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Effects of HMW-GS Ax1 or Dx2 absence on the glutenin polymerization and gluten micro structure of wheat (*Triticum aestivum* L.)

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ABSTRACT

Wheat (*Triticum aestivum* L.) dough strength and extensibility are mainly determined by the polymerization of glutenin. The number of high-molecular-weight glutenin subunits (HMW-GS) differs in various wheat varieties due to the silencing of some genes. The effects of Ax1 or Dx2 subunit absence on glutenin polymerization, dough mixing properties and gluten micro structure were investigated with two groups of near-isogenic lines. The results showed that Ax1 or Dx2 absence decreased the accumulation rate of glutenin polymers and thus delayed the rapid increase period for glutenin polymerization by at least ten days, which led to lower percentage of polymeric protein in mature grain. Ax1 or Dx2 absence significantly decreased the dough development time and dough stability, but increased the uniformity of micro structure. Lacunarity, derived from quantitative analysis of gluten network, is suggested as a new indicator for wheat quality.

1. Introduction

Bread wheat is one of the crucial cereals for human diet. The processing quality of wheat flour for production of bread and other food products is mainly determined by the gluten proteins (Shewry, 2003). According to their solubility in various solvents, the gluten proteins are classified into monomeric gliadins and polymeric glutenins. The polymeric glutenins consist of high- and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS) (Wieser, 2007), and HMW-GS are recognised as the most important components determining the strength and elasticity of wheat gluten (Shewry, 2003), even though HMW-GS represent only 5–10% of the protein in wheat grain. The HMW-GS variation is encoded by genes located at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. Given each locus contains genes linked together encoding an x-type and a y-type HMW-GS (Shewry, Halford, & Lafiandra, 2003), it may be expected that bread wheat could contain up to six different HMW-GS. In fact, only three, four or five subunits are present in most of cultivars of bread wheat, due to the silencing of some genes. Many studies reported that variation in the amount of HMW-GS has great effects on the rheological properties of wheat dough (Gao, Appelbee, Mekuria, Chalmers, & Mather, 2012; León et al., 2009; Ma, Yan, Huang, Chen, & Zhao, 2012; Ragupathy et al., 2008; Rakszegi et al., 2005). However, the influence of variation in the number of HMW-GS on the formation of wheat quality needs to be studied further.

Size distribution of glutenin polymer can be indicated by the percentage of SDS-unextractable polymeric protein relative to total polymeric protein (UPP%), and it is positively correlated with dough rheological properties (Gupta, Khan, & MacRitchie, 1993). The formation of glutenin polymers is affected by both environmental and genetic factors and their interactions (Ferrise, Bindi, & Martre, 2015; Johansson, Kuktaite, Andersson, & Prieto-Linde, 2005; Liu et al., 2016; Malik, Kuktaite, & Johansson, 2013; Naeem & MacRitchie, 2005) during grain development. The studies focusing on the effects of allelic variation on the polymerization of glutenins demonstrated that near-isogenic lines (NILs) with strong gluten-associated HMW-GS (Bx17 + By18 at *Glu-B1* and Dx5 + Dy10 at *Glu-D1*) show earlier polymerization and higher UPP% than those of the NILs with weak gluten-associated HMW-GS (Bx7 + By8 at *Glu-B1* and Dx2 + Dy12 at *Glu-D1*) (Liu et al., 2016; Naeem and MacRitchie, 2005). However, the allelic silencing at *Glu-A1* or *Glu-D1* has not been characterized in terms of the effects on polymerization of glutenin during grain filling stage.

Numerous studies aimed at finding statistical relationships between glutenin composition and quality-related parameters (Eagles, Eastwood, Hollamby, Martin, & Cornish, 2004; Eagles et al., 2006; He, Liu, Xia, Liu, & Peña, 2005; Ohm, Ross, Peterson, & Ong, 2008). A series of studies by our laboratory demonstrated that variations of the HMW-GS composition controlled by *Glu-A1*, *Glu-B1* and *Glu-D1* loci give distinguishing rheological properties of wheat dough by affecting micro structure of gluten (Gao et al., 2016; Li et al., 2016; Liu et al., 2016). The contribution of single HMW-GS to gluten micro structure and bread-making quality is worth to verify to better understand the formation of wheat quality. In the previous research, the micro structure of

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Table 1
Quantitative analysis of the proportion of high-molecular-weight glutenin subunit(s) encoded by *Glu-1* loci relative to total HMW-GS and protein component ratios in the four wheat lines.

Group	Line	*Glu-A1* (%)	*Glu-B1* (%)	*Glu-D1* (%)	HMW-GS/LMW-GS ratio	Glutenin/gliadin ratio
I	HMW-A1a	0.00 b	65.85 ± 0.86 a	34.14 ± 0.85 a	0.25 ± 0.01 b	0.56 ± 0.02 b
	HMW-A1p	13.27 ± 0.07 a	58.34 ± 0.41 b	28.42 ± 0.38 b	0.30 ± 0.03 a	0.64 ± 0.03 a
II	HMW-D1a	16.28 ± 1.20 a	66.54 ± 4.6 a	17.18 ± 0.80 b	0.15 ± 0.01 b	0.46 ± 0.01 b
	HMW-D1p	14.60 ± 1.54 b	60.03 ± 5.3 a	25.18 ± 0.30 a	0.27 ± 0.01 a	0.51 ± 0.00 a

Values are expressed as mean ± standard deviation. Results followed by a different letter in the same column within the same group are significantly different ($P < 0.05$).

