Wheat storage proteins primarily include polymeric glutenins and monomeric gliadins, among which high-molecular-weight glutenin subunit (HMW-GS) is a key factor in dough quality (Payne 1987). HMW-GS are encoded by Glu-1 loci on the long arms of homologous chromosomes 1A, 1B and 1D in common wheat. Each of these loci encodes two closely linked genes that encode one larger x-type and one smaller y-type HMW-GS (Payne 1987, Shewry et al. 1992). The typical primary structures of a HMW-GS include a signal peptide (removed from a mature protein), an N-terminal domain, a large repetitive domain and a C-terminal domain (Payne 1987). Within the HMW-GS, cysteine residues are highly conserved in both number and position (Shewry et al. 1992). For example, 1Dx5 + 1Dy10 are associated with good dough quality, whereas 1Dx2 + 1Dy12 have negative effects on dough quality (Flavell et al. 1989). Studies have shown that the HMW-GS with longer repetitive domains, higher expression amounts and extra cysteine residues can result in excellent dough quality (Gao et al. 2012, Gianibelli et al. 2001).

Previous studies have indicated that Glu-1 loci showed high levels of allelic variations and may thus be potential gene sources to improve wheat quality (Liu et al. 2003, Sun et al. 2006, Zhang et al. 2008). The Aegilops section Sitopsis includes Aegilops speltoides (SS, 2n = 2x = 14), Aegilops searsii (Ss, 2n = 2x = 14), Aegilops bicornis (SbSb, 2n = 2x = 14), Aegilops longissima (SlSl, 2n = 2x = 14) and Aegilops sharonensis (SshSsh, 2n = 2x = 14) (Wang et al. 2013). These species are involved in the evolution of some polyploid Aegilops species, such as Aegilops kotschyi, Aegilops peregrine and Aegilops syriacum. In addition, the S genome of Ae. speltoides is considered the progenitor of the B genome of bread wheat (Garg et al. 2014). Although several HMW-GS genes in S genome have been cloned and characterized (Garg et al. 2014, Ma et al. 2013), their effects on dough quality have not yet been studied. In this study, we characterized a new HMW-GS from wheat-Ae. searsii derivative lines via sodium dodecyl sulfonate polyacrylamide gel electrophoresis (SDS-PAGE) and genomic in situ hybridization (GISH) and confirmed that the Ae. searsii-derived HMW-GS will be potentially useful for quality improvement in wheat.
Materials and Methods

Plant materials

_Ae. searsii_ S2114 and the wheat cultivar known as Chinese Spring are maintained at the School of Life Sciences, Guizhou Normal University, China. GL1402 is a chromosome substitution line that was generated by S2114 × Chinese Spring. Briefly, _Ae. searsii_ was crossed with Chinese Spring, and immature embryos were cultured according to Mujeeb-Kazi _et al._ (1996). When their roots appeared, seedlings were transferred to soil and grown in a chamber at 25°C under 16-h light/8-h darkness. Then, the F₁ plants were backcrossed and selfed to generate the BC₄F₄ generation (Supplemental Fig. 1). GL1402 was selected from the BC₄F₄ population for further study. Chinese Spring and GL1402 were grown in a well-fertilized field of Tongren, Guizhou, China, according to a completely randomized design with three replications.

SDS-PAGE and GISH

The HMW-GS of _Ae. searsii_, GL1402 and Chinese Spring were extracted from mature seeds. Each seed was crushed and extracted in 1 mL of 60% (v/v) isopropanol at 65°C for 30 min to remove the gliadins. After centrifugation at 12000 rpm for 10 min, the supernatant was discarded, and the precipitation was mixed with 1-propanol containing 1% (w/v) DTT and then left at 65°C for 30 min. A 10-μL supernatant was loaded into a 10% SDS-PAGE gel (Ma _et al._ 2013). Separation was carried out at 10 mA for 12 h, after which the gel was stained by coomassie brilliant blue R250 for 3 h and washed in distilled water until the bands appeared clearly.

Root tip cells were prepared for chromosome analysis by the acetocarmine squash method and were used for genomic in situ hybridization (GISH) analysis according to Chen _et al._ (1998). To determine the presence of _Ae. searsii_ chromosomes, the total genomic DNA of _Ae. searsii_ was labeled with tetramethyl-rhodamine-5-dUTP according to the random primer labelling method. Chromosomes were counter-stained with 4′,6-diamidino-2-phenylindole (DAPI) and observed using a fluorescence microscope (BX61, Olympus, Japan). Images were captured by a cooled CCD camera (CoolSnap fx, Photometrix).

Dough quality analysis

Grains of Chinese Spring and GL1402 were milled into flour using a Brabender Quadratam Junior mill according to the AACC approved method 26-21A (AACC 2000) for quality testing. The flour protein content was determined according to Beljkaš _et al._ (2010). Mixograph properties were determined according to AACC approved methods 54-40A (AACC 2000). A 10 g mixograph (National Mfg. Co., Lincoln, NE) was used to analyze the mixing properties of flour. The middle peak time (MPT), middle peak height (MPH), middle peak width (MPW), right peak slope (RPS), band height (MT × H), and width at 8 min (MT × W) were used as the quality parameters. GMP concentration was measured according to Zhang _et al._ (2013).

