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Influence of high-molecular-weight glutenin subunit composition at *Glu-B1* locus on secondary and micro structures of gluten in wheat (*Triticum aestivum* L.)

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ABSTRACT

Glutenin is one of the critical gluten proteins that affect the processing quality of wheat dough. High-molecular-weight glutenin subunits (HMW-GS) affect rheological behavior of wheat dough. This research demonstrated the effects of four variations of HMW-GS composition at the *Glu-B1* locus on secondary and micro structures of gluten and rheological properties of wheat dough, using the bread wheat Xinong 1330 and its three near-isogenic lines (NILs). Results indicated that the Amide I bands of the four wheat lines shifted slightly, but the secondary structure, such as content of α-helices, β-sheets, disulfide bands, tryptophan bands and tyrosine bands, differed significantly among the four NILs. The micro structure of gluten in NIL 2 (Bx14 + By15) and NIL 3 (Bx17 + By18) showed more cross linkage, with two contrasting patterns. Correlation analysis demonstrated that the content of β-sheets and disulfide bonds has a significant relationship with dough stability, which suggests that the secondary structures could be used as predictors of wheat quality.

1. Introduction

Bread wheat (*Triticum aestivum* L.) can be processed into different foods and is one of the major constituents of the human diet. The gluten proteins of wheat are recognised as the most important components governing the bread-making quality of wheat. Based on their solubility in aqueous alcohol, gluten proteins are classified as gliadins or glutenins. The gliadins are single-chain polypeptides and present as monomeric proteins, whereas the glutenins are multiple-chain polymeric proteins in which individual polypeptides are stabilised by a network formed through inter-chain disulfide and hydrogen bonds (Wieser, 2007). The glutenins are further divided into low- and high-molecular-weight subunits (LMW-GS and HMW-GS). These proteins interact to make dough elastic, which allows dough to trap the gas bubbles produced by yeast, which in turn causes bread to rise (Shewry & Halford, 2002). Variation in HMW-GS composition has been reported to account for up to 70% of the genetic variation for dough properties in wheat: 45–70% in Europe (Branlard & Dardevet, 1985; Payne, Holt, Krattiger, & Carrillo, 1988), 35–60% in China (Liu et al., 2005), and 30–50% in southern Australia (Eagles, Hollamby, & Eastwood, 2002).

The HMW-GS genes are encoded at the *Glu-1* loci on the long arm of group-1 chromosomes (1A, 1B and 1D) (Shewry, Halford, & Lafiandra, 2003). At the *Glu-B1* locus, many alleles have been discovered (McIntosh et al., 2012) including one gene encoding an 'x-type' (Bx) HMW-GS and one gene encoding a 'y-type' (By) HMW-GS (Shewry, Halford, & Lafiandra, 2003). Both x-type and y-type of HMW-GS have a typical three-domain structure consisting of highly conserved N- and C-terminal non-repetitive domains flanking a long central repetitive domain (Shewry, Halford, & Lafiandra, 2003). α-Helices are present at the N- and C-terminals while β-turns and intermolecular β-sheets are present in the repetitive domain (Gilbert et al., 2000). Secondary and tertiary structures of gluten proteins change when treated with various temperatures (Georget & Belton, 2006; Wang et al., 2014), or different additives (Ferrer, Gómez, Añón, & Puppo, 2011; Gómez, Ferrer, Añón, & Puppo, 2013; Kaur, Singh, Kaur, Ahlawat, & Singh, 2014). For the secondary structure of gluten proteins, the content of β-sheets and β-turns was found to be positively correlated with the viscoelasticity of dough, while the content of α-helices showed a negative correlation (Georget & Belton, 2006). The number and

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distribution of cysteine residues differ between x-type and y-type HMW-GS, which affects the structure of glutenin polymers (Lindsay, Tamás, Appels, & Skerritt, 2000). Thus, the secondary structure of native gluten could be used as a predictive factor for bread-making quality.

In this research, secondary and tertiary structures and microstructure of gluten proteins from four near-isogenic lines (NILs) contrasting in HMW-GS composition encoded by the *Glu-B1* locus were characterised, together with dough rheological properties of these NILs, to study the influence of HMW-GS composition on the secondary structure of gluten and rheological behavior of wheat dough.

2. Materials and methods

2.1. Plant materials

NILs were created by initially crossing Xinong 1330 (Ax1, Bx7 + By9, Dx2 + Dy12) with wheat cultivar Fa 721 (null, Bx7 + By8, Dx5 + Dy10), Xiaoyan 6 (Ax1, Bx14 + By15, Dx2 + Dy12) and Fa 746 (null, Bx17 + By18, Dx3 + Dy12), respectively. The F_2 progenies were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) to select the various HMW-GS compositions and then backcrossed with Xinong 1330 six times. The backcross F_1 progenies with the appropriate HMW-GS were selected. BC_6F_8 homozygous NILs were checked for glutenin and gliadin by SDS–PAGE and A-PAGE. Wheat cultivar Xinong 1330 and its three HMW-GS NILs (NIL 1, NIL 2, and NIL 3) were selected for this research. BC_6F_8 and BC_6F_9 plants were planted at Yangling (108° 4′ E, 34° 16′ N), Shaanxi Province of China with two crop cycles in 2010–2011 and 2011–2012. Grains were dried and stored for 60 d before testing and milling. After hand-cleaning, grains were tempered for 20 h at 15% (w/w) moisture level and milled in a Brabender Quadrumat Senior mill (Brabender OHG, Duisburg, Germany). Lankao Teaizao (Ax1, Bx7 + By9, Dx2 + Dx12), Chinese Spring (Bx7 + By8, Dx2 + Dx12), Shaannong 981 (Ax1, Bx14 + By15, Dx2 + Dx12) and Huacheng 3366 (Bx17 + By18, Dx2 + Dx12) were included as controls to determine the HMW-GS composition.