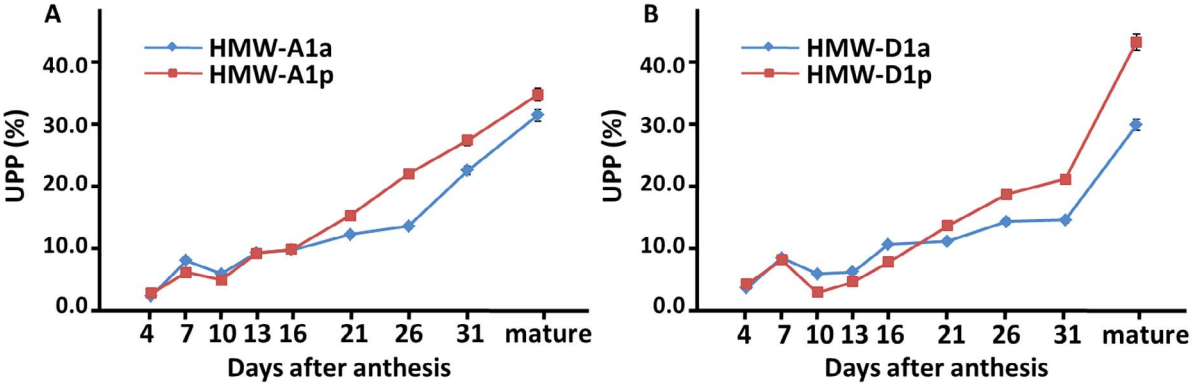

Fig. 1. Percentage unextractable polymeric protein (UPP%) versus days after anthesis for the four wheat lines. Data are present as mean of two years.

gluten and/or dough can only be qualitatively studied by image description (Gao et al., 2016; Landriscina et al., 2017; Wang et al., 2014). A novel method named protein network analysis was reported recently (Bernklau, Lucas, Jekle, & Becker, 2016), allowing quantifying the micro structure of dough or gluten with parameters.

In this research, wheat NILs with an HMW-GS absence/presence at individual *Glu-A1* or *Glu-D1* locus were characterized, to determine the effects of single HMW-GS absence on polymerization of glutenin during grain development, dough mixing properties and micro structure of gluten. The relationship between UPP%, dough mixing properties and novel parameters generated from protein network analysis were determined.

2. Materials and methods

2.1. Plant materials

Four wheat NILs were used in this study. Two NILs with HMW-GS Ax1 absence and presence at *Glu-A1* locus, designated as HMW-A1a and HMW-A1p, were created from a cross between Xinong 1718 (Ax1, Bx7 + By9, Dx2 + Dy12) and Fa 721 (null, Bx7 + By8, Dx5 + Dy10). The other two wheat NILs with HMW-GS Dx2 absence and presence at *Glu-D1* locus, designated as HMW-D1a and HMW-D1p, were created from a cross between Xinong 2208 (Ax1, Bx7 + By9, Dx2 + Dy12) and Fa 746 (null, Bx17 + By18, Dx3 + Dy12). The F_2 progenies were analysed by SDS polyacrylamide gel electrophoresis (SDS–PAGE) to select various HMW-GS compositions, followed by backcrossing with rotational parent (Xinong 1718 or Xinong 2208) for six times. The backcross progeny was detected by SDS-PAGE and the NILs with appropriate composition were selected. The glutenin and gliadin composition of the BC_6F_{11} homozygous NILs were confirmed by SDS–PAGE and acid-PAGE (A-PAGE). The wheat varieties Chinese Spring (null, Bx7 + By8, Dx2 + Dy12) and Lankao Teaizao (Ax1, Bx7 + By9, Dx2 + Dy12), were included as controls to distinguish the HMW-GS composition.

Field experiments were performed at Yangling (108 °4′E, 34 °160′N), Shaanxi Province, China, during 2014–2015 and 2015–2016 growing seasons. Grain materials at different stages after anthesis were collected according to Liu et al. (2016). The mature grains were sun-dried and stored for 60 d before analysis. Grains were tempered at 15% (w/w) moisture for 20 h and milled in a Brabender Quadrumat Senior mill.

2.2. Separation and quantification of gliadin and glutenin

The gliadins were extracted according to the method established in our lab (Gao et al., 2016). The gliadins were separated by 8% A-PAGE according to Yan, Hsam, Yu, Jiang, and Zeller (2003). The glutenins were extracted and subjected to electrophoresis according to the method described by Gao et al. (2012). Discontinuous SDS-PAGE procedure was performed by using a 5% (w/v) stacking gel and a 10% (w/v) separating gel.

