GUIDELINES FOR PREPARATION OF CONFERENCE PAPERS

GUIDELINES

Papers are required to include an abstract, and if the authors would like to include more detail on the research they may also include an Introduction, Methods, Results, Discussion, Conclusions and References in a more traditional paper format.

- Papers should be prepared in Word using Times New Roman 12 point font
- Select A4 page size with a 2.5 cm margin all around.
- The title should be typed in BOLD CAPITALS, centred, leave a gap of one line after.
- Authors' names should be in lower case, centred, leaving a gap of one line after.
- Underline the name of the presenting author.
- Authors' affiliations and addresses should appear after a one-line space in lower case, aligned to the left, with multiple affiliations indicated by superscripted numbers.
- Leave a double space and proceed with the paper, using single spacing and the writing justified without indentation.

SAMPLE PAPER

HMW GLUTENIN SUBUNITS FROM 1S^{l} GENOME OF *AEGILOPS LONGISSIMA*

Wujun Ma[1], Yueming Yan[2], Shunli Wang[1,2], Zitong Yu[1,2], H Weißgerber[3], FJ Zeller[3], SLK Hsam[3], Dean Diepeveen[1], Shahidul Islam[1], Rudi Appels[1]

[1]Australian Export Grain Innovation Centre, 3 Baron-Hay Court, Perth, WA 6151, & School of Veterinary and Life Sciences, Murdoch Unversity, South St, Perth WA 6150;
[2]College of Life Science, Capital Normal University, 100048 Beijing, China
[3]Division of Plant Breeding and Applied Genetics, Technical University of Munich, D-85350 Freising-Weihenstephan, Germany

ABSTRACT

A Chinese Spring (CS) substitution line was produced in which a chromosome (1S^{l}) from *Aegilops longissima* substituted one chromosome (1B) of CS with a significantly increase of dough rheological property. A range of studies have been carried out in aspects of high molecular weight glutenin coding genes, protein accumulations, protein bodies, starch granules and protein disulfide isomerase (PDI) and PDI-like protein expressions (PDI and PDI-like protein can control diversified metabolic functions) in order to uncover the mechanism underlying the superior dough and breadmaking quality of this substitution line.

RESULTS

Quality analysis revealed that the dough maximum resistance (Rmax) of CS-1S^{l}(1B) increased more than 200%. However, no significant difference of dough extensibility was observed between two lines. Baking experiments indicated that CS-1S^{l}(1B) had large extension surface with an average value of 128.4 cm^2 while CS only had 42 cm^2. Particularly, the loaf volume significantly increased by 27.1%, from 550 cm^3 in CS to 699 cm^3 in CS-1S^{l}(1B).

SDS-PAGE indicated that two novel HMW-GS subunits were present in CS-1S^{l}(1B) but without the Glu-B1 allele 1Bx7+1By8, demonstrating that two 1S^{l}-encoded novel subunits replaced 1Bx7 and 1By8 subunits. The electrophoretic mobilities of 1S^{l}-encoded x- and y-type subunits were close to those of 1Dx2.2 and 1By16, respectively, and therefore designated as

1S^{l}x2.3* and 1S^{l}y16*. Accurate molecular masses of 1S^{l}x2.3* and 1S^{l}y16* were determined by MALDI-TOF-MS to be M_r 97797 Da and 780165 Da, respectively. Some differences in the LMW-GS and gliadin compositions between CS and CS-1S^{l}(1B) were also found. HPLC quantification results of total protein, gliadin, glutenin protein in CS and CS-1S^{l}(1B) were 30714.7 and 38200.3 (AU)^{b}, 9993 and 10273 (AU)^{b}, 16382.4 and 21736.3 (AU)^{b}, respectively. The gliadin/glutenin ratios in CS and CS-1S^{l}(1B) were 0.610 and 0.473, respectively. The total peak areas of HMW-GS, LMW-GS and alpha/beta-gliadin, gamma-gliadin, omega-gliadin in CS were 20459.20, 30786.20, 215463, 45939 and 90920 mAU*min (mini-Absorb unit * minute), respectively. While in the substitution line, the total peak areas of the five proteins were 34733.4, 38317.4, 184347, 31410 and 79710 mAU*min. The HMW-GS/LMW-GS ratios in CS and CS-1S^{l}(1B) were 0.660 and 0.897, respectively. Since HMW-GS are major determinant for dough elasticity, the introduced 1S^{l}x2.3* and 1S^{l}y16* subunits could be responsible for the superior breadmaking quality of substitution line CS-1S^{l}(1B).

