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Characterization of agronomic and quality traits and HSW-G5 compositions from the progenies of common wheat (*Triticum aestivum* L.) with different protein content

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ABSTRACT. High molecular weight glutenin subunits (HMW-GS) play an essential role in wheat processing quality. In this study, we evaluated the genetic pattern with HMW-GS composition between generations and examined whether agronomic and quality traits were correlated with each other. A wheat (*Triticum aestivum* L.) cultivar with high protein content and 2 cultivars with low protein content were subjected to a reciprocal cross. Sixteen agronomic and 4 quality characteristics were investigated. A total of 216 seeds from each F2 generation were chosen randomly and analyzed for HMW-GS composition using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Agronomic and quality characteristics were not significantly different

between reciprocal crosses, indicating no cytoplasmic effect on the characteristics studied. The separation ratio of 2 HMW-GS loci was 9:3:3:1, indicating no linkage between any 2 loci. The novel HMW-GS N was detected in cultivar R145, which did not follow the Mendelian segregation ratio. A Glu-A1a(1) band was not detected in 1 individual from Tian8901xR145. Average grain weight per spike was significantly correlated with quality characteristics and may be a suitable criterion for selecting high protein content in wheat breeding programs.

Key words: Common wheat; High-molecular weight glutenin subunit; Quality; Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the oldest cultivated crops in world and an important crop that offers substantial protein to human beings. Grain protein has a significant influence on processing quality, such as dough rheological properties, baking quality, and end-product attributes (Buck et al., 2007). The level of global protein yield in wheat has remained relatively stable, despite an increase in productivity of this crop (Zlatska, 2005). Wheat quality improvement is a major challenge for many breeding programs.

Wheat flour proteins can be classified as albumin, globulin, prolamin, or glutenin (Du-Pont et al., 2005). Glutenin, a major class of storage proteins in the wheat endosperm, is classed into 2 groups: high molecular weight (HMW) glutenin and low molecular weight glutenin (LMW) (Payne et al., 1984). The HMW-glutenin subunits (GS) of common wheat are encoded by the Glu-A1, Glu-B1, and Glu-D1 loci on the long arms of homoeologous chromosome 1, each locus contains 2 tightly linked genes corresponding to a subunit of HMW and a subunit of LMW, termed x-type and y-type, respectively (Payne et al., 1980; Lawrence and Shepherd, 1981). The HMW-GS are particularly important for determining dough elasticity; the presence of specific HMW subunits is positively correlated with good bread-making quality (Anjum et al., 2007).

Since last century, great effort has been invested in identifying genetic information on HMW-GS and the relationships between quality traits and agronomic traits in order to predict bread wheat quality and provide theoretical guidance for wheat quality breeding (MacRitchie et al., 1990; Weegels et al., 1996). Alveograph and baking tests are labor-intensive and require large quantities of flour (Oury et al., 2010). Thus, agronomic characteristics that are correlated with quality characteristics offer indirect information for predicting wheat grain quality during early generations of the breeding process, although quality characteristics are significantly affected by environmental conditions (Noorka et al., 2009).

The objectives of this study were to investigate whether cytoplasm affects HMW-GS composition in progeny, as well as the relationships between agronomic characteristics and quality characteristics.

MATERIAL AND METHODS

Plant materials and progeny production

Three common wheat cultivars, differing significantly in protein content (protein con-

tent was high in 1 cultivar and low in the other 2), were used in this study (Table 1). Reciprocal crosses were made between the high protein content parent and 2 low protein content parents in 2007. The 4 resulting F1 hybrids were planted in Kungming, Yunnan, China and self-pollinated to produce F2 generations. Three parents, their F1 hybrids, and F2 generations were sown into the experimental field of Huazhong Agricultural University in Wuhan, Hubei, China, with rows that were 1 m long and 20 cm wide, with 10 plants in each row. Seeds were harvested from a single plant in May 2008.

Five plants from each parent and F1 hybrids and all plants from the F2 generations were investigated for the agronomic and quality characteristics.