Total amount of gliadin and glutenin were calculated using the electrophoresis samples, with the known quantities of albumin from bovine serum as control. The ratio of glutenin to gliadin was calculated. The gliadin and glutenin samples were further separated by reversed phase high-performance liquid chromatography (RP-HPLC) analysis according to the method described by Li et al. (2016). Each sample was analysed in duplicate. Based on the peak area of RP-HPLC traces, proportion of HMW-GS encoded by the three *Glu-1* loci relative to total HMW-GS, and HMW/LMW ratio were calculated.

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

A Diode Array 7200 NIR spectrometer instrument (Perten Instrument AB, Sweden) was applied to determine the protein content, moisture, wet gluten content, and Zeleny sedimentation for grains of the four wheat lines. The wavelength ranged from 950 nm to 1650 nm, and reflectance was set up as data collection mode. Each sample was evaluated in duplicate and each replicate gives a mean evaluation based on the internal Perten calibration.

2.4. Glutenin polymerization analysis during grain development

Size exclusion (SE)-HPLC analysis was conducted to determine UPP % in grains at different stages (4 d, 7 d, 10 d, 13 d, 16 d, 21 d, 26 d, and 31 d after anthesis and mature). The SDS extractable and unextractable proteins were determined according to the method described by Gao

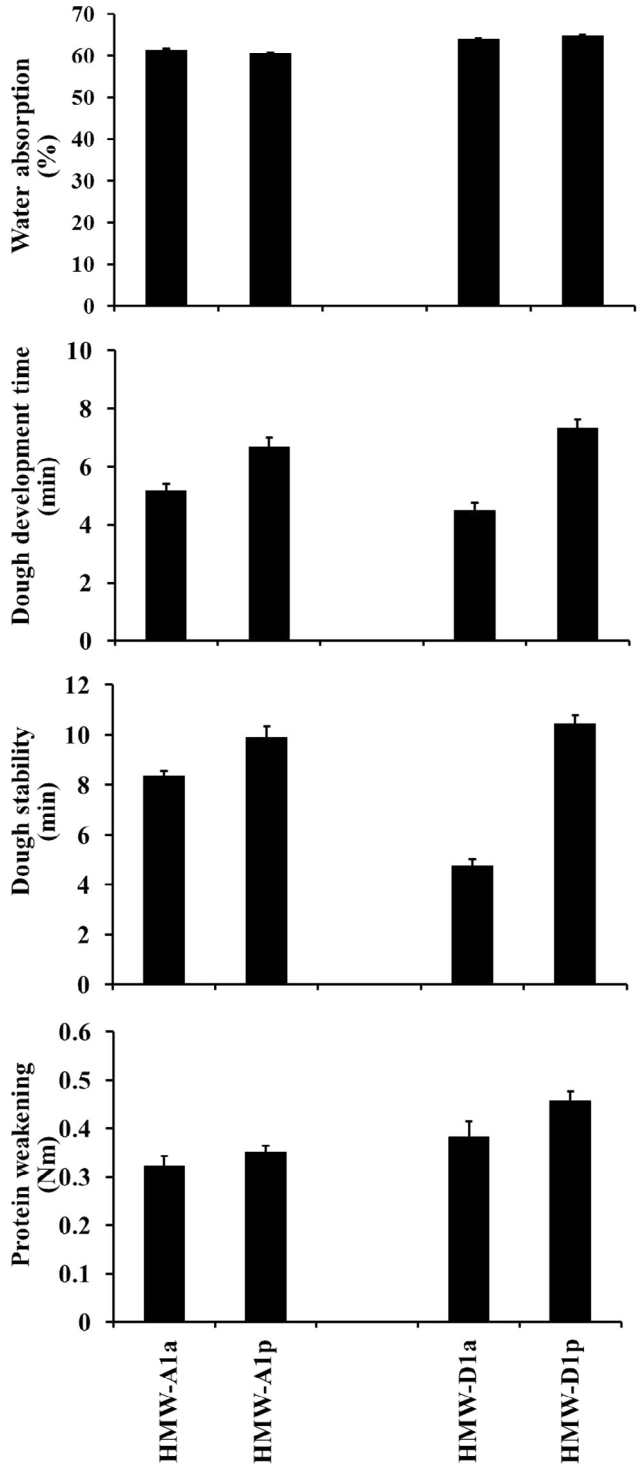

Fig. 2. Dough mixing properties determined by Mixolab. Data are present as mean of two years.

et al. (2012). Each sample was tested in duplicate and UPP% was calculated as the ratio of the peak area for unextractable polymers relative to the total area of both the extractable and unextractable polymers. The dynamic changes of UPP% were analysed by the mean of two-year values at different stages during grain development.

2.5. Dough mixing property analysis

Dough mixing properties were studied using the Mixolab instrument (Chopin, Tripette and Renaud, France), which measures the torque (expressed in Nm) produced by passage of the dough between the two kneading arms under controlled temperature, according to the method described by Rosell, Collar, and Haros (2007). The variables recorded by Mixolab contain water absorption reflecting percentage of water required to get a constant torque of 1.1 Nm, dough development time (min), dough stability (min), and C2 reflecting protein weakening. Each wheat sample was tested in triplicate and the mean values were calculated.