2.2. Extraction and fractionation of gliadin and glutenin

Gliadins were extracted from 30 mg of flour milled from BC_6F_8 homozygous NILs and homogenized in 150 μl of 60% (v/v) aqueous ethanol on a Zeleny type rocker (40 cycles per min) for 40 min. Samples were centrifuged at 13,000*g* for 5 min, the supernatant containing gliadins was collected and the pellet was subsequently used for glutenin extraction. Glutenins were extracted following the method described by Gao, Appelbee, Mekuria, Chalmers, and Mather (2012). Gliadins and glutenins were separated by A-PAGE and SDS–PAGE according to Yan, Hsam, Yu, Jiang, and Zeller (2003) and Gao et al. (2012), respectively.

2.3. Separation and identification of gliadin and glutenin

Total gliadin and glutenin were determined using A-PAGE and SDS–PAGE samples with known quantities of albumin from bovine serum as standard. The glutenin samples were further prepared according to the method reported by Singh, Shepherd, and Cornish (1991). The amounts of HMW-GS and LMW-GS were determined twice by reversed phase high-performance liquid chromatography (RP-HPLC) according to the method described by Vawser and Cornish (2004). Subunit peaks were identified following previously established protocols (Cinco-Moroyoqui & MacRitchie, 2008; Vawser & Cornish, 2004). The HMW/LMW ratio and proportion of HMW-GS encoded by three *Glu-1* loci relative to total HMW-GS were calculated from the RP-HPLC trace. Each sample was tested in duplicate.

2.4. Near-infrared reflectance (NIR) analysis of grains

NIR analysis was conducted using a Diode Array 7200 NIR spectrometer (Perten Instrument AB, Sweden). The collected wavelength range was from 950 to 1650 nm and the data collection mode was reflectance. Each sample was tested in duplicate. NIR parameters evaluated were moisture, protein content, starch content, gluten content, hardness, Zeleny sedimentation and SDS sedimentation.

2.5. Extraction of gluten

Gluten samples were obtained by washing Brabender-milled wheat flour according to Wellner, Mills, and Brownsey (2005) with minor modification. Thirty grams of flour was mixed with 18.6 ml of water for 30 min. The dough was washed with distilled water until no starch was left. The gluten was kept in water and freeze-dried and the dry gluten mass was ground in a mortar. Ten milligrams of gluten from each sample was immersed in distilled water without stirring and equilibrated at 4 °C for at least 24 h.

2.6. FT-Raman Spectroscopy analysis of gluten

FT-Raman Spectroscopy was conducted in duplicate for each sample to determine the parameters related to secondary and tertiary structures of gluten proteins according to Ferrer et al. (2011). Spectra were recorded at 25 °C with a laser power of 500 mW and a spectral resolution of 6 cm^{-1}. Each spectrum was obtained after collecting and averaging 1000 scans in order to obtain spectra with high signal-to-noise ratios. FT-Raman spectra were plotted as intensity (arbitrary units) against Raman shift in wavenumber units (cm^{-1}). In Amide I region, a straight baseline passing through the ordinates at 1700 and 1600 cm^{-1} was adjusted in order to calculate this band intensity. To calculate the secondary structure components of gluten, the Amide I region was truncated and deconvoluted using a nonlinear least-squares curve-fitting procedure, including mixed Gaussian–Lorentzian components. The spectra were analyzed using the Omnic software package (version 6.1a, Thermo Nicolet Corp). The resulting fitted curve was analyzed taking into account the band assignment for the secondary structure previously reported in the literature (Kaur et al., 2014; Wang et al., 2014). The targeted structures were β-sheets: 1680–1695 cm^{-1}, β-turns: 1670–1680 cm^{-1}, α-helices: 1650–1660 cm^{-1}, antiparallel β-sheets: 1627–1635 cm^{-1} and intermolecular β-sheets due to protein aggregation: 1613–1620 cm^{-1}. In order to calculate the percentage contribution of the different types of conformations to the area of all the components, bands assigned to a given conformation were summed and divided by the total Amide I area. The number obtained was taken as the proportion made up by the polypeptide chain in the corresponding conformation. Band assignment of the major vibrational motions of the side chains, such as inter-chain disulfide bands (497 cm^{-1}), tryptophan bands (880 cm^{-1}) and tyrosine bands ($I_{850/830}$), was based on comparison to Raman data reported in previous research (Ferrer et al., 2011; Gómez et al., 2013; Navarra, Tinti, Leone, Militello, & Torreggiani, 2009). Of them, the tryptophan band at 880 cm^{-1} was used as an indicator of the strength of hydrogen bonding and $I_{850/830}$ as a monitor of the hydrogen bonding of the phenolic hydroxyl group. A decrease in $I_{850/830}$ ratio has been reported to reflect an increase in buriedness, suggesting possible involvement of tyrosyl residues in intermolecular or intramolecular interactions (Meng, Ma, & Phillips, 2003). Duplicate samples were harvested in each year and the mean values are reported.

2.7. Microstructure of gluten samples

The microstructure of gluten samples was observed by scanning electron microscopy (SEM). Gluten samples were prepared following the method described by Gómez et al. (2013). Dehydrated samples were coated with gold particles in a sputter coater (Pelco, Redding, California, USA). Images were taken in a JOEL JSM 35 CF scanning electron microscope (Tokyo, Japan) with a 5 kV acceleration voltage.

2.8. Rheological properties analysis: Farinograph and Extensograph

Flour samples were mixed for standard Farinograph and Extensograph analysis (Brabender GmbH & Co. KG, Germany). The Farinograph characteristics (dough development time and dough stability) were determined according to American Association of Cereal Chemists (AACC) Method 54-21. The Extensograph characteristics (area beneath the curve or energy value, extensibility and maximum resistance (R_{max})) were analyzed according to AACC Method 54-10.

2.9. Statistical analysis

Student's *t*-test (Spiegel, 1972) was used to determine significant differences among NILs. Pearson correlation analysis was carried out using SPSS19.0 (SPSS, Inc., Chicago, IL, USA) to determine the relationship between the different variables.