The complete open reading fragms (ORFs) encoded 1S^{l}x2.3* and 1S^{l}y16* subunits were isolated and cloned from CS-1S^{l}(1B) by AS-PCR. The full length ORFs of *1S^{l}x2.3** and *1S^{l}y16** were 2829 bp and 2250 bp, encoding 941 and 749 amino acid residues, respectively. Their deduced molecular weights (97851 Da and 78118 Da) were well consistent with those determined by MALDI-TOF-MS, suggesting no post-translational modifications present in both subunits.

Comparison analysis of the deduced protein sequences showed that 1S^{l}x2.3* had typical characteristics of x-type HMW-GS, including a signal peptide of 21 amino acid residues, an N-terminal domain of 86 amino acid residues, followed by a repetitive domain of 792 amino acid residues and a C-terminal domain of 42 amino acid residues. The repetitive domain of 1S^{l}x2.3* contained 31 hexapeptides (consensus PGQGQQ and SGQGQQ), 11 nonapeptides (consensus GYYPTSPQQ and GYYPTSLQQ) and 24 tripeptide (consensus GQQ) motifs. Four cysteine residues were distributed at conserved positions as other x-type HMW-GS: three in the N-terminal domain (at positions 31, 43 and 58) and one in the C-terminal domain (at position 929). Our results demonstrate that 1S^{l}x2.3* is the largest HMW-GS among the HMW-GS x-type subunits encoded by 1B. Among all reported HMW-GSs, it is only a little smaller than two subunits on the D genome, 1Dx2.2 (2919 bp) and 1Dx2.2* (3078 bp). 1S^{l}y16* appeared to be the largest y-type HMW-GS in *T. aestivum* since it is larger than the previously reported largest y-type subunit, 1By16 (2220 bp). It contained a signal peptide of 21 amino acid residues, an N-terminal domain of 104 amino acid residues, followed by a repetitive domain of 582 amino acid residues and a C-terminal domain of 42 amino acid residues. The repetitive domain contains 25 hexapeptide (consensus PGQGQQ and SGQGQQ) and 8 nonapeptide (consensus GHYPASQQQ or GYYPTSLQQ) motifs. Seven cysteine residues were present at conserved positions as other y-type subunits: three in the N-terminal domain (at positions 31, 43, and 65, 66 and 76), one in the repetitive domain (at position 635) and one in the C-terminal domain (at position 737).

The coding sequences of 1S^{l}x2.3* and 1S^{l}y16* subunit genes were aligned with other 17 x-type and 17 y-type HMW-GS genes, respectively. The SNPs and InDels present in the two 1S^{l}-encoded HMW-GS genes were identified. A total of 11 SNPs were detected at different positions in each gene while 4 InDels were present in *1S^{l}x2.3** and only one deletion was detected in *1S^{l}y16**. Apparently, a 97 bp insertion resulted in the increase of *1S^{l}x2.3** gene size. In addition, more than half of the detected SNPs were nonsynonymous in both genes.