2.6. Preparation of gluten samples

Gluten samples of the four wheat NILs were obtained by hand washing according to the method reported by Gao et al. (2016). The method of preparation of gluten influences the protein structure in products of the gluten (Rasheed et al., 2015). Taking the influence into account, the freshly-washed gluten samples were divided into two lots. One lot was directly kept for micro structure analysis. The other lot of the freshly-washed gluten samples was lyophilized with a freeze dryer for two days and kept for micro structure analysis.

2.7. Micro structure of gluten samples

Micro structure of both freeze-dried and freshly-washed gluten samples of each NIL were observed by scanning electron microscopy (SEM) followed the method described by Liu et al. (2016).

Micro structure of freshly-washed gluten samples was also measured by confocal laser scanning microscopy (CLSM) according to the method reported by Bernklau et al. (2016) with minor modifications. To stain the samples for protein visualization, the freshly-washed gluten samples were soaked in 1 mL of Rhodamine B solution (0.01 g/100 mL water) for 1 h. The gluten samples were transferred to an object carrier and sealed with a cover glass. Samples were analysed by an Olympus IX83 inverted microscope with FLUOVIEW FV1200 biological confocal laser scanning system (Olympus, Tokyo, Japan) with a semiconductor laser LD559 and an objective lens (UPlanSApo 20×, NA = 0.75). Ten images of each sample were taken with a resolution of 800 × 800 pixel and a size of 635 × 635 μm.

2.8. CLSM image processing and analysis

The CLSM images were analysed with a software, AngioTool64 version 0.6a (National cancer institute, National institute of health, Maryland, USA), which is usually applied in medical science for image analysis to identify the variations in vascular networks (Zudaire, Gambardella, Kurcz, & Vermeren, 2011). According to a recent study (Bernklau et al., 2016), the gluten micro structure can be quantified with several parameters, such as protein area, protein junctions, junctions density, protein end-points, lacunarity, branching rate and end-point rate.

2.9. Statistical analyses

The statistical analyses were performed using SPSS (version 22.0, SPSS Inc., Chicago, IL, USA). All data were subject to one-way analysis of variance (ANOVA) and significant differences ($p < 0.05$) among the investigated parameters were calculated using Duncan's multiple range tests. Pearson's correlation coefficients were calculated to determine the relationship between UPP%, dough mixing properties and parameters related to gluten networks.

3. Results and discussion

3.1. Absence of HMW-GS encoded by Glu-A1 or Glu-D1

The gliadins and glutenins of the four wheat lines were separated on A-PAGE and SDS-PAGE, respectively (Supplementary Fig. S1). It is