3. Results and discussion

3.1. Variation of HMW-GS composition encoded by Glu-B1

To investigate the variation of gluten subunit composition, the gliadins and glutenins of Xinong 1330 and its three NILs were separated on A-PAGE and SDS–PAGE (Supplementary Fig. S1), respectively, and LMW glutenins were further separated by RP-HPLC (Supplementary Fig. S2). The gliadins and LMW-GS of the four lines were identical (Supplementary Figs. S1 and S2). The HMW-GS were Ax1 encoded by *Glu-A1* and Dx2 and Dy12 by *Glu-D1* for all four NILs. The only differences among the NILs were in HMW-GS encoded by *Glu-B1* (Supplementary Fig. S1b): Bx7 + By9, Bx7 + By8, Bx14 + By15 and Bx17 + By18 in Xinong 1330, NIL 1, NIL 2 and NIL 3, respectively. As elasticity properties of wheat are mainly determined by HMW-GS (Shewry, Halford, Tatham, et al., 2003), studies were focused on the effects on functional properties of variation of HMW-GSs and their composition (Jin et al., 2013; Shewry, Halford, Tatham, et al., 2003). Wheat NILs contrasting in HMW-GS encoded by different alleles of *Glu-B1* have been used as ideal materials to study the effects of HMW-GS on functional properties of wheat flour (Deng, Tian, & Sun, 2005; Jin et al., 2013; Jonnala et al., 2010; Liu et al., 2005; Naeem & MacRitchie, 2005), because of their identical genetic background. At the *Glu-B1* locus, many allelic variants have been detected (McIntosh et al., 2012). The present study focuses on the effects of four variants at *Glu-B1* locus on wheat gluten structures and rheological properties of the four NILs. The four variants at *Glu-B1* locus account for more than 90% frequency of HMW-GS alleles in Chinese varieties (Liu et al., 2005). The comparison of the differences in contribution to dough strength made by Bx14 + By15 and Bx17 + By18 is first reported in the current study.

3.2. Proportion of HMW-GS encoded by Glu-1 loci relative to the total protein

There were no significant difference in moisture, protein content, starch content or wet gluten content among the four NILs (Supplementary Table S1). However, there were significant differences among the samples in Zeleny sedimentation and SDS-sedimentation values, indicators of dough strength. As it has also been suggested that dough strength can be affected by the expression level of HMW-GS (Vawser & Cornish, 2004), to ensure that the differences in gluten structure and/or dough rheological properties observed were not caused by the expression level of HMW-GS,

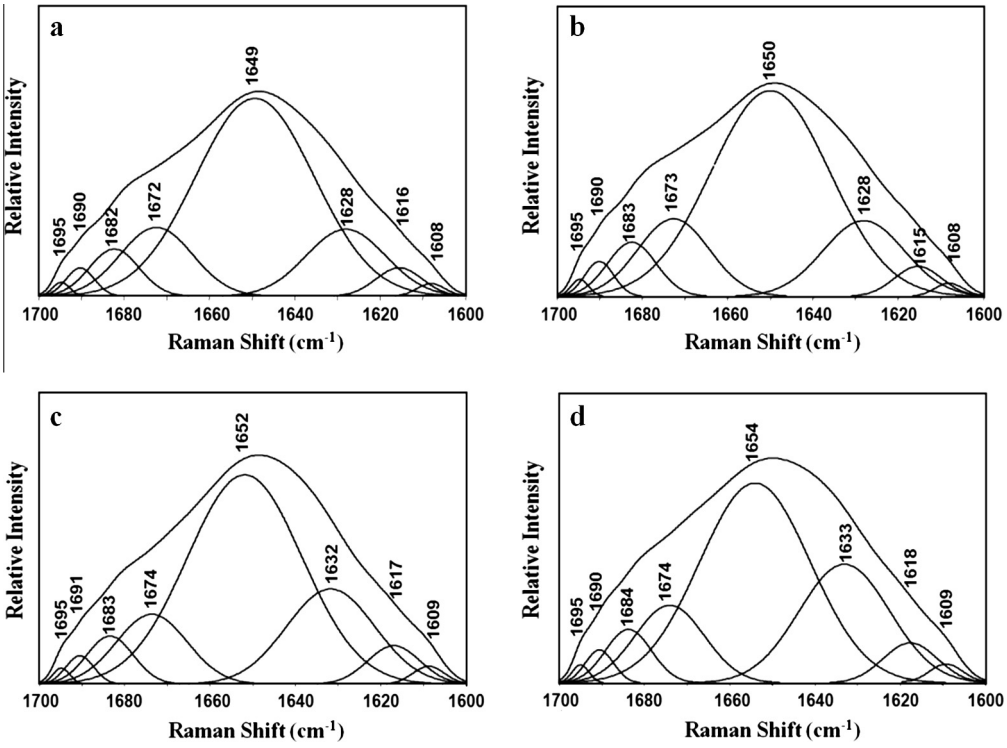

Fig. 1. Amide I band of Xinong 1330 (a) and three near-isogenic lines: NIL 1 (b), NIL 2 (c) and NIL 3 (d).

Table 1
FT-Raman Spectroscopy determination of secondary structure percentages of Xinong 1330 and the three near-isogenic lines.

