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Genetic Diversity of HMW-GS and the Correlation of Grain Quality Traits in Bread Wheat (*Triticum aestivum* L.) in Hubei Province, China

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Abstract: High-molecular-weight glutenin subunits (HMW-GS) are an important component of total cereal proteins in wheat. It is closely related to the processing quality of flour. Here, we analyzed allelic variations at the *Glu-1* locus in 163 wheat accessions from Hubei Province, China with SDS-PAGE. Among the 15 alleles detected, alleles 1, 7+8, and 2+12 were the major alleles, and 7, 6+8, and 2+10 were rare alleles. The breeding lines had higher genetic diversity than the commercial varieties. Alleles 7 and 6+8 significantly reduced the grain protein content and wet gluten content of wheat. The “1, 7+9, 5+10” and “1, 14+15, and 2+12” allelic combinations significantly increased the grain protein content, hardness index, test weight, and wet gluten content of wheat. Alleles 7+9, 14+15, and 5+10 were identified as alleles related to high wheat quality. The “1, 7, 5+10”, “1, 6+8, 5+10”, “null, 7+9, 2+12”, “1, 14+15, 2+12”, and “1, 7+9, 5+10” allelic combinations had greater effects on wheat grain quality traits. These results demonstrate the effects of HMW-GS on wheat grain quality traits and provide a reference for the genetic improvement of wheat quality.

Keywords: wheat; HMW-GS; allelic combinations; genetic diversity; grain quality traits



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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. Wheat flour is used to produce a variety of products, such as bread, buns, noodles, cakes biscuits, etc. [1]. Based on their functions, proteins in wheat kernels can be classified into metabolic proteins related to the nutritional quality and storage proteins associated with the processing quality [2]. The processing quality of wheat flour is largely determined by the properties of seed storage proteins (SSP) [3,4], which mainly consist of polymeric glutenins and monomeric gliadins. The former is important for dough extensibility, while the latter is critical for dough strength and elasticity [5,6]. Furthermore, based on their molecular weights, wheat glutenins can be divided into low-molecular-weight glutenin subunits (LMW-GS) and high-molecular-weight glutenin subunits (HMW-GS) [7].

Previous studies have demonstrated that HMW-GS, which accounts for about 10% of the total glutenin content in wheat, is an important factor for the processing quality of wheat and can determine about 40–75% of the baking quality of bread [6–8]. HMW-GS is coded by the *Glu1* locus on the long arm of homoeologous group I chromosomes, where the three allelic variants are designated *Glu-A1*, *Glu-B1*, and *Glu-D1* [9,10]. Each locus

encodes one X- and one Y-type subunit, including *Glu-1Ax*, *Glu-1Ay* (usually silent), *Glu-1Bx*, *Glu-1By*, *Glu-1Dx*, and *Glu-1Dy*, respectively [5,11]. It has been demonstrated that alleles 1 and 2* at the *Glu-A1* locus have a greater contribution to wheat bread-making quality than the null allele [12]. At the *Glu-B1* locus, allele 14+15 has a positive effect on dough elasticity [13], and allele 17+18 is positively correlated with dough extensibility [14]. Ragupathy et al. [15] found that allele Bx7^{OE} significantly improves dough strength and thereby promotes bread-making quality. At the *Glu-D1* locus, the allele 5+10 has a positive effect on flour quality [16].

Wheat grain quality consists of complex quantitative traits controlled by multiple genes. Grain starch, grain protein content, hardness index, test weight, wet gluten content, and SDS sedimentation value are important indicators of wheat processing quality [17]. Starch is the main component of wheat endosperm, accounting for 65–80% of the final dry weight of wheat kernels, and grain yield can be improved by increasing starch accumulation [18]. Wheat protein content is an important determinant of bread baking quality [19,20].