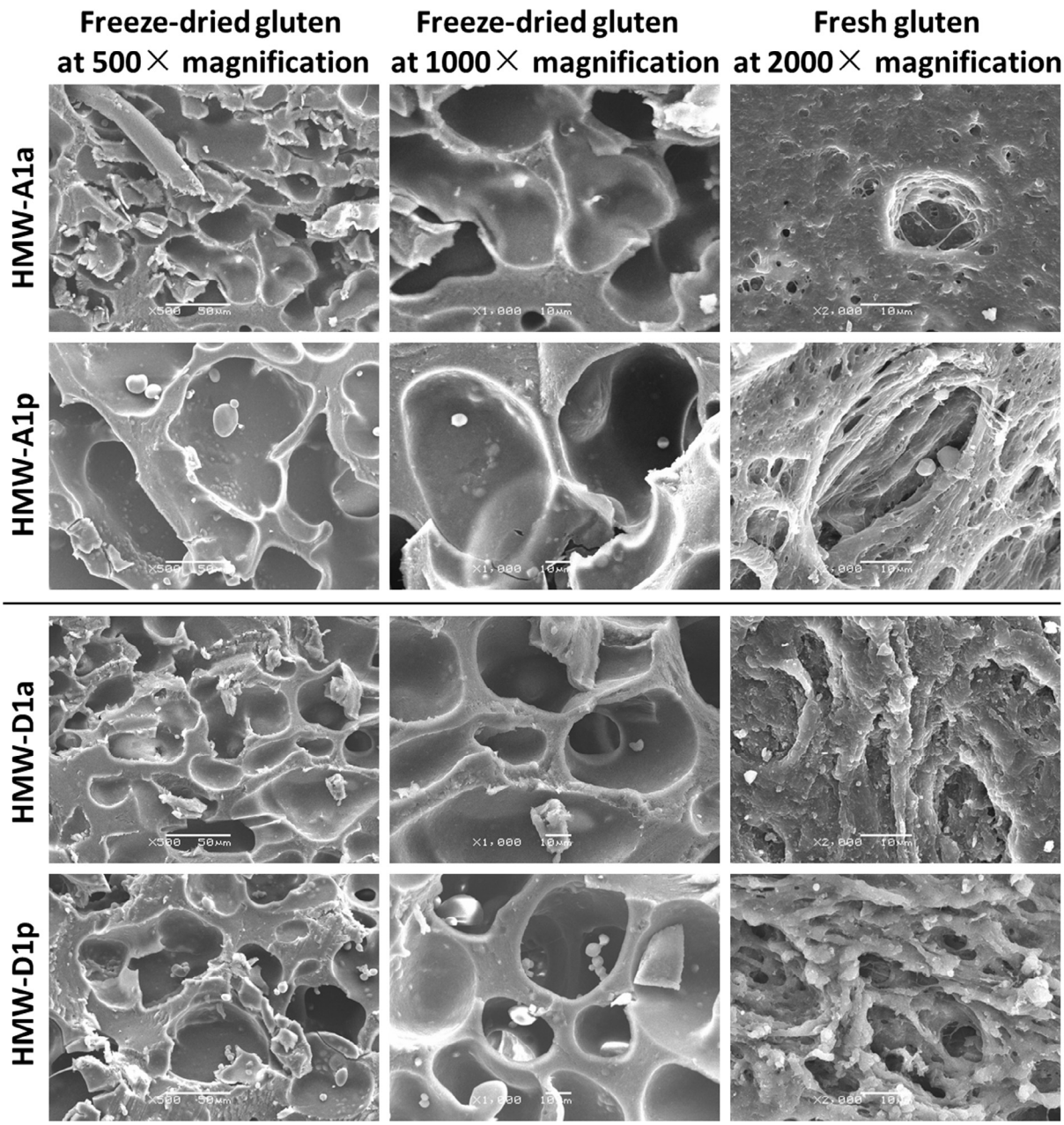

Fig. 3. Scanning electron microscopy images of freeze-dried and freshly washed gluten samples of the four wheat lines. Images in the left and middle columns show the micro structures of the freeze-dried gluten samples at 500× and 1000× magnification, respectively. Images in the right column show the micro structures of the freshly-washed gluten samples at 2000× magnification.

shown that the HMW-GS composition of HMW-A1a (null, Bx7 + By8, Dx2 + Dy12) differs from that of HMW-A1p (Ax1, Bx7 + By8, Dx2 + Dy12) by the absence of Ax1 subunit, and the difference between HMW-GS composition of HMW-D1a (Ax1, Bx7 + By9, Dy12) and HMW-D1p (Ax1, Bx7 + By9, Dx2 + Dy12) is the absence/presence of Dx2 subunit. The gliadins and LMW-GS of wheat lines were further illustrated by RP-HPLC. In agreement with the results of A-PAGE and SDS-PAGE analyses, the RP-HPLC results demonstrated HMW-A1a and HMW-A1p have the identical elution profiles for the gliadins and LWM-GS composition. Furthermore, the gliadins and LMW-GS of HMW-D1a and HMW-D1p are identical (Supplementary Figs. S2 and S3). Based on the identical background between wheat lines, HMW-A1a and HMW-A1p were designated as Group I, and HMW-D1a and HMW-D1p were designated as Group II. As gluten contains various protein subunits, which have cooperative effect on the end-use quality of wheat dough (Delcour et al., 2012; Wieser, 2007), the identical background of gliadins and LMW-GS enable to study the influence of HMW-GS composition on the formation of wheat quality. Many allelic variations of HMW-GS have been identified at the *Glu-A1* and *Glu-D1* loci (McIntosh et al., 2012). As two genes encoding HMW-GS link together at each locus, the previous studies mainly focused on the effects of HMW-GS composition at different loci on wheat processing quality (Gao et al., 2016; Jin et al., 2013; Li et al., 2016; Liu et al., 2016). The influence of few individual HMW-GS (Ax1, Dx5 and Dy10) on dough rheological properties was studied by using transgenic wheat lines (León et al., 2009). In the present research, the effects of absence of single subunit Ax1 or Dx2 on glutenin polymerization and gluten structures were studied.