Agronomic characteristic evaluation

Sixteen agronomic characteristics were investigated in both parents and F1 generations including plant height (excluding awns), main spike length, length from the main spike to flag leaf pillow, length of the first internode, number of effective spikes (spike with more than 5 grains), number spikelets of the main spike, number of grains per plant, grain weight per plant, average grain weight per spike, 1000-kernel weight, flag leaf length, flag leaf width, area of flag leaf (leaf width x leaf length x 0.83), photosynthesis rate, stomatal conductance, and transpiration rate. Ten characteristics were investigated in the F2 generation, including plant height, main spike length, length from the main spike to flag leaf pillow, length of the first internode, number of effective spikes, number spikelets on the main spike, number of grains per plant, grain weight per plant, average grain weight per spike, and 1000-kernel weight.

Photosynthesis rate, stomatal conductance, and transpiration rate were determined using the Li-6400 Photosynthesis System (Li-Cor Biosiences, Lincoln, NE, USA; photosynthetically active radiation was set to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during the time from anthesis to grain filling.

Quality evaluation

Total protein, starch, and wet gluten content as well as Zeleny sedimentation value were determined using the Infratec™ 1241 Grain Analyzer (FOSS, Eden Prairie, MN, USA) after harvest. The scanning temperature was controlled from 21°-25°C. The absorption wavelength range of the samples was 850-1050 nm.

Extraction and identification of HMW-GS

A total of 216 seeds were randomly selected from each of the 4 F2 generations. Each seed was cut in half. The embryo-less half of the kernel from the F2 progeny was wrapped in filter paper and crushed using a hammer.

The HMW-GS extraction was prepared according to Liu et al. (2005) with the following modifications: whole meal was weighed in a 1.5-mL centrifuge tube and extracted in 1 mL 50% 2-propanol (v/v) for 5 min under continuous vortex mixing, followed by incubation for 20 min at 65°C, vortexing for 5 min, and centrifugation for 5 min at 1100 rpm. The supernatant was discarded. This procedure was repeated 3 times to remove the gliadin proteins. Solution B1 (2% dithiothreitol, 40% Tris-HCl, 50% 2-propanol, 2% sodium dodecyl sulfate (SDS), pH 8.0) was added to the residue at a 1 mg/5 µL ratio. The mixture was incubated for 30 min at 65°C. Equal amounts of solution B2 (1.4% 4-vinylpyridine, 40 mL Tris-HCl, 50% 2-propanol,

2% SDS, pH 8.0) was added to the mixture, followed by incubation for 15 min at 65°C. After 5 min vortexing and 4000 rpm centrifugation, a 100 µL supernatant was mixed into a 100 µL extraction solution (2% SDS, 6.25 mL Tris-HCl, 44% glycerol, 0.05% bromophenol blue, 5% β-mercaptoethanol, pH 6.8) and incubated at 100°C for 5 min, followed by centrifugation for 10 min at 4000 rpm.

The HMW-GS extraction was analyzed using 12% SDS-PAGE electrophoresis. Chinese spring (7+8, 2+12) was used as a control. PageRuler unstained protein ladder (#SM0661, Fermentas, Vilnius, Lithuania) was used as the molecular weight marker. Bovine serum albumin was also used to determine the concentrations of the subunits. HMW-GS were identified based on the numbering system developed by Payne and Lawrence (1983). Gels were scanned using the AlphaImager EP (NatureGene Corp., Beijing, China).

Statistical analysis

The data was analyzed using SAS System for Windows v6.12 (SAS Institute Inc., Cary, NC, USA) and the Excel software. Correlations between the agronomic and quality characteristics were determined based on Pearson's correlation coefficient values.

RESULTS

Agronomic characteristics and quality characteristics of parents and F1 hybrids

The 3 parents varied significantly in the 7 characteristics studied, including plant height, main spike length, number of grains per plant, grain weight per plant, average grain weight per spike, width of flag leaf, and length of flag leaf ($P \leq 0.01$). Quality characteristics of the 3 parents also significantly differed ($P \leq 0.01$) (Table 1). No significant difference between F1 reciprocal crosses was found for the agronomic or quality characteristics.

Table 1. Origins, quality characteristics, and HMW-GS compositions of Tian8901, R145, and E51125.