Preparation of CSB

The recipe of CSB consisted of 100 g of wheat flour, 0.8 g of dehydrated yeast and 50 mL of water. After mixing and kneading manually for 5 min to form the mixture into dough, the dough rested at 28°C for 5 min. Then, the dough was moulded by hand and fermented at 30°C for 30 min. Finally, the dough was steamed for 20 min using a steam oven. CSB samples were cooled to room temperature before analysis.

Volume and texture properties of CSB

CSB volume was determined according to the rapeseed displacement method (AACC 2000). Specific volume was expressed as mL/g. Texture profile analysis (TPA) was used following the approach of Fu _et al._ (2015).

Statistical analysis

All tests were performed at least two replicate determinations. Analysis of variance (ANOVA) was performed using the SPSS software with one-way analysis of variance and Tukey’s Honest Significant Difference test. _P < 0.05_ was used to define the significance of differences between the samples.

Results and Discussion

Chromosome constitution and HMW-GS composition

The GL1402 used in the present study was registered as a disomic substitution line. GISH analysis showed that GL1402 carried a pair of _Ae. searsii_ chromosomes (Fig. 1a). In addition to the contribution to dough quality, HMW-GS is an important genetic marker because it is located only on the long arms of homologous chromosome 1. The chromosome composition could be directly reflected combined with GISH and HMW-GS analysis (Fig. 1a, 1b).

Mixograph parameters and GMP quantity

The grain protein content and parameter values of the mixograph are shown in Table 1. The protein content of GL1402 was higher than that of Chinese Spring (Table 1). Mixograph analysis indicated a significant increase in MPT and MT × W in GL1402 and a decrease in MPH, MPW and MRS. Previous reports indicated that the HMW-GS fractions and mixograph parameters showed significant correlations (He _et al._ 2005, Liu _et al._ 2005, Zhang _et al._ 2009). Wheat possessing 1Bx7 + 1By9 and 1Dx5 + 1Dy10 always had a significantly better MPT, RPS, MT × H, and MT × W than did wheat with 1Bx14 + 1By15 and 1Dx2 + 1Dy12. The present study of mixograph parameters suggests that the 1Sx’-2114 + 1Sy’-2114 had a better MPT, RPS and MT × W than 1Bx7 + 1By8 did. Therefore, GL1402 might provide better dough strength than Chinese Spring. The yields of GMP are shown in Fig. 2. GL1402 had a significantly
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higher GMP content than Chinese Spring did. Previous studies have found that GMP quantity correlates with wheat flour quality (Pritchard 1993, Weegels et al. 1996). Don et al. (2003) indicated that the GMP content was controlled by HMW-GS composition and that the GMP content in flour was significantly correlated with the quality parameters. The results demonstrated that wheat that possessed 1Sx-2114 + 1Sy-2114 showed better dough quality and a higher quantity of GMP than did wheat with 1Bx7 + 1By8 (Table 1, Fig. 2).

Physical properties of CSB

Table 2 shows the physical properties of CSB, including the loaf volume, loaf height and moisture influenced by the storage proteins in 1S+ chromosome. It demonstrates that the CSB loaf volume, loaf height and specific volume of GL1402 increased significantly compared to those of Chinese Spring. However, the moisture showed no significant change. The textural properties of CSB were also analyzed (Table 3). The hardness, gumminess, chewiness and resilience were significantly decreased in GL1402. The height of steamed bread is generally related to dough elasticity and flexibility: Zhang et al. (2007) reported the correlations of steamed bread score with dough strength. In this study, GL1402 showed excellent CSB quality traits with good textural properties. Hard wheat with medium to strong dough strength is suitable for CSB processing (Huang et al. 1996). Zhang et al. (2014) suggested that wheat functionality for CSB can be improved by changing the HMW-GS composition. In this work, the HMW-GS from A. searsii greatly improved the quality of CSB.

In conclusion, the wheat-Ae. searsii substitution line greatly altered the dough rheological properties, with the substitution line showing excellent dough quality. The substitution line was thus proposed for use in achieving superior

| Table 1. Comparison of dough properties between GL1402 and its recurrent parent Chinese Spring |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Protein content (%)            | MPT (min)       | MPH (cm)        | MPW (cm)        | RPS (°)         | MT × H (cm)     | MT × W (cm)     |
| Chinese Spring                 | 12.4 ± 0.12     | 1.7 ± 1.02      | 52.9 ± 0.98     | 28.6 ± 1.32     | −4.9 ± 0.02     | 38.4 ± 1.44     |
| GL1402                          | 15.6 ± 0.09     | 4.3 ± 1.22      | 49.1 ± 1.01     | 14.5 ± 1.15     | −2.1 ± 0.04     | 37.5 ± 1.87     |

Means ± standard deviations (n = 3). Means followed by different letters within the same column were significantly different from each other (p < 0.05)

MPT, middle peak time; MPH, middle peak height; MPW, middle peak width; RPS, right peak slope; MT × H, band height; MT × W, width at 8 min.
CSB quality. Finally, the substitution line can be used as an important genetic resource to improve the overall quality in wheat processing.

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