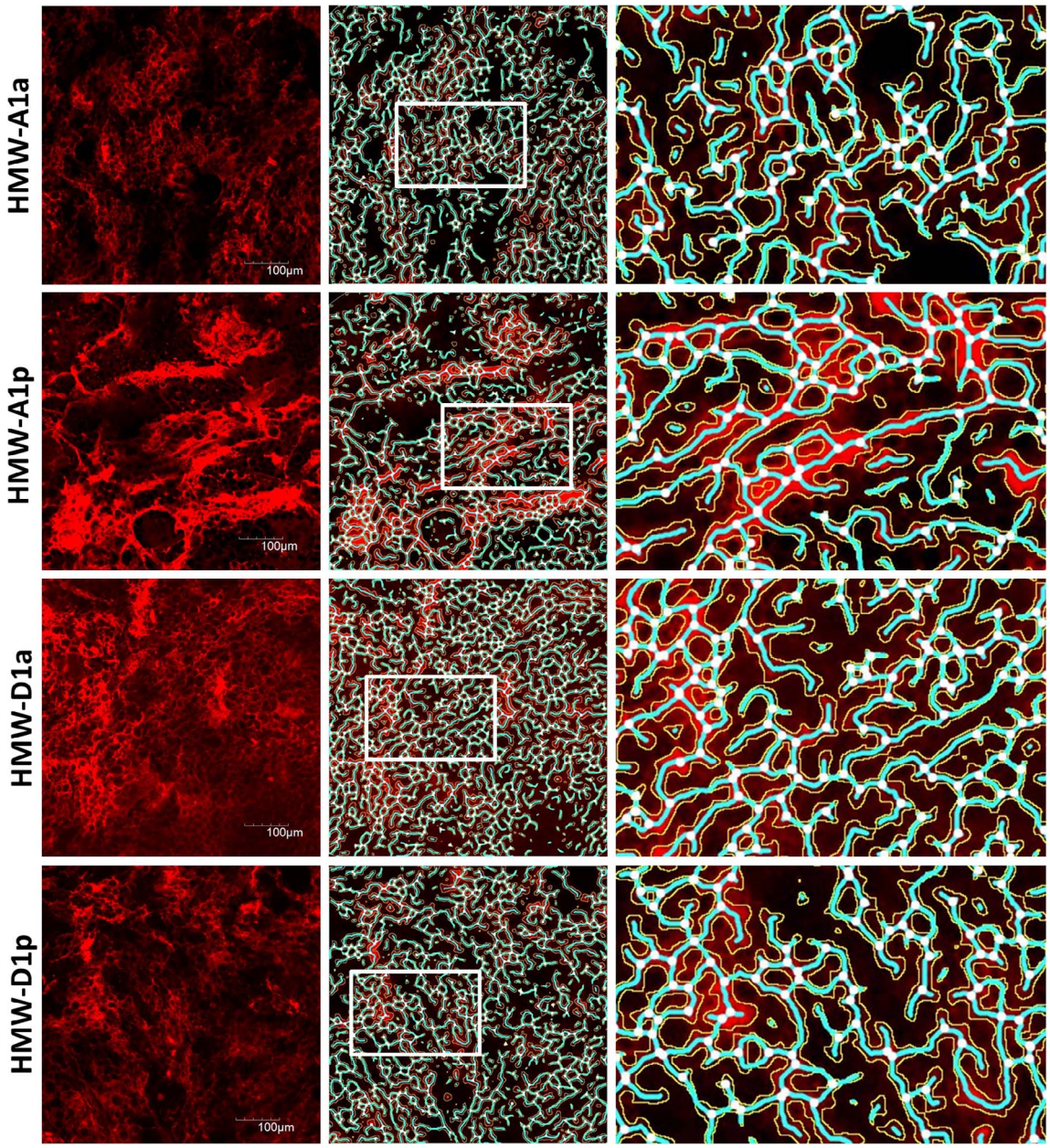

Fig. 4. Protein network analysis of gluten samples of the four wheat lines. The gluten samples were stained with Rhodamin B to visualize proteins and analysed by confocal laser scanning microscopy (CLSM). The images in the left column are the original CLSM pictures, with the scale bar of 100 μm. The images in the middle column are the pictures processed with AngioTool. The images in the right column are the enlarged areas selected from the processed images highlighted with the white boxes, with junctions shown in white, protein skeleton shown in white and protein outline/area shown in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Changes in the proportion of HMW-GS relative to the total protein and proportion of HMW-GS encoded by individual locus relative to the total HMW-GS

Results of NIR analysis (Supplementary Table S1) showed that there is no significant difference in moisture and protein content, but it is significantly different in wet gluten and Zeleny sedimentation value between HMW-A1a and HMW-A1p. HMW-D1a is only significantly different from HMW-D1p in Zeleny sedimentation value. Compared to HMW-A1p and HMW-D1p, HMW-A1a and HMW-D1a have lower ratios of HMW-GS/LMW-GS and glutenin/gliadin (Table 1), respectively, which indicated that the absence of individual subunit caused the decrease of total HMW-GS amount, referring to the similar protein content within each group. The expression levels of HMW-GS encoded by each locus relative to the total HMW-GS show significant difference between wheat lines within each group (Table 1). Because of the absence of Ax1 subunit, the proportion of HMW-GS encoded by *Glu-B1* and *Glu-D1* were dramatically increased from 58.34% to 65.85% and from 28.42% to 34.14%, respectively. The absence of Dx2 subunit caused a decrease of HMW-GS encoded by *Glu-D1*, from 25.18% to 17.18%, and thus it caused an increase of HMW-GS encoded by both *Glu-A1* and *Glu-B1*, from 14.60% to 16.28% and from 60.03% to 66.54%, respectively. It has been well accepted that the HMW-GS/LMW-GS and glutenin/gliadin ratios in gluten are significantly relative to the rheological properties of wheat dough (Wieser & Kieffer, 2001). It can be indicated from the ratios tested in this study that the absence of either Ax1 or Dx2 has negative effects on dough strength, which is consistent with the result of Zeleny sedimentation value, a dough strength indicator, evaluated by NIR analysis. It has been also suggested that the expression level of HMW-GS affects dough strength (Vawser & Cornish, 2004). For the case in this study, the absence Ax1 or Dx2 caused significant increase of HMW-GS encoded by the other two loci, but the increase

Table 2
Parameters determined by AngioTool reflecting the protein network of gluten in the four wheat lines.