Name	Origin	HMW-GS composition	Means ± SD			
			Protein content (%)**	Starch content (%)**	Wet gluten content (%)**	Zeleny sedimentation value (mL)**
Tian8901	Tianjing, China	1.7 + 8.5 + 10	15.26 ± 0.9343[a]	52.963 ± 1.2863[a]	29.1328 ± 1.3583[a]	46.1585 ± 2.060[a]
R145	France	1.14 + 15.2 + 12.N	11.56 ± 0.4879[b]	57.7212 ± 1.2707[b]	24.5309 ± 0.7353[b]	32.4448 ± 1.6536[b]
E51125	Hubei, China	7 + 9,5 + 10	11.12 ± 0.2168[b]	58.6634 ± 0.9032[b]	24.4008 ± 0.3374[b]	32.2592 ± 2.1621[b]

Means with the same letter were not significantly different **Significance at P = 0.01 level of probability, respectively.

Correlation analyses of quality and agronomic characteristics

Starch was negatively correlated with protein, wet gluten, and Zeleny sedimentation value. Positive correlations among protein, wet gluten, and Zeleny were detected with coefficients higher than 0.9 ($\alpha = 0.01$, data not shown). A significant correlation was observed between quality characteristics in all 4 F2 crosses.

Correlation analysis between agronomic characteristics and quality characteristics showed that most agronomic characteristics were negatively correlated with protein content.

Plant height, main spike length, length from the main spike to flag leaf pillow, length of the first internode, number of grains per plant, grain weight per plant, average grain weight per spike, and 1000-kernel weight were negatively correlated with protein content, wet gluten content, and Zeleny sedimentation value, but were positively correlated with starch content. Main spike length, number of grains per plant, and number of effective spikes were positively correlated with protein content, wet gluten content, and Zeleny sedimentation value, but negatively correlated with starch content. Of these characteristics, average grain weight per spike was significantly correlated with quality characteristics at the level of 0.001.

HMW-GS composition

Parents and F1 hybrids

The high protein content cultivar Tian8901 was reciprocally crossed with 2 low protein content cultivars, R145 and E51125. The F1 hybrids and 216 seeds from each F1 cross were analyzed by SDS-PAGE. The HMW-GS compositions of the 3 parents are shown in Table 1. A novel HMW-GS was detected in cultivar R145, with a molecular weight of 83 kD, which was referred to as N (Figure 1, lane 3). N showed 2 closely linked bands on 12% SDS-PAGE.

The HMW-GS compositions of F1 hybrids were also analyzed. The results revealed that the HMW-GS bands from both parents expressed in the F1 hybrids. The subunits in the F1 hybrids were genetically codominant in all 4 crosses and showed a dosage effect. The bands of the same subunit differed in the F1 hybrids between reciprocal crosses. The bands from female parents were darker than those from male parents, indicating that the effect of the subunit from female parents was higher than that from male parents (Figure 1). This result was consistent with those of Uhlen and Ringlund (1987).