Year	Line	α-Helices	Intermolecular β-sheets	β-Sheets	β-Turns	Antiparallel β-sheets
2010–2011	Xinong 1330	60.33 ± 0.03a	3.44 ± 0.01c	13.71 ± 0.01d	12.83 ± 0.01c	8.69 ± 0.08c
	NIL 1	58.43 ± 0.07b	3.35 ± 0.07c	14.69 ± 0.12c	13.30 ± 0.14a	9.37 ± 0.09b
	NIL 2	53.44 ± 0.17c	4.65 ± 0.07b	18.50 ± 0.28b	13.40 ± 0.00a	9.70 ± 0.17a
	NIL 3	48.46 ± 0.23d	4.89 ± 0.01a	24.05 ± 0.07a	13.00 ± 0.13b	9.26 ± 0.01b
2011–2012	Xinong 1330	60.41 ± 0.04a	3.38 ± 0.03c	13.71 ± 0.02d	12.88 ± 0.04c	8.77 ± 0.02c
	NIL 1	58.42 ± 0.02b	3.36 ± 0.03c	14.66 ± 0.04c	13.17 ± 0.07b	9.48 ± 0.20a
	NIL 2	53.60 ± 0.06c	4.66 ± 0.01b	18.39 ± 0.09b	13.44 ± 0.04a	9.59 ± 0.04a
	NIL 3	48.29 ± 0.13d	4.87 ± 0.01a	24.01 ± 0.02a	13.16 ± 0.08b	9.29 ± 0.01b

Values followed by a different letter in the same column are significantly different ($P < 0.05$).

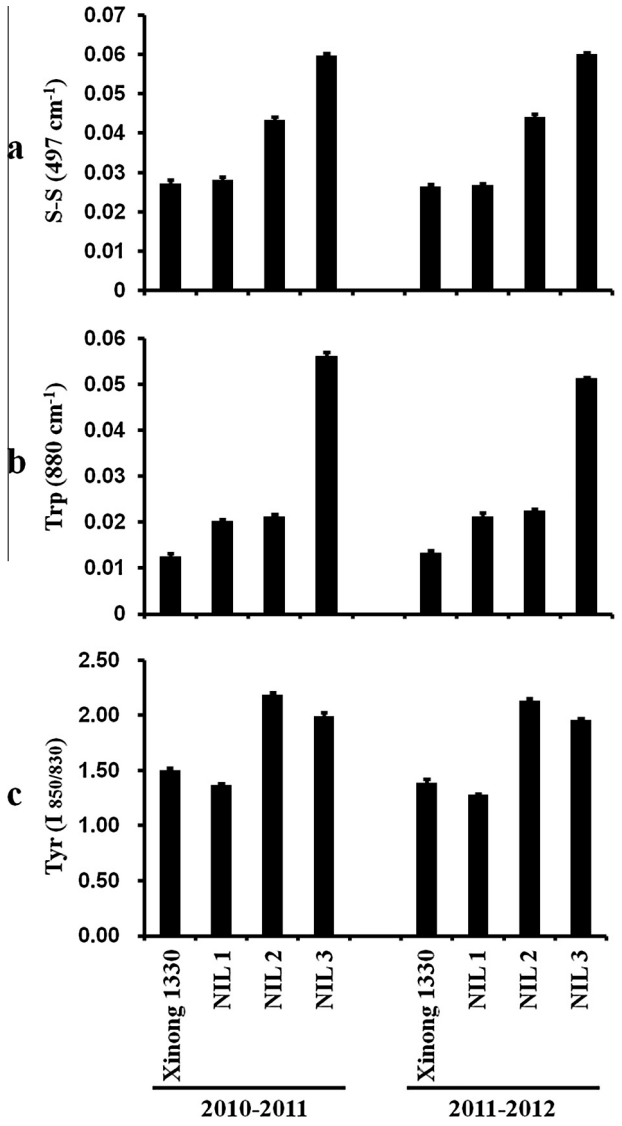

Fig. 2. Side chain vibrations analysis of Xinong 1330 and three near-isogenic lines NIL 1–3. (a) Normalized intensity of inter-chain disulfide band appeared at 497 cm^{-1}. (b) Normalized intensity of tryptophan band appeared at 880 cm^{-1}. (c) Intensity ratios $I_{850/830}$ indicating tyrosine residues. Normalized intensity values correspond to the mean values of three independent experiments. Vertical bars represent confidence intervals at $P < 0.05$.

quantitative analysis of HMW-GS, relative to total protein of the four NILs was conducted in the current study. This analysis showed that the glutenin/gliadin ratio and HMW-GS/LMW-GS ratio ranged from 0.63 to 0.67 and from 0.29 to 0.33, respectively, and differences in the ratios were not significant among the four NILs. The HMW-GS encoded by *Glu-A1*, *Glu-B1*, *Glu-D1* represented 9.8–10.6%, 57.2–58.3%, and 31.4–32.5% relative to the total amount of HMW-GS, respectively among the four NILs (Supplementary Table S2). The results demonstrated the expression level of HMW-GS encoded by different *Glu-1* loci was similar among the four NILs, which ensured the differences reported here were caused by the HMW-GS composition itself.

3.3. Secondary and tertiary structures of gluten

To analyze the protein backbone conformation that gives information on the secondary and tertiary structures, Amide I bands (1700–1600 cm^{-1}) were obtained from the gluten of each NIL (Fig. 1). The results show that the pattern and intensity of the Amide I bands are identical among the four NILs in 2011 and 2012. However, the Amide I bands of NIL 1 (1650 cm^{-1}), NIL 2 (1652 cm^{-1}) and NIL 3 (1654 cm^{-1}) show slight shifts towards higher wavenumbers, compared to that of Xinong 1330 (1649 cm^{-1}). The shift of the Amide I bands indicates changes in the α-helix and β-sheet content of the gluten samples, which is correlated with the results of quantitative analysis of the secondary structure in the Amide I band region (Table 1). The results show that Xinong 1330 has the highest α-helix content, followed by NIL 1, NIL 2 and NIL 3. NIL 3 has the highest intermolecular β-sheet and β-sheet content, followed by NIL 2, while both NIL 2 and NIL 3 are significantly different from those of Xinong 1330 and NIL 1. NIL 2 has the highest β-turn and antiparallel β-sheet content, followed by NIL 1, NIL 3 and Xinong 1330. The information concerning inter-chain disulfide bands, tryptophan bands and tyrosine bands is reported in Fig. 2. The results demonstrate that NIL 3 has the highest intensities of disulfide and tryptophan bands, followed by NIL 2, NIL 1 and Xinong 1330. NIL 2 has the highest intensity ratio $I_{850/830}$, followed by NIL 3, Xinong 1330 and NIL 1, which indicates NIL 2 had the lowest intensity of tyrosine band among the four NILs.