RP-HPLC analysis of the synthesis and accumulation characteristics of HMW-glutenins in CS and CS-1S^{l}(1B) demonstrated that glutenin proteins synthesis initiated at 10 DPA and then steadily increased until grain maturity. The important stages for HMW-glutenin synthesis were 15-20 DPA during which the HMW-glutenin proteins accumulated to a considerable amount. Compared with CS, CS-1S^{l}(1B) expressed a higher HMW-glutenin amount from 10 to 25 DPA and finally reached the highest amount at 25 DPA. The GMP content result showed that it was higher in CS-1S^{l}(1B) (3.39%) than that in CS (3.18%). This suggested that high amount of glutenin polymeric protein requires a high HMW-GS accumulation during grain development. The accumulation pattern of LMW-GS was similar between CS and CS-1S^{l}(1B), which had a stable increase based on the results of RP-HPLC. Taking together with the results from qRT-PCR, CS-1S^{l}(1B) had higher HMW-GS expression amounts than CS in both transcriptional and translational levels.

Fluorescence microscopy analysis showed that both CS and CS-1S^{l}(1B) had similar morphologies of the endosperm cells and similar ontogeny of PBs. The PBs immunolabelled with anti-HMW-GS were detected at 7 DPA in both lines; they grew in size by fusion among themselves and finally reached the maximum size at 15 and 19 DPA in CS and CS-1S^{l}(1B), respectively. At 15 DPA, microscopy observations demonstrated that PBs did not remain as separate particles but coalesced to form large aggregates in CS, whereas in CS-1S^{l}(1B) the PBs were kept getting larger from 15 DPA to 19 DPA. At 19 DPA and 22 DPA, no PBs could be observed by fluorescence microscopy in CS, while large and normal PBs could be detected in CS-1S^{l}(1B). These suggest that the HMW-GS in CS began to form a matrix entrapping starch granules from 19 DPA and 22 DPA in CS and CS-1S^{l}(1B), respectively. The average area of PBs labeled with anti-HMW-GS in CS-1S^{l}(1B) was much more than those in CS. This suggests that more HMW-GS was accumulated in CS-1S^{l}(1B) than that in CS.

The PB sizes were studied by TEM experiments on endosperm sections at 7, 11 and 19 DPA. At 7 DPA, the diameter of PB was 0.4 - 0.5 μm in CS and 0.6 - 2.0 μm in CS-1S^{l}(1B). At 11 DPA, the average diameter of PB was 0.85 and 1.20 μm in CS and CS-1S^{l}(1B), respectively. At 19 DPA, few PBs were detected in CS which was consistent with the fluorescence microscopy analysis result, whereas the diameter of PB was more than 2 μm in CS-1S^{l}(1B). This result further verified larger and more PBs in CS-1S^{l}(1B) than that in CS.

DISCUSSION

It is well known that HMW glutenin subunits play an important role in determinating the elastic properties of glutenin complex (Payne 1987; Ma et al 2005). 1S^{l}x2.3* had 941 amino acid (AA) residues with an extra 102 amino acid residue insertion in the central repetitive domain (603-704th), including 8 hexapeptides and 6 nonapeptides. The 1S^{l}y16*, containing 749 amino acid residues, which appeared to be the largest y-type HMW subunit among the characterized y-type subunits so far. A long repetitive domain is considered to have a positive influence on wheat flour quality (Belton 1999; Masci et al 1998; Masci et al 2000) because it can form more -β-turns structure conferring elasticity to the protein molecule (Gianibelli et al 2001; Tatham et al 1985). Insertion in the central repetitive domain could directly affect the functional properties (Hassani et al 2005), suggesting that the extra 102 amino acid residue insertion in the central repetitive domain of 1S^{l}x2.3* subunit may play a positive role in dough visco-elastic properties. D'Ovidio and Anderson (1994) suggested that y-type HMW-GS are among the main components responsible for differences in the technological characteristics of flour. It was deduced that the largest y-type subunit 1S^{l}y16* could have positive effects on dough-making quality of the flour. Theoretically, high glutamine content can stabilize the polymeric structure of glutenin through forming more hydrogen bonds (Gilbert et al 2000) so that larger HMW-GS