Group	Line	Protein area (μm^2)	Protein percentage area (%)	Protein junctions	Junction density ($\times 10^{-3}$)	Total protein length (μm)	Protein end-points	Lacunarity ($\times 10^{-2}$)	Branching rate ($\times 10^{-2}$)	End-point rate ($\times 10^{-3}$)
I	HMW-A1a	375320.8 ± 3215.4 a	59.40 ± 2.51 a	3026.9 ± 26.4 b	4.8 ± 0.3 b	63380.91 ± 3281.0 a	1028.57 ± 31.9 a	4.2 ± 0.2 a	0.8 ± 0.0 b	2.7 ± 0.0 a
	HMW-A1p	355682.1 ± 5281.0 b	59.52 + 2.29 a	3594.9 ± 52.1 a	6.0 ± 0.1 a	62664.65 ± 2662.3 a	966.31 ± 21.3 b	3.8 ± 0.1 b	1.0 ± 0.1 a	2.7 ± 0.1 a
II	HMW-D1a	395550.3 ± 7859.2 a	62.64 ± 1.23 a	3570.0 ± 26.3 b	5.7 ± 0.1 b	67478.37 ± 2114.3 a	1024.76 ± 21.4 a	5.1 ± 0.3 a	0.9 ± 0.0 b	2.6 ± 0.0 a
	HMW-D1p	374881.2 ± 6851.9 b	59.13 ± 2.22 a	3762.5 ± 44.2 a	5.9 ± 0.0 a	66272.85 ± 1326.1 a	942.72 ± 19.6 b	3.0 ± 0.1 b	1.0 ± 0.0 a	2.5 ± 0.1 a

Values are expressed as mean ± standard deviation. Results followed by a different letter in the same column within the same group are significantly different ($P < 0.05$).

rate of HMW-GS encoded by each locus seemed to be random.

3.3. Dynamic changes of UPP% during grain development

The effects of HMW-GS absence on glutenin polymerization during grain development are shown by the mean of values in the two years (Fig. 1). It shows an increase trend indicating the UPP accumulation during grain development in all the four lines, except a slight decrease from 7 d to 10 d after anthesis. This could be explained by the faster accumulation of EPP than that of UPP during this period. There was a rapid UPP% accumulation period for HMW-A1p from 16 d after anthesis to mature. However, for HMW-A1a, the rapid UPP% accumulation period started from 26 d after anthesis and for HMW-D1a and HMW-D1p, the fastest accumulation of UPP% was gained from 31 d after anthesis to mature, but the increase rate of UPP% in HMW-D1p is higher than that in HMW-D1a from 10 d after anthesis (Fig. 1). It can be indicated that the absence of either Ax1 or Dx2 decreased the accumulation rate at the crucial period for the formation of glutenin polymers, and thus decreased the glutenin polymerization and dough strength at mature stage. The results suggested that the absence of HMW-GS had negative impact on the timing of polymerization, accumulation rate of UPP% and the UPP% at maturity, which was consistent with the previous studies demonstrating that wheat lines with alleles related to strong dough start to form larger polymers several days earlier than those with weak dough alleles (Liu et al., 2016; Naeem et al., 2005). The decrease rate of UPP% at mature stage caused by the absence of Ax1 and Dx2 were 9.25% and 29.2%, respectively, which may suggest that the absence of Dx2 has more negative effect on dough strength. This could be explained by the previous studies suggesting that the variations of *Glu-D1* may have more impact on dough properties than those of *Glu-A1* (Liu et al., 2005).

3.4. Dough mixing properties of wheat NILs

Dough mixing properties reflecting gluten strength were measured by Mixolab (Fig. 2). The results showed that the absence of either Ax1 or Dx2 subunit significantly reduces dough development time and dough stability, which indicated that the dough strength of HMW-D1p was the strongest, followed by that of HMW-A1p, HMW-A1a and HMW-D1a. The prediction of processing quality of dough based on dough mixing properties is consistent with the result of UPP% in this study, which both confirmed the absence of either Ax1 or Dx2 subunit has a negative effect on the formation of wheat quality. No significant difference is found in protein weakening between HMW-A1a and HMW-A1p. However, compared to HMW-D1p, HMW-D1a has lower value for protein weakening. Given that protein weakening is positively correlated with SDS sedimentation volume, gluten index, dough extensibility and loaf volume (Dhaka, Gulia, & Khatkar, 2012), it can be predicted that, compared to Ax1, the absence of Dx2 would have more effect on protein reduction and thus dough processing quality.