Dough properties may be affected by the secondary structure of the gluten. According to Mondher, Barbara, Abdelfattah, Franck, and Mohamed (2005) and Georget and Belton (2006), the content of β-sheets is positively correlated to the viscoelasticity of dough and the content of α-helices is negatively correlated to this characteristic. It has also been reported that the band appearing at 497 cm^{-1} is related to the inter-chain disulfide bond, which is the main factor determining dough properties (Ferrer et al., 2011; Lindsay et al., 2000). Furthermore, as demonstrated by previous studies (Belton, 2005; Georget & Belton, 2006), both hydrogen bonding and disulfide bonds are crucial factors in gluten rheology, and the intermolecular disulfide bonds associated with β-sheets act synergistically, which stabilises the gluten polymers. It is to be noted that both disulfide, including intermolecular and intramolecular disulfide bonds, and tryptophan bonds do not only come from HMW-GS, but also come from gliadins or LMW-GS, which may affect dough properties. However, in this study, the

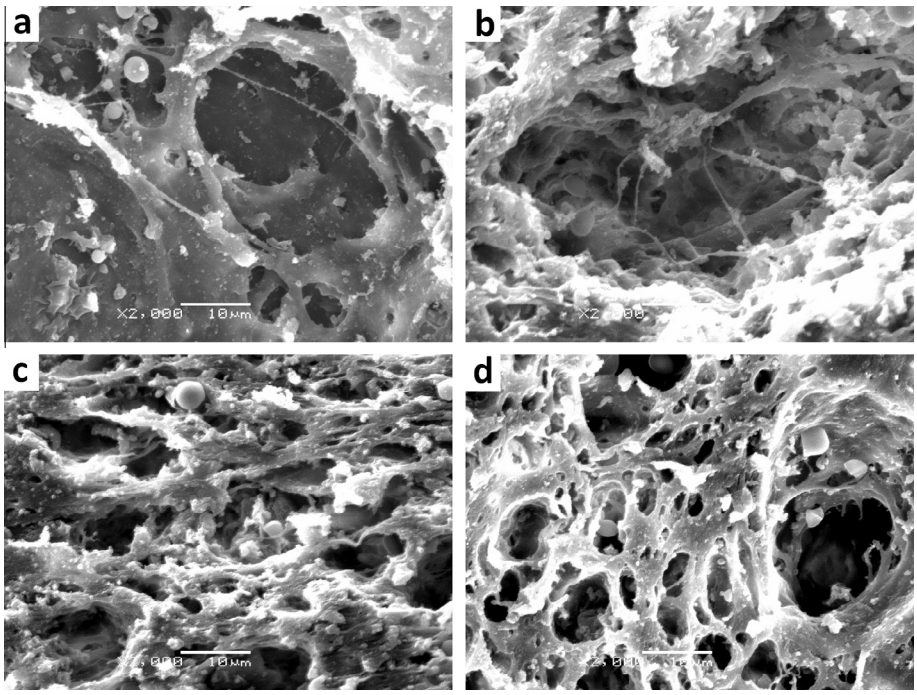

Fig. 3. Scanning electron microscopy of gluten extracted from Xinong 1330 and three near-isogenic lines. (a) Xinong 1330, (b) NIL 1, (c) NIL 2, (d) NIL 3. Magnification: 2000×. Bars: 10 μm.

significant differences in secondary structure are not mainly caused by gliadins or LMW-GS with the identical components and similar proportion among the four NILs. Thus, based on data on the secondary structure of gluten in this study, the stability of gluten structure was ranked as NIL 3 > NIL 2 > NIL 1 > Xinong 1330. Bx17 + By18 gave the highest stability compared to the HMW-GS compositions carried by the other three NILs.

3.4. Microstructure of the gluten network

The microstructure of the gluten network examined using SEM is shown in Fig. 3. It is observed that the apertures of Xinong 1330 (Fig. 3a) and NIL 1 (Fig. 3b) are significantly larger than those of NIL 2 (Fig. 3c) and NIL 3 (Fig. 3d). Gluten of NIL 3 in Fig. 3d has more cross-links than that of NIL 2 in Fig. 3c, followed by those of NIL 1 and Xinong 1330 in Fig. 3b and a, which is consistent with the results of disulfide bonds determined by FT-Raman Spectroscopy analysis. The degree of connectivity for NIL 3 is greater than that for NIL 2, but both NIL2 and NIL 3 showed higher levels than Xinong 1330 and NIL 1. Compared to Xinong 1330 in Fig. 3a, NIL 2 in Fig. 3c presents a more fibrous structure and NIL 3 in Fig. 3d presents a more laminar gluten network. It was notable from Fig. 3c and d that NIL 2 represented a completely different type of the gluten network from NIL 3. This could be explained by the differences in the structures of various glutenin subunits. As glutenin subunits act as a structural backbone to the glutenin macropolymer, glutenin subunits are stabilised into a network by inter-chain disulfide and hydrogen bonds (Shewry, Tatham, Forde, Kreis, & Miflin, 1986). Different glutenin subunits cause changes in the secondary and tertiary structures of gluten. It has been demonstrated that HMW-GS may have two types of networks when gluten is extended (Belton, 2005). In one type of network, the HMW-GSs act as a chain extender and link together via head-to-tail disulfide bonds to form a linear chain (Pirozi, Margiotta, Lafiandra, & MacRitchie, 2008); in the other type, the HMW-GS act as chain brancher by linking through disulfide bonds (Köhler, Keck-Gassenmeier, Wieser, & Kasarda, 1997) to promote a highly cross-linked gluten network that dramatically increases dough strength and improves baking properties (Darlington et al., 2003; Lafiandra, D'Ovidio, Porceddu, Margiotta, & Colaprico, 1993; Tatham, Field, Keen, Jackson, & Shewry, 1991). The gluten network observed in NIL 2 appears to be more like the first type, as it is more linear than that of NIL 3. This can be explained by the cysteine number of the HMW subunit Bx14. Compared with typical x-type glutenin subunits, which have four cysteines (three at the N-terminal and one at the C-terminal), subunit Bx14 has only two cysteines, one at each terminal (Li et al., 2004). Therefore, subunit Bx14 is more likely to form a linear chain by intermolecular disulfide bonds with other subunits, which gives high dough strength by increasing the extensibility of the wheat dough (Deng et al., 2005). For NIL 3, the gluten network was more well-distributed than those of Xinong 1330 and NIL 1. This may be attributed to the high content of disulfide bonds and β-sheets discussed above, both of which may act more synergistically in NIL 3 than those in other wheat lines.