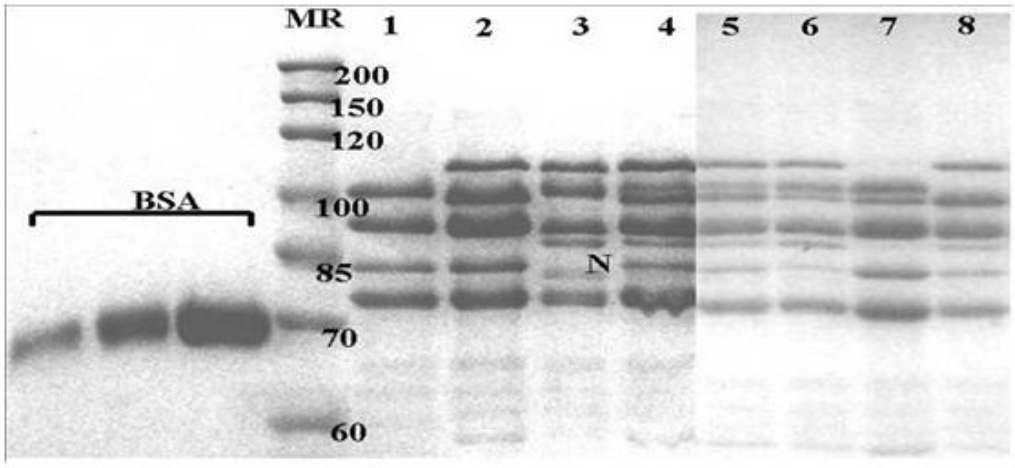

Figure 1. High molecular weight glutenin subunit was detected in the progeny of Tian8901xR145. BSA: Bovine serum albumin, *lane MR*: Protein molecular marker, *lane 1*: Chinese spring, *lane 2*: Tian8901, *lane 3*: R145, *lane 4*: Tian8901xR145 F1 hybrid, *lanes 5-8*: Tian8901xR145 F2 progenies, the sample in *lane 7* missing Glu-A1a(1) band, N: high molecular weight glutenin subunit N.

HMW-GS composition of F2 generations

Nine types of HMW-GS composition were observed in Tian8901xR145 and R145x-Tian8901 crosses, and 6 types in Tian8901xE51125 and E51125xTian8901 crosses. Their subunit compositions and percentage are listed in Table 2.

The ratio of present and absent for each HMW-GS bands was close to 3:1 in all F2 populations, including Glu-A1a(1), Glu-D1a(2+12), Glu-D1d(5+10), Glu-B1b(7+8), Glu-B1h(14+15), and Glu-B1c(7+9). No significant difference was observed between reciprocal crosses for each HMW-GS band ($P > 0.05$) (Table 3). The HMW-GS N in cultivar R145 did not fit Mendelian segregation ratio in the Tian8901xR145 and R145xTian8901 F2 generations. One individual from the Tian8901xR145 population did not show the Glu-A1a(1) band (Figure 1). The ratio of any 2 HMW-GS loci segregation were as follows 9:3:3:1 ratio, indicating no linkage between any 2 loci. The ratio of female parent type, heterozygous type, and male parent type was 1:14:1 in the Tian8901xR145 and R145x-Tian8901 crosses, while the ratio was 3:12:1 in the Tian8901xE51125 cross and 1:12:3 in the E51125xTian8901 cross.

Table 2. Subunit compositions and percentage in 4 crosses.

Subunit composition				Percentage (%)	
				Tian8901xR145	R145xTian8901
2 + 12	5 + 10	7 + 8	14 + 15	25.93	26.39
	5 + 10	7 + 8	14 + 15	13.43	12.04
2 + 12		7 + 8	14 + 15	10.19	9.26
2 + 12	5 + 10		14 + 15	12.96	15.74
2 + 12	5 + 10	7 + 8		11.57	13.43
	5 + 10		14 + 15	11.11	6.02
	5 + 10	7 + 8		5.56	5.09
2 + 12			14 + 15	5.56	6.02
2 + 12		7 + 8		3.7	6.02
				Tian8901xE51125	E51125xTian8901
5 + 10		7 + 8	7 + 9	41.2	37.96
5 + 10		7 + 8	7 + 9	10.19	10.65
5 + 10			7 + 9	16.67	18.98
5 + 10		7 + 8		17.13	21.3
5 + 10			7 + 9	7.41	7.87
5 + 10		7 + 8		7.41	3.24

Table 3. Presence and absence of separating subunits in different crosses.

Subunit	Present		Absent		Chi-square	
	Tian8901xR145	R145xTian8901	Tian8901xR145	R145xTian8901	Tian8901xR145	R145xTian8901
2+12	152	166	64	50	2.228395	0.302469
5+10	173	170	43	46	2.722222	1.388889
7+8	152	156	64	60	2.228395	0.746914
14+15	171	163	45	53	1.783951	0.006173
	Tian8901xE51125	E51125xTian8901	Tian8901xE51125	E51125xTian8901	Tian8901xE51125	E51125xTian8901
1	162	169	54	47	0.006173	1.04321
8	164	158	52	58	0.055556	0.302469
9	163	163	53	53	0.006173	0.006173