as well as LMW-GS rich in glutamines have a greater positive effect on dough strength than smaller subunits (Békés et al 1995; Li et al 2008). It was found that the total glutamine content of $1S^{l}$x2.3*and $1S^{l}$y16* was similar with the subunit pair 1Dx5 + 1Dy10 that confers superior wheat flour processing qualities (Ma et al 2005). This may be another mechanism for the positive effects of $1S^{l}$x2.3* and $1S^{l}$y16*. Another important factor to affect dough elasticity is the proportion of the consensus hexapeptides and nonapeptides in the repetitive domain. Masci et al (2000) reported that a rather large and regular repeated sequence domain is helpful in increasing the viscosity and elasticity of doughs through inter-molecular interactions. Higher proportion of repeats of the consensus type in 1Dy10 than that of 1Dy12, which may produce a more regular pattern of repetitive β-turns in the protein, contributed to better dough elasticity. Compared to 1Dy10, $1S^{l}$y16*showed a similar repetitive domain, including 22 consensus hexapeptide (PGQGQQ) and 3 consensus (SGQGQQ) motifs. The $1S^{l}$x2.3*subunit had 23 consensus (PGQGQQ) and 8 consensus (SGQGQQ) hexapeptide motifs, as well as 24 tripeptide (consensus GQQ) motifs. Both subunits had no consensus nonapeptide motifs, suggesting that the consensus hexapeptides were more important than nonapeptides in determining the dough elasticity.

1. It has shown that the expression and accumulation patterns of storage proteins are associated with gluten quality properties. Higher accumulation of HMW-GS and LMW-GS at earlier grain developmental stage may contribute to the superior gluten quality of wheat (Panozzo et al 2001). The expression pattern of gliadin, LMW-GS and HMW-GS genes were similar and 10-18 DPA was the key dates of storage protein genes expression. The probable reason was that simultaneous storage protein can easily build up glutenin polymeric structure by the inter- or intra- molecular disulphide bonds (Panozzo et al 2001; Pistón et al 2004; Pistón et al 2006; Altenbach and Kothari (2007); Laudencia-Chingcuanco et al 2007; Li et al 2010). Different storage proteins in the different cultivars may have different accumulation patterns, and different environmental conditions can also alter the accumulation rates, which consequently cause flour quality variations Altenbach et al 2007; Laudencia-Chingcuanco et al 2007; Li et al 2010).

In the current study, more HMW-GS genes were transcribed in CS-$1S^{l}$(1B), which takes shorter time to reach the maximum accumulation during grain development. This implies that good quality wheat may require more HMW-GS gene transcription at the mRNA level. Earlier synthesized HMW-GS mRNA may facilitate the accumulation of glutenin protein in advance, making an adequate time available to form the polymeric protein. In parallel of this, some enzymes (such as glutamine synthetase, PDI) at early development stages were highly active, which facilitate the folding of gluten proteins and formation of more regular glutenin polymers (Liu et al 2012). It was noteworthy that the HMW-GS genes were still transcribed in the late stage of grain development in CS-$1S^{l}$(1B). This may result in more HMW-GS accumulation required for large glutenin polymer formations. The protein expression pattern was similar with the gene expression pattern at the mRNA level. More HMW-GSs and LMW-GSs were accumulated in CS-$1S^{l}$(1B) than in CS according to the results of RP-HPLC. This suggested more glutenin transcription and expression in the good quality wheat than that of poor quality wheat.

REFERENCES

Altenbach SB, Kothari KM (2004) Transcript profiles of genes expressed in endosperm tissue are altered by high temperature during wheat grain development. J Cereal Sci 40: 115–126.

Altenbach SB, Kothari KM (2007) Omega gliadin genes expressed in *Triticum aestivum* cv. Butte 86: Effects of post-anthesis fertilizer on transcript accumulation during grain development. J Cereal Sci 46: 169–177.