3.5. Quality properties of grain and rheological properties of dough

There were considerable variations for Zeleny sedimentation and SDS sedimentation among the four NILs. NIL 2 had the highest Zeleny sedimentation value, while NIL 3 had the highest SDS sedimentation value among the NILs (Supplementary Table S1). As suggested by Najafian (2012) based on correlation among bread-making quality assessment indices, grain hardness and SDS-sedimentation volume are the most reliable and practical indices in assessment of wheat grain quality. Based on the NIR results, it is predicted that the contribution ranks of HMW-GS encoded by *Glu-B1* locus to bread-making quality is Bx17 + By18 > Bx14 + By15 > Bx7 + By8 > Bx7 + By9. These results are partly consistent with previous reports (Deng et al., 2005; Jin et al., 2013; Marchylo, Lukow, & Kruger, 1992).

The rheological properties were examined with Farinograph and Extensograph measurements (Fig. 4). For the Farinograph, it was notable that NIL 2 had the greatest dough development time,

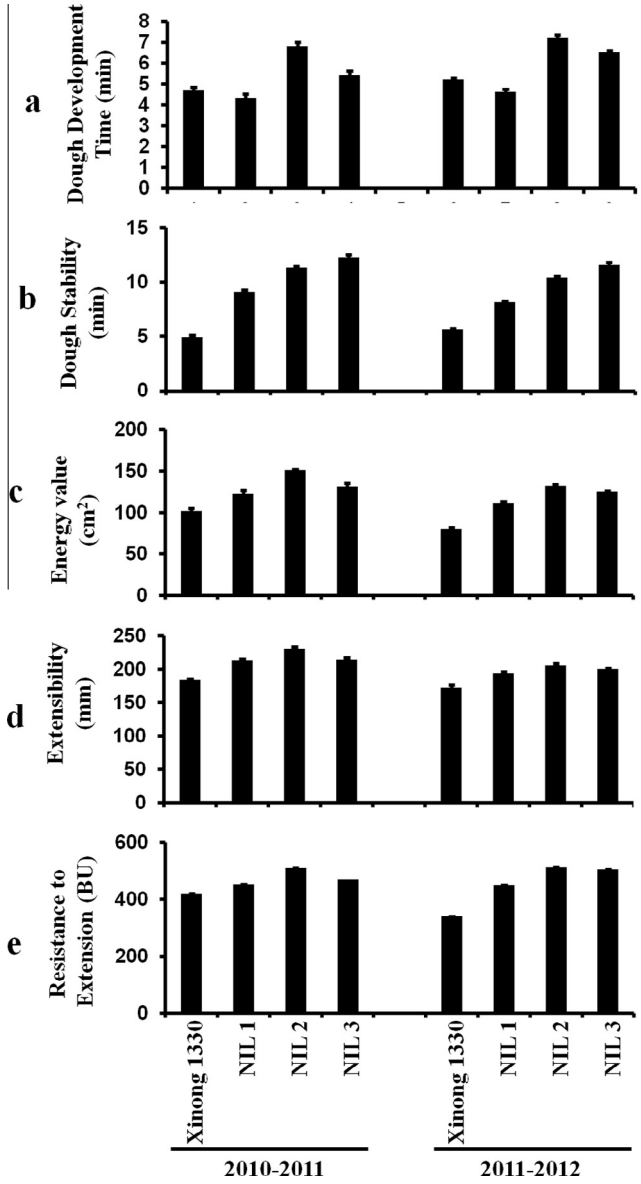

Fig. 4. Rheological properties of dough determined by Farinograph and Extensograph for Xinong 1330 and three near-isogenic lines. (a) Dough development time. (b) Dough stability. (c) Energy value of dough relaxed in 135 min. (d) Extensibility of dough relaxed in 135 min. (e) R_{max} of dough relaxed in 135 min.

yet NIL 3 had the highest dough stability. For the Extensograph, NIL 2 showed the greatest energy value, extensibility and R_{max}, which confirmed the inference based on the microstructure of the gluten network. Moreover, Xinong 1330 had the lowest value in all parameters among the four NILs. The contribution to dough stability time of HMW-GS encoded by the *Glu-B1* locus was ranked as Bx17 + By18 > Bx14 + By15 > Bx7 + By8 > Bx7 + By9, which is consistent with the prediction derived from the NIR data.

3.6. Correlation analysis of the secondary structures and dough rheological properties

The correlation matrix between the secondary structure and dough rheological properties is reported in Table 2. The results show that the secondary structure of gluten was significantly correlated with the dough rheological parameters. The content of α-helices was negatively correlated with that of β-sheets ($r = -0.993$) and intermolecular β-sheets ($r = -0.942$), which could be explained by the transformation between α-helices and β-sheets under certain conditions (Georget & Belton, 2006). The contents of intermolecular and antiparallel β-sheets were correlated with parameters from the Farinograph and Extensograph, which indicated that they played a crucial role in both dough stability and extensibility. It is suggested that β-sheets is correlated with dough stability and β-turns is correlated with extensibility, respectively. The content of disulfide bonds was significantly correlated with dough stability, which further confirmed the effects of cysteine number on dough properties. These correlation results provide the strong evidence that the content of intermolecular and antiparallel β-sheets in gluten are major parameters in determining dough quality when applying secondary structures of gluten as quality predictors.