DISCUSSION

HMW-GS composition

In the present study, HMW-GS bands from both parents of each cross were completely expressed in the F1 hybrids. The band density of the same subunit differed in the F1 hybrids between reciprocal crosses. Those from female parents were darker than those from male parents, indicating that the maternal parent had a greater effect on the expression of HMW-GS in F1 hybrids than the paternal parent (Figure 1, 2, 3, and 4). This is because the sperm fuses with 2 polar nuclei to form a triploid (3n) nucleus known as the primary endosperm nucleus during double fertilization. F1 hybrid subunits were genetically codominant. HMW-GS are encoded by loci on the long arms of homoeologous group 1 chromosomes (Payne et al., 1980; Galili and Feldman, 1984), while there were some new loci reported to be the regulators of the HMW glutenins (Wanous et al., 2003; Jin et al., 2009; Storlie et al., 2009). In the present study, segregation of HMW-GS loci in 4 crosses followed the Mendelian law of independent segregation, suggesting no linkage between any 2 loci. Our results corresponded well with the previous hypothesis that HMW-GS genes exhibit simple co-dominant Mendelian inheritance (Payne, 1987; Payne et al., 1981, 1987).

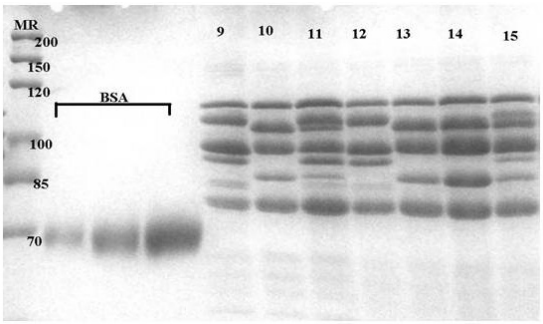

Figure 2. High molecular weight glutenin subunit was detected in the progeny of R145xTian8901.BSA: Bovine serum albumin, *lane MR*: Protein molecular marker, *lane 9*: R145, *lane 10*: Tian8901, *lane 11*: F1 R145xTian8901 hybrid, *lanes 12-15*: R145xTian8901 F2 progenies.

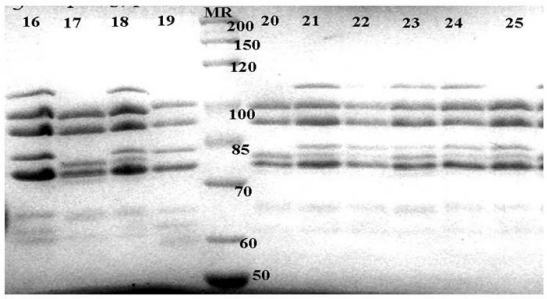

Figure 3. High molecular weight glutenin subunit was detected in the progeny of Tian8901xE51125. BSA: Bovine serum albumin, *lane MR*: protein molecular marker, *lane 16*: Tian8901, *lane 17*: E51125, *lane 18*: Tian8901xE51125 F1 hybrid, *lane 19*: Chinese spring, *lanes 20-25*: Tian8901xE51125 F2 progenies.

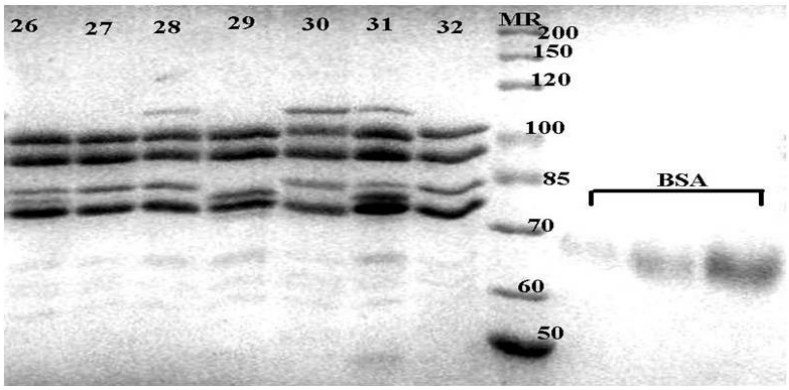

Figure 4. High molecular weight glutenin subunit was detected in the progeny of E51125xTian8901. BSA: Bovine serum albumin, *lane MR*: Protein molecular marker, *lanes 26-28*: E51125xTian8901 F2 progenies, *lane 29*: E51125, *lane 30*: Tian8901, *lane 31*: E51125xTian8901 F1 hybrid, *lane 32*: Chinese spring.