As an important component of wheat grain proteins, HMW-GS is closely related to flour processing quality. However, there has been no systematic research concerning the effects of HMW-GS on wheat grain quality traits in the wheat-growing region of Hubei Province, China. In this study, we analyzed the genetic diversity of HMW-GS alleles and allelic combinations in 90 commercial varieties and 73 breeding lines in Hubei Province and comprehensively investigated the relationship between wheat grain quality traits. The findings provide a reliable basis for the selection of high-quality germplasm resources and genetic improvement of specialized high-quality wheat.

2. Materials and Methods

2.1. Plant Materials

A total of 90 commercial common bread wheat varieties from Hubei Province, China, and 73 breeding lines selected by Huazhong Agricultural University were used in this study (Supplementary data Table S1). Commercial varieties were derived from germplasm resources with validated high yield and quality in Hubei Province, and the breeding lines were bred lines with rich genetic backgrounds and quality improvement potential that were selected over the past years. These wheat accessions were planted at a Huazhong Agricultural University Experimental farm (48° N 30 °C, 36° E 114 °C) and Ezhou Experimental farm (36° N 30 °C, 71° E 114 °C) in 2021–2022. A completely randomized block design with three replications of three rows each (3 m long and 20 cm apart) was used to identify wheat HMW-GS and grain quality traits. Chinese Spring and Zhongyou 9507 were used as the standard cultivars for the identification of HMW-GS alleles.

2.2. SDS-PAGE Analysis

The SDS-PAGE method reported by Wang et al. [21] was used to identify the HMW-GS compositions. First, 40 mg of flour was added with 600 µL of SDS-PAGE buffer [62.5 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 0.002% (w/v) bromophenol blue], and well shaken for 30 min. The sample was then immediately placed in a 90 °C boiling water bath for 5 min, centrifuged at 13,000 rpm for 5 min, and the supernatant was aspirated for SDS-PAGE analysis. SDS-PAGE was conducted with 12% (w/v) running gels and 4% (w/v) stacking gels. The gels were run at 25 mA for 5 h (Liuyi-Beijing mini-cell apparatus). After electrophoresis, the gel was stained in Coomassie Brilliant Blue solution (R-250) for 8–10 h, and then 40% (v/v) methanol and 7% (v/v) glacial acetic acid were used for decontamination. The HMW-GS composition was identified using the classification method of Payne and Lawrence [22].

2.3. Quality Tests

Near-infrared spectroscopy (NIRS DS2500, FOSS Corporation, Denmark) was used to determine the grain starch (GS), grain protein content (GPC), falling number (FN), hardness

index (HI), weakness index (WI), test weight (TW), wet gluten content (WGC), and SDS sedimentation value (SDSS) of wheat kernels [23,24].

2.4. Statistical Methods

Genetic diversity indicators, including the genetic diversity index (H), number of alleles (Alleles), average number of alleles (A), and effective number of alleles (A_e), were calculated according to a previously reported method [25]. Correlation analysis and analysis of variance (ANOVA) were performed on the data using SPSS software (v.25.0). Graphics were drawn using the Origin v.2021(9.7) software.

3. Results

3.1. Allelic Variations at the *Glu-1* Locus

Fifteen HMW-GS alleles were detected at locus *Glu-1*, with three for *Glu-A1*, eight for *Glu-B1*, and four for *Glu-D1* in 163 wheat accessions from Hubei (Table 1). At the *Glu-A1* locus, *Glu-A1a* (allele 1), *Glu-A1b* (allele 2*), and *Glu-A1c* (allele Null) were detected. Allele 1 had the highest frequency (58.90%) in the breeding lines; the null allele had the highest frequency (53.33%) in commercial varieties; and allele 2* had the lowest frequency in both commercial varieties (2.22%) and breeding lines (2.74%). At the *Glu-B1* locus, allele 7 was not detected in commercial varieties, but it was observed in 8.22% of the breeding lines. Allele 7+8 was present at frequencies of 51.11% and 46.58%, and allele 7+9 was detected at frequencies of 36.67% and 24.66% in commercial varieties and breeding lines, respectively. Allele 6+8 was less frequently detected in both classes of wheat accessions. Alleles 13+16 and 13+19 were only detected in a few commercial varieties (1.11%). Allele 14+15 was detected at frequencies of 6.67% and 13.70%, and allele 17+18 was detected at frequencies of 2.22% and 1.37% in commercial varieties and breeding lines, respectively. At the *Glu-D1* locus, four alleles 2+10, 2+12, 5+10, and 5+12 were observed in commercial varieties and breeding lines. Allele 2+12 was detected at the highest frequency in both commercial varieties and breeding lines (58.89% and 53.42%, respectively); while allele 2+10 was detected in breeding lines at a frequency of 1.37%; and allele 5+12 was detected in both commercial varieties (1.11%) and breeding lines (4.11%) (Table 1).