3.5. Observation and quantitative analysis of micro structure of gluten

The micro structures of freeze-dried and freshly-washed gluten from the four wheat lines using SEM are shown in Fig. 3. Compared to HMW-A1p and HMW-D1p, HMW-A1a and HMW-D1a exhibit more uniform apertures, respectively, according to the images taken from the freeze-dried gluten at 500× and 1000× magnification. HMW-A1a and HMW-D1a show laminar gluten network, while HMW-A1p and HMW-D1p show more fibrous structures, according to the images taken from the freshly-washed gluten samples. It has been accepted that dough with strong strength exhibited apertures with various sizes and heterogeneous distribution in the gluten structure (Kaur, Singh, Kaur, Ahlawat, & Singh, 2014; Liu et al., 2016), which can be embedded with different types of starch granules with different size (Shevkani, Singh, Bajaj, & Kaur, 2017). The absence of either Ax1 or Dx2 increased the

uniformity, which suggested the capacity of embedding starch granules with different size could be decreased in the gluten micro structures of HMW-A1a and HMW-D1a.

The freshly-washed gluten samples were further analysed with CLSM (Fig. 4) and the images analysed with 'AngioTool' allowed quantitative analysis on the micro structures of gluten networks (Table 2). The results showed that, no difference was found in the protein percentage area, total protein length and end-point rate between the two NILs within each group. Compared to HMW-A1p and HMW-D1p within each group, either HMW-A1a or HMW-D1a exhibits significantly lower values in protein junctions, junction density and branching rate, respectively. Referring to the definition of the variables (Bernklau et al., 2016), junction density and branching rate are derived from protein junctions. These results indicated that less protein junctions formed in the gluten network because of the absence of Ax1 or Dx2 subunit, reflecting less cross linkage formed in the gluten network. However, HMW-A1a and HMW-D1a have significantly higher values in protein area, protein end-points and lacunarity. Higher protein area indicates less aperture area, reflecting the connectivity of the gluten network. Protein end-points describe the open-ended protein threads, which was suggested to be used as an indicator for cohesion of a network (Bernklau et al., 2016). Based on the results of protein end-points, it can be indicated that the absence of Ax1 or Dx2 decreased the viscosity of wheat gluten. The result of lacunarity showed that the gluten network of HMW-A1a and HMW-D1a were more uniform than those of HMW-A1p and HMW-D1p, respectively, which is agreed with the result observed by SEM. In the studies referring to wheat dough mixing properties, the protein network has been regarded as an important aspect reflecting dough processing quality, and micro structures of gluten have been analysed by different microscopy systems (Beck, Jekle, & Becker, 2012; Ferrer, Gómez, Añón, & Puppo, 2011; Gao et al., 2016; Kuktaite et al., 2011; Wang et al., 2014). However, the microscopy images have been rarely quantitatively analysed. The computational tool 'AngioTool' implements quantitative analysis of the gluten network and allows to comparing the micro structures of different gluten samples with parameters, such as protein junctions, lacunarity, protein end-points and branching rate. The protein network analysis can be further validated and used to predict dough processing properties. As gluten proteins provide a framework filled with starch granules, size distribution of both starch granules and protein polymers affect each other and finally co-affect dough properties (Delcour et al., 2012; Shevkani et al., 2017). Lacunarity of gluten network, indirectly reflecting the size distribution of starch granules, could be further applied as indicators of dough properties.

3.6. Correlation analysis of UPP%, dough mixing properties, and the parameters of protein network analysis

Correlation coefficients between UPP%, dough mixing properties and the parameters of protein network analysis were calculated and listed in Table S2. According to the definitions of parameters given by Bernklau et al. (2016), junction density and branching rate were calculated by the number of protein junctions divided by explant area and protein area, respectively. It was not surprising to find junction density is significantly correlated with protein junctions and branching rate. However, it was found that lacunarity is positively correlated with UPP % ($r = 0.959$), dough development time ($r = 0.953$) and dough stability ($r = 0.925$). Given that the previous studies suggested UPP% as an indicator of glutenin polymer assembly (Ferrise et al., 2015), and dough development time and dough stability as indicators of gluten strength (Singh et al., 2016), we propose that lacunarity derived from the protein network analysis can be used as an indicator reflecting the protein network structure and thus wheat dough mixing properties. It was also found that protein end-points is negatively correlated with dough development time ($r = -0.969$), and end point rate is significantly negatively correlated with water absorption ($r = -0.950$) and protein weakening ($r = -0.970$). We propose that protein end-points and end-point rate could be used as potential parameters to indicate dough property on protein network level. Future work needs to be conducted on more wheat materials to validate the proper parameters as wheat quality indicators.

4. Conclusion

Two groups of wheat NILs with identical genetic background allow to comparing the differences caused by either Ax1 or Dx2 subunit in glutenin polymerization during grain development, dough mixing properties and micro structure of gluten. We verified that the absence of either Ax1 or Dx2 slow down the process of glutenin polymerization during grain development, and decreased UPP% in mature grain, indicating weak dough strength, which were further confirmed by the dough mixing property analysis. The quantitative protein network analysis enable to quantify the micro structure of wheat gluten with several parameters, of which are positively correlated with UPP% and dough mixing properties. Lacunarity, derived from quantitative analysis of protein network, may be used as new indicator for wheat quality.

Conflict of interest

The authors declare no competing financial interest.

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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.2017.07.165.

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