In the present study, 1 individual in the Tian8901xR145 F2 generation did not show the Glu-A1a(1) band (Figure 1, lane 7), while both parents showed this band. Previous studies found that the missing mechanism was associated with the presence of a premature stop codon within the coding region (De Bustos et al., 2000; Wan et al., 2002; Sun et al., 2004). This may be because of the insertion of a transposon-like element in the coding region (Harberd et al., 1987; Lafiandra et al., 1997; Yuan et al., 2009). Lafiandra et al. (1997) reported that an ancestral active subunit 1 gene was silenced by the insertion of the 8-kb transposon-like fragment into the linked y-type gene. The cause of the missing Glu-A1a(1) in our study requires further study.

HMW-GS were controlled by genes at the long arms of the chromosomes 1D and 1B. Six genes, 1Ax, 1Ay, 1Bx, 1By, 1Dx, and 1Dy reportedly control HMW-GSs (Bietz et al., 1975). Common wheat typically expresses 3-5 HMW subunits because of gene silencing (Payne and Lawrence, 1983; Payne et al., 1987; Anjum et al., 2007). However, some cultivars contain more than 5 subunits (Johansson et al., 1993; Buonocore et al., 1996; Anjum et al., 2000). In the present study, cultivar R145 was found to have 6 subunits, including subunit N with 2 closely linked bands detected on 12% SDS-PAGE. Further study is required to determine the influence of the glutenin subunit N on processing quality. Subunit N did not fit the Mendelian segregation ratio. The presence to absence ratio was observed to be 116:100 and 138:78 in the Tian8901xR145 and R145xTian8901 F2 generations, respectively.

Agronomic and quality characteristics

Cytoplasmic inheritance studies on agronomic characteristics and quality characteristics often show contradictory results. In the present study, neither agronomic characteristics nor quality characteristics showed a significant difference between reciprocal crosses, indicating no cytoplasmic effect on these characteristics. This result is consistent with those of Atienza et al. (2007) who reported that reciprocal F1 lines did not differ for any of the agronomic traits evaluated with the exception of anthesis date, but disagreed with those of Ekiz et al. (1998) and Rajcan et al. (2002) who reported significant differences in reciprocal crosses for 1000-kernel weight, protein content, grain hardness, and days to maturity in some crosses.

Correlation between different characteristics generally results from the presence of linked genes and the epistatic effect of different genes (Mohsin et al., 2009; Yucel et al., 2009). In the present study, positive correlations among protein, wet gluten, and Zeleny were detected with coefficients > 0.9 ($\alpha = 0.01$, data not shown). The result is consistent with those of various previous studies (Cesevičienė et al., 2009; Hrušková and Švec, 2009).

Most agronomic characteristics were negatively correlated with protein; only main spike length, number of grains per plant, and number of effective spike were positively correlated, which corresponds well with the results of previous studies (McNeal et al., 1982; Holland et al., 2001; Khattak et al., 2005). We also found significant correlation between average grain weight per spike and quality characteristics at the level of 0.001. This result is consistent with the finding of Dağustü (2008). A decrease in average grain weight per spike was directly associated with increased protein content, wet gluten content, and Zeleny sedimentation value.

In conclusion, the HMW-GS composition of different generations was examined. Separations followed Mendelian laws of independent assortment, suggesting no linkage between any 2 loci. This result agrees with those of previous reports. A novel glutenin subunit identified in this study did not fit the Mendelian segregation ratio and contained 2 closely linked bands in the F2 generations. One individual in the F2 generation did not show the Glu-A1a(1) band, while both the parents showed the Glu-A1a(1) band. Correlation analyses found that decreased average grain weight per spike was directly associated with increased protein content, wet gluten content, and Zeleny sedimentation value.

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