4. Conclusion

The wheat NILs studied here provided the identical genetic background needed to compare the quality difference caused by variations in HMW-GS composition. The results of this research demonstrate that the different HMW-GS compositions encoded by *Glu-B1* affected the proportion of secondary structure, and the microstructure of gluten and the rheological properties of wheat dough. The HMW-GS, especially with the different number of the cysteine residues, gave distinguishing patterns of the microstructure of gluten. Among the four near-isogenic lines, NIL 2 with Bx14 + By15, possessed the highest content of β-turns and the greatest viscoelasticity, while NIL 3 with Bx17 + By18, possessed

Table 2
Pearson correlation coefficients between different secondary structures and dough rheological properties.

	Intermolecular β-sheets	β-Sheets	β-Turns	Antiparallel β-sheets	S–S (497 cm^{-1})	Trp (760 cm^{-1})	Tyr ($I_{850/830}$)	Dough development time	Dough stability	Energy value	Extensibility	R_{max}
α-Helices	−0.942**	−0.993**	−0.306	−0.468	−0.990**	−0.913**	−0.792*	−0.616	−0.907**	−0.669	−0.569	−0.711*
Intermolecular β-sheets	1.000	0.916**	0.387	0.492	0.948**	0.740*	0.947**	0.814*	0.855**	0.722*	0.594	0.743*
β-Sheets		1.000	0.192	0.362	0.994**	0.945**	0.745*	0.557	0.860**	0.587	0.490	0.632
β-Turns			1.000	0.927**	0.211	0.009	0.485	0.553	0.616	0.765*	0.742*	0.772*
Antiparallel β-sheets				1.000	0.356	0.225	0.520	0.509	0.762*	0.836**	0.819*	0.820*
S–S (497 cm^{-1})					1.000	0.909**	0.804*	0.627	0.849**	0.603	0.493	0.648
Trp (760 cm^{-1})						1.000	0.495	0.270	0.766*	0.414	0.355	0.464
Tyr ($I_{850/830}$)							1.000	0.896**	0.755*	0.768*	0.644	0.748*
Dough development time								1.000	0.581	0.576	0.414	0.645
Dough stability									1.000	0.850**	0.814*	0.825*
energy value										1.000	0.962**	0.928**
Extensibility											1.000	0.806*

* And ** indicate significant correlations at $P < 0.05$ and $P < 0.01$, respectively.

the highest content of β-sheets and the greatest wheat dough strength.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.11.085.

References

Belton, P. (2005). New approaches to study the molecular basis of the mechanical properties of gluten. *Journal of Cereal Science, 41*, 203–211.

Branlard, G., & Dardevet, M. (1985). Diversity of grain protein and bread wheat quality. II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. *Journal of Cereal Science, 3*, 345–354.

Cinco-Moroyoqui, F. J., & MacRitchie, F. (2008). Quantitation of LMW-GS to HMW-GS ratio in wheat flours. *Cereal Chemistry, 85*, 824–829.

Darlington, H., Fido, R., Tatham, A. S., Jones, H., Salmon, S. E., & Shewry, P. R. (2003). Milling and baking properties of field grown wheat expressing HMW subunit transgenes. *Journal of Cereal Science, 38*, 301–306.

Deng, Z. Y., Tian, J. C., & Sun, G. X. (2005). Influence of high molecular weight glutenin subunit substitution on rheological behaviour and bread-baking quality of near-isogenic lines developed from Chinese wheats. *Plant Breeding, 124*, 428–431.

Eagles, H. A., Hollamby, G. J., & Eastwood, R. F. (2002). Genetic and environmental variation for grain quality traits routinely evaluated in southern Australian wheat breeding programs. *Australian Journal of Agricultural Research, 53*, 1047–1057.

Ferrer, E. G., Gómez, A. V., Añón, M. C., & Puppo, M. C. (2011). Structural changes in gluten protein structure after addition of emulsifier. A Raman spectroscopy study. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 79*, 278–281.

Gómez, A. V., Ferrer, E. G., Añón, M. C., & Puppo, M. C. (2013). Changes in secondary structure of gluten proteins due to emulsifiers. *Journal of Molecular Structure, 1033*, 51–58.

Gao, X., Appelbee, M., Mekuria, G., Chalmers, K., & Mather, D. E. (2012). A second 'overexpression' allele at the *Glu-B1* high-molecular-weight glutenin locus of wheat: Sequence characterisation and functional effects. *Theoretical and Applied Genetics, 124*, 333–343.

Georget, D. M. R., & Belton, P. S. (2006). Effects of temperature and water content on the secondary structure of wheat gluten studied by FTIR spectroscopy. *Biomacromolecules, 7*, 469–475.

Gilbert, S. M., Wellner, N., Belton, P. S., Greenfield, J. A., Siligardi, G., Shewry, P. R., & Tatham, A. S. (2000). Expression and characterization of a highly repetitive peptide derived from a wheat seed storage protein. *Acta Biochimica et Biophysica Sinica, 1479*, 135–146.

Jin, H., Zhang, Y., Li, G., Mu, P., Fan, Z., Xia, X., & He, Z. (2013). Effects of allelic variation of HMW-GS and LMW-GS on mixograph properties and Chinese noodle and steamed bread qualities in a set of Aroona near-isogenic wheat lines. *Journal of Cereal Science, 57*, 146–152.

Jonnala, R. S., MacRitchie, F., Smail, V. W., Seabourn, B. W., Tilley, M., Lafiandra, D., & Urbano, M. (2010). Protein and quality characterization of complete and partial near-isogenic lines of waxy wheat. *Cereal Chemistry, 87*, 538–545.

Köhler, P., Keck-Gassenmeier, B., Wieser, H., & Kasarda, D. D. (1997). Molecular modeling of the N-terminal regions of high molecular weight glutenin subunits 7 and 5 in relation to intramolecular disulfide bond formation. *Cereal Chemistry, 74*, 154–158.