Békés F, Gras PW, Gupta RB (1995) The effects of purified cereal polypeptides on the mixing properties of dough. In: Williams, Y.A. and Wrigley, C.W. (eds), Capturing the benefits of research for consumers. R. Aust. Chem. Inst. Cereal Chem. Div. pp: 92–98.
Belton PS (1999) On the elasticity of wheat gluten. J Cereal Sci 29: 103–107.
D'Ovidio R, Anderson O (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread making quality. Theor Appl Genet 88: 759–763.
Gilbert SM, Wellner N, Belton PS, Greenfielda JA, Siligardic G, et al. (2000) Expression and characterization of a highly repetitive peptide derived from a wheat seed storage protein. Biochimi Biophys Acta 1479: 135–146.
Gianibelli MC, Larroque, OR, MacRitchie F, Wrigley CW (2001) Biochemical, genetic and molecular characterization of wheat glutenin and its component subunits. Cereal Chem 78: 635–646.
Hassani ME, Gianibelli MC, Shariflou MR, Sharp PJ (2005) Molecular structure of a novel y-type HMW glutenin subunit gene present in *Triticum tauschii*. Euphytica 141: 191–198.
Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol 38: 141–153.
Laudencia-Chingcuanco DL, Stamova BS, You FM, Lazo GR, Beckles DM, et al. (2007) Transcriptional profiling of wheat caryopsis development using cDNA microarrays. Plant Mol Biol 63: 651–668.
Li XH, Wang AL, Xiao YH, Yan YM, He ZH, et al. (2008) Cloning and characterization of a novel low molecular weight glutenin subunit gene at the *Glu-A3* locus from wild emmer wheat (*Triticum turgidum* L. var. *dicoccoides*). Euphytica 159: 181–190.
Li XH, Wang K, Wang SL, Gao LY, Xie XX, et al. (2010) Molecular characterization and comparative transcriptional analysis of LMW-m-type genes from wheat (*Triticum aestivum* L.) and *Aegilops* species. Theor Appl Genet 121: 845–856.
Liu W, Zhang YZ, Gao X, Wang K, Wang SL, et al. (2012) Comparative proteome analysis of glutenin synthesis and accumulation in developing grains between superior and poor quality bread wheat cultivars. J Agr Food Chem 92: 106–115.
Ma W, Appels R, Bekes F, Larroque O, Morell MK, et al. (2005) Genetic characterisation of dough rheological properties in a wheat doubled haploid population: additive genetic effects and epistatic interactions. Theor Appl Genet 111: 410–422.
Panozzo J, Eagles HA, Wootton M (2001) Changes in protein composition during grain development in wheat. Aust J Agr Res 52: 485–493.
Pistón F, Martín A, Dorado G, Barro F (2004) Cloning and characterization of a gamma-3 hordein mRNA (cDNA) from *Hordeum chilense* (Roem. et Schult.). Theor Appl Genet 108: 1359–1365.
Pistón F, Dorado G, Martín A, Barro F (2006) Cloning of nine γ-gliadin mRNAs (cDNAs) from wheat and the molecular characterization of comparative transcript levels of γ-gliadin subclasses. J Cereal Sci 43: 20–128.
Masci S, D'Ovidio R, Lafiandra D, Kasarda DD (1998) Characterization of a low-molecular-weight glutenin subunit gene from bread wheat and the corresponding protein that represents a major subunit of the glutenin polymer. Plant Physiol 118: 1147–1158.
Masci S, D'Ovidio R, Lafiandra D, Kasarda DD (2000) A 1B coded low-molecular-weight glutenin subunit associated with quality in durum wheats show strong similarity to subunits present in some bread wheat cultivars. Theor Appl Genet 100: 396–400.
Tatham AS, Miflin BJ, Shewry PR (1985) The â–turn conformation in wheat gluten proteins: relationship to gluten elasticity. Cereal Chem 62: 405–412.