Table 1. Detection frequencies of the alleles and HMW-GS at *Glu-1* locus in wheat. — representation as none.

Locus	Subunit	Allele	No. of Varieties	Commercial Varieties		Breeding Lines	
				No. of Varieties	Frequency (%)	No. of Lines	Frequency (%)
<i>Glu-A1</i>	1	<i>Glu-A1a</i>	83	40	44.44	43	58.90
	2*	<i>Glu-A1b</i>	4	2	2.22	2	2.74
	Null	<i>Glu-A1c</i>	76	48	53.33	28	38.36
<i>Glu-B1</i>	7	<i>Glu-B1a</i>	6	0	—	6	8.22
	7+8	<i>Glu-B1b</i>	80	46	51.11	34	46.58
	7+9	<i>Glu-B1c</i>	51	33	36.67	18	24.66
	6+8	<i>Glu-B1d</i>	5	1	1.11	4	5.48
	13+16	<i>Glu-B1f</i>	1	1	1.11	0	—
	13+19	<i>Glu-B1g</i>	1	1	1.11	0	—
	14+15	<i>Glu-B1h</i>	16	6	6.67	10	13.70
<i>Glu-D1</i>	17+18	<i>Glu-B1i</i>	3	2	2.22	1	1.37
	2+12	<i>Glu-D1a</i>	92	53	58.89	39	53.42
	5+10	<i>Glu-D1d</i>	66	36	40.00	30	41.10
	2+10	<i>Glu-D1e</i>	1	0	—	1	1.37
	5+12	<i>Glu-D1h</i>	4	1	1.11	3	4.11

3.2. Frequencies of HMW-GS Compositions at the *Glu-1* Loci

A total of 25 allelic combinations were detected at the *Glu-1* locus (Table 2). The “null, 7+8, 2+12” allelic combination was the most frequently detected in commercial varieties

(26.67%), followed by the “null, 7+9, 2+12” and “1, 7+8, 5+10” allelic combinations (12.22% and 10.00%, respectively). Six allelic combinations had frequencies lower than 5%, among which “1, 6+8, 5+10”, “1, 13+16, 2+12”, “1, 13+19, 5+10” and “null, 7+9, 5+12” had the lowest frequency (1.11%). The “1, 17+18, 5+10” and “2*, 7+9, 5+10” allelic combinations were detected at a frequency of 2.22%. The “null, 7+8, 2+12” allelic combination appeared the most frequently in breeding lines (15.07%), followed by “1, 7+8, 2+12”, “1, 7+8, 5+10”, and “1, and 7+9, 2+12” with frequencies of 12.33%, 10.96%, and 10.96%, respectively. Fourteen allelic combinations were detected at frequencies lower than 5%, among which “1, 7, 2+12”, “1, 7+9, 2+10”, “1, 17+18, 5+10”, “2*, 7+8, 5+10”, “2*, 7+9, 5+10”, “null, 7, 2+12”, “null, 7, 5+10”, “null, 14+15, 5+10” had the lowest frequency (1.37%). The “1, 7, 2+12”, “1, 7, 5+10”, and “1, 7+8, 5+12” allelic combinations were only detected in breeding lines, while “1, 7+9, 5+10”, “1, 13+16, 2+12”, and “1, 13+19, 5+10” were specific to commercial varieties.

Table 2. Combination type and score of wheat HMW-GS. ? symbol indicates that previous research did not define a score value.

Number	HMW-GS Compositions	Alleles	Number of Varieties	Commercial Varieties		Breeding Lines		Score
				Number	Frequency (%)	Number	Frequency (%)	
1	1/7/2+12	aaa	1	0	—	1	1.37	6
2	1/7/5+10	aad	3	0	—	3	4.11	8
3	1/7+8/2+12	aba	17	8	8.89	9	12.33	8
4	1/7+8/5+10	abd	17	9	10.00	8	10.96	10
5	1/7+8/5+12	abh	3	0	—	3	4.11	?
6	1/7+9/2+12	aca	14	6	6.67	8	10.96	7
7	1/7+9/5+10	acd	6	6	6.67	0	—	9
8	1/7+9/2+10	ace	1	0	—	1	1.37	?
9	1/6+8/5+10	add	5	1	1.11	4	5.48	8
10	1/13+16/2+12	afa	1	1	1.11	0	—	8
11	1/13+19/5+10	agd	1	1	1.11	0	—	?
12	1/14+15/2+12	aha	6	3	3.33	3	4.11	8
13	1/14+15/5+10	ahd	5	3	3.33	2	2.74	10
14	1/17+18/5+10	aid	3	2	2.22	1	1.37	10
15	2*/7+8/5+10	bbd	1	0	—	1	1.37	10
16	2*/7+9/5+10	bcd	3	2	2.22	1	1.37	9
17	N/7/2+12	caa	1	0	—	1	1.37	4
18	N/7/5+10	cad	1	0	—	1	1.37	6
19	N/7+8/2+12	cba	35	24	26.67	11	15.07	6
20	N/7+8/5+10	cbd	7	5	5.56	2	2.74	8
21	N/7+9/2+12	cca	13	11	12.22	2	2.74	5
22	N/7+9/5+10	ccd	13	7	7.78	6	8.22	7
23	N/7+9/5+12	cch	1	1	1.11	0	—	7
24	N/14+15/2+12	cha	4	0	—	4	5.48	6
25	N/14+15/5+10	chd	1	0	—	1	1.37	8

3.3. Genetic Diversity of HMW-GS at the *Glu-1* Locus

Table 3 shows the statistical analysis of the genetic diversity index of commercial varieties and breeding lines. Commercial varieties (0.518 and 0.599) had a higher and lower genetic diversity index than breeding lines (0.505 and 0.694) at *Glu-A1* and *Glu-B1*, respectively. Moreover, at the *Glu-D1* locus, commercial varieties (0.493) had a lower genetic diversity index than breeding lines (0.544). These results indicated that the *Glu-B1* locus had more abundant allelic variations than *Glu-A1* and *Glu-D1*. The genetic diversity (*H_b*) of commercial varieties and breeding lines was 0.537 and 0.581, respectively. The number and average (*A*) of alleles were the same for commercial and breeding lines, which were 13 and 4.333, respectively. Therefore, the number of effective alleles was 2.180 and 2.492 for commercial varieties and breeding lines, respectively. Breeding lines had higher genetic diversity and a larger number of effective alleles than commercial varieties.

Table 3. Genetic diversity index of HMW-GS at the *Glu-1* locus.

Indicator Type	Commercial Variety	Breeding Line
H_{a1}	0.518	0.505
H_{a2}	0.599	0.694
H_{a3}	0.493	0.544
H_b	0.537	0.581
Alleles	13	13
A	4.333	4.333
A_e	2.180	2.492

H : Genetic diversity index; H_{a1} : H value of *Glu-A1*; H_{a2} : H value of *Glu-B1*; H_{a3} : H value of *Glu-D1*. H_b : The average value of H at the three loci of *Glu-1*. Alleles: number of alleles. A : Average number of alleles. A_e : Effective number of alleles.

3.4. Analysis of Wheat Grain Quality Traits

The grain quality traits of commercial varieties and breeding lines were determined (Figure 1). Among the eight indicators of wheat grain quality, commercial varieties had significantly higher values of five indicators: grain protein content (GPC), falling number (FN), hardness index (HI), wet gluten content (WGC), and SDS sedimentation (SDSS) while significantly lower values of two indicators: grain starch (GS) and weakness index (WI) than breeding lines ($p < 0.01$). In terms of test weight (TW), there was no significant difference between the two classes of wheat accessions.

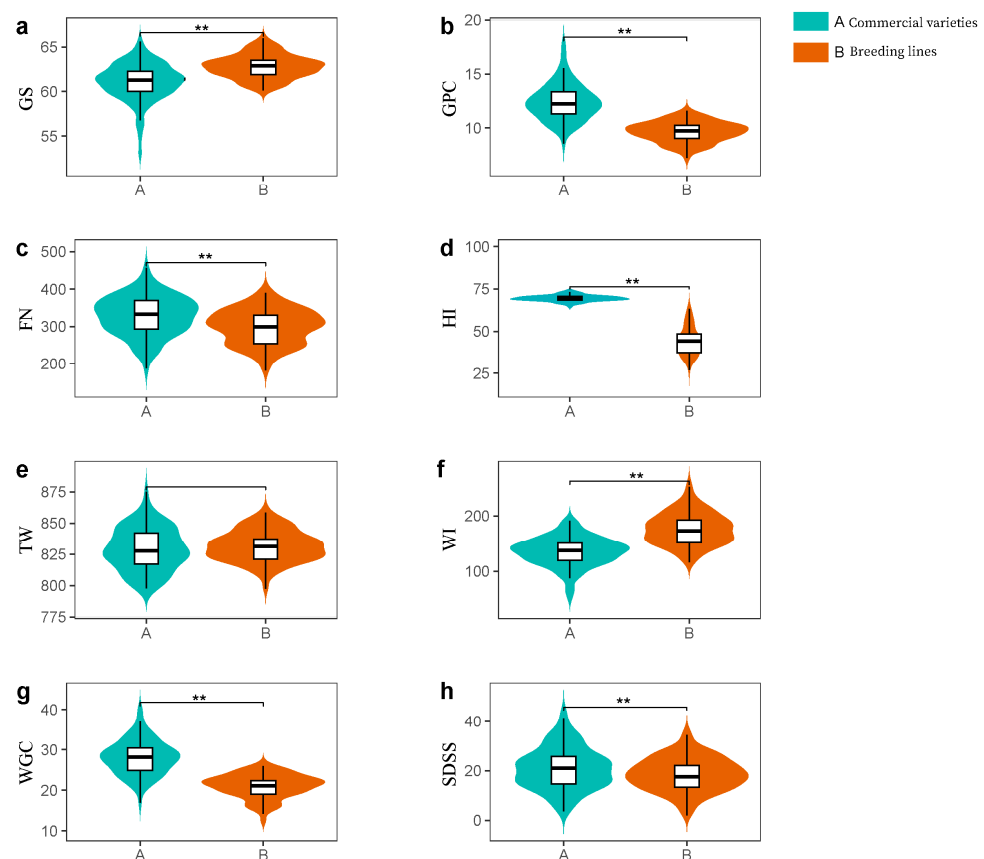


Figure 1. Differences in grain quality traits between commercial varieties and breeding lines. (a): grain starch (GS); (b): grain protein content (GPC); (c): falling number (FN); (d): hardness index (HI); (e): test weight (TW); (f): weakness index (WI); (g): wet gluten content (WGC); (h): SDS sedimentation (SDSS). **: Significant at $p < 0.05$ and 0.01 , respectively.

Correlation analyses were performed for wheat quality traits of commercial varieties and breeding lines (Figure 2). WI had a positive correlation with GS ($r = 0.65$, $p < 0.01$), but

was negatively correlated with HI, WGC, GPC, SDSS, and FN ($-0.74 \sim -0.51$, $p < 0.01$). GS had a negative correlation with GPC, FN, HI, WGC, and SDSS ($r = -0.83 \sim -0.48$, $p < 0.01$). HI displayed a positive correlation with WGC (0.74), GPC (0.71), SDSS (0.36), and FN ($r = 0.34 \sim 0.74$, $p < 0.05$ or 0.01). WGC showed a positive correlation with GPC (0.99), SDSS (0.74), and FN ($r = 0.57 \sim 0.99$, $p < 0.01$), and GPC had a positive correlation with SDSS (0.73, $p < 0.01$) and FN (0.55, $p < 0.01$).

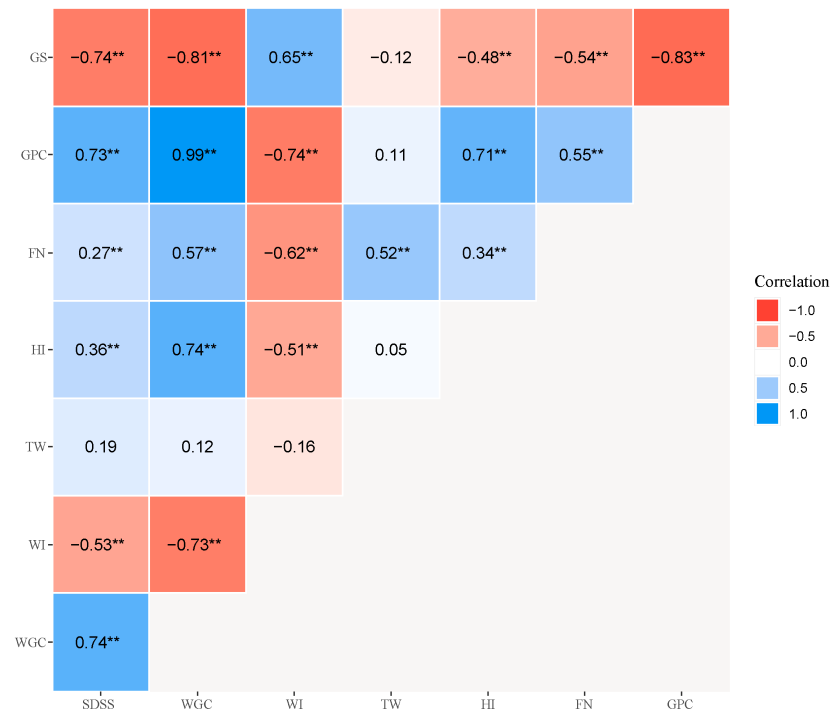


Figure 2. Correlations of wheat grain quality traits. GS, grain starch; GPC, grain protein content; FN, falling number; HI, hardness index; WI, weakness index; TW, test weight; WGC, wet gluten content; and SDSS, SDS sedimentation. **, significant at $p < 0.05$ and 0.01 , blue indicates positive correlation, while red indicates negative correlation. respectively.

In order to determine the effect of alleles in the *Glu-1* locus on wheat grain quality traits, the main types of alleles at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci were analyzed separately in relation to grain quality traits (Figure 3). Because the genotypes *Glu-B1d*, *Glu-B1i*, and *Glu-D1h* have a small sample size, while genotypes 13+16 and 13+19 in *Glu-B1* and 2+10 have only one sample each, which is insufficient for calculating statistical parameters. At the *Glu-A1* locus, allele 1 had significantly higher TW than alleles null and 2* ($p < 0.05$) (Figure 3A). At the *Glu-B1* locus, allele 6+8 had a significantly higher value of GS than the alleles 7+8, 7+9, and 14+15, allele 7+8 had a significantly higher value of GS than allele 7+9. Alleles 7 and 6+8 had significantly lower GPC than alleles 7+8, 7+9, and 14+15 ($p < 0.05$) (Figure 3B). Moreover, allele 7+8 had significantly higher TW than allele 7+9, and alleles 7 and 6+8 had significantly lower WGC than alleles 7+8 and 7+9 ($p < 0.05$). At the *Glu-D1* locus, SDSS significantly differed between alleles 2+12 and 5+10 ($p < 0.05$) (Figure 3C). Compared to allele 2+12, allele 5+10 showed significantly lower SDSS ($p < 0.05$). Alleles 7+9, 14+15, and 5+10 were identified as alleles related to high wheat quality.