Kaur, A., Singh, N., Kaur, S., Ahlawat, A. K., & Singh, A. M. (2014). Relationships of flour solvent retention capacity, secondary structure and rheological properties with the cookie making characteristics of wheat cultivars. *Food Chemistry, 158*, 48–55.

Lafiandra, D., D'Ovidio, R., Porceddu, E., Margiotta, B., & Colaprico, G. (1993). New data supporting high M_r glutenin subunit 5 as the determinant of quality differences among the pairs 5 + 10 vs. 2 + 12. *Journal of Cereal Science, 18*, 197–205.

Li, W., Wan, Y., Liu, Z., Liu, K., Liu, X., Li, B., ... Wang, D. (2004). Molecular characterization of HMW glutenin subunit allele *1Bx14*: Further insights into the evolution of *Glu-B1-1*alleles in wheat and related species. *Theoretical and Applied Genetics, 109*, 1093–1104.

Lindsay, M. P., Tamás, L., Appels, R., & Skerritt, J. H. (2000). Direct evidence that the number and location of cysteine residues affect glutenin polymer structure. *Journal of Cereal Science, 31*, 321–333.

Liu, L., He, Z. H., Yan, J., Zhang, Y., Xia, X. C., & Peña, R. J. (2005). Allelic variation at the Glu-1 and Glu-3 loci, presence of the 1B.1R translocation, and their effects on mixographic properties in Chinese bread wheats. *Euphytica, 142*, 197–204.

Marchylo, B. A., Lukow, O. M., & Kruger, J. E. (1992). Quantitative variation in high molecular weight glutenin subunit 7 in some Canadian wheats. *Journal of Cereal Science, 15*, 29–37.

McIntosh, R., Yamazaki, Y., Dubcovsky, J., Rogers, J., Morris, C., Somers, D., ... Devos, K. (2012). *Catalogue of gene symbols for wheat*. Japan: Wheat Genetic Resource Database Komugi-Natl. BioResource Res. Project. Available at <http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp>.

Meng, G., Ma, C.-Y., & Phillips, D. L. (2003). Raman spectroscopic study of globulin from *Phaseolus angularis* (red bean). *Food Chemistry, 81*, 411–420.

Mondher, M., Barbara, R., Abdelfattah, B., Franck, M., & Mohamed, M. (2005). Effects of some additives on wheat gluten solubility: A structural approach. *Food Chemistry, 92*, 7–15.

Naeem, H. A., & MacRitchie, F. (2005). Polymerization of glutenin during grain development in near-isogenic wheat lines differing at *Glu-D1* and *Glu-B1* in greenhouse and field. *Journal of Cereal Science, 41*, 7–12.

Najafian, G. (2012). Study of relationship among several bread making quality assessment indices in hexaploid wheat (*Triticum aestivum* L.) using correlation analysis. *Quality Assurance and Safety of Crops & Foods, 4*, 136–158.

Navarra, G., Tinti, A., Leone, M., Militello, V., & Torreggiani, A. (2009). Influence of metal ions on thermal aggregation of bovine serum albumin: Aggregation kinetics and structural changes. *Journal of Inorganic Biochemistry, 103*, 1729–1738.

Payne, P. I., Holt, L. M., Krattiger, A. F., & Carrillo, J. M. (1988). Relationships between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. *Journal of Cereal Science, 7*, 229–235.

Pirozi, M. R., Margiotta, B., Lafiandra, D., & MacRitchie, F. (2008). Composition of polymeric proteins and bread-making quality of wheat lines with allelic HMW-GS differing in number of cysteines. *Journal of Cereal Science, 48*, 117–122.

Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: Structures, properties and role in grain utilization. *Theoretical and Applied Genetics, 53*, 947–958.

Shewry, P. R., Halford, N. G., & Lafiandra, D. (2003). Genetics of wheat gluten proteins. *Advances in Genetics, 49*, 111–184.

Shewry, P. R., Halford, N. G., Tatham, A. S., Popineau, Y., Lafiandra, D., & Belton, P. S. (2003). The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. *Advances in Food and Nutrition Research, 45*, 219–302.

Shewry, P. R., Tatham, A. S., Forde, J., Kreis, M., & Miflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science, 4*, 97–106.

Singh, N. K., Shepherd, K. W., & Cornish, G. B. (1991). A simplified SDS–PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science, 14*, 203–208.

Spiegel, R. (1972). *Theory and problems of statistics in SI units*. New York: McGraw-Hill.

Tatham, A. S., Field, J. M., Keen, J. N., Jackson, P. J., & Shewry, P. R. (1991). Purification and characterization of HMW glutenin subunits encoded by chromosome 1B of durum wheat (*Triticum durum*). *Journal of Cereal Science, 14*, 111–116.

Vawser, M. J., & Cornish, G. B. (2004). Over-expression of HMW glutenin subunit *Glu-B1* 7x in hexaploid wheat varieties (*Triticum aestivum*). *Australian Journal of Agricultural Research, 55*, 577–588.

Wang, P., Xu, L., Nikoo, M., Ocen, D., Wu, F., Yang, N., ... Xu, X. (2014). Effect of frozen storage on the conformational, thermal and microscopic properties of gluten: Comparative studies on gluten-, glutenin- and gliadin-rich fractions. *Food Hydrocolloids, 35*, 238–246.

Wellner, N., Mills, E. N. C., & Brownsey, G. (2005). Changes in protein secondary structure during gluten deformation studied by dynamic Fourier transform infrared spectroscopy. *Biomacromolecules, 6*, 255–267.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*, 115–119.

Yan, Y. M., Hsam, S. L. K., Yu, J. Z., Jiang, Y., & Zeller, F. J. (2003). Genetic polymorphisms at *Gli-D*^{t} gliadin loci in *Aegilops tauschii* as revealed by acid polyacrylamide gel and capillary electrophoresis. *Plant Breeding, 122*, 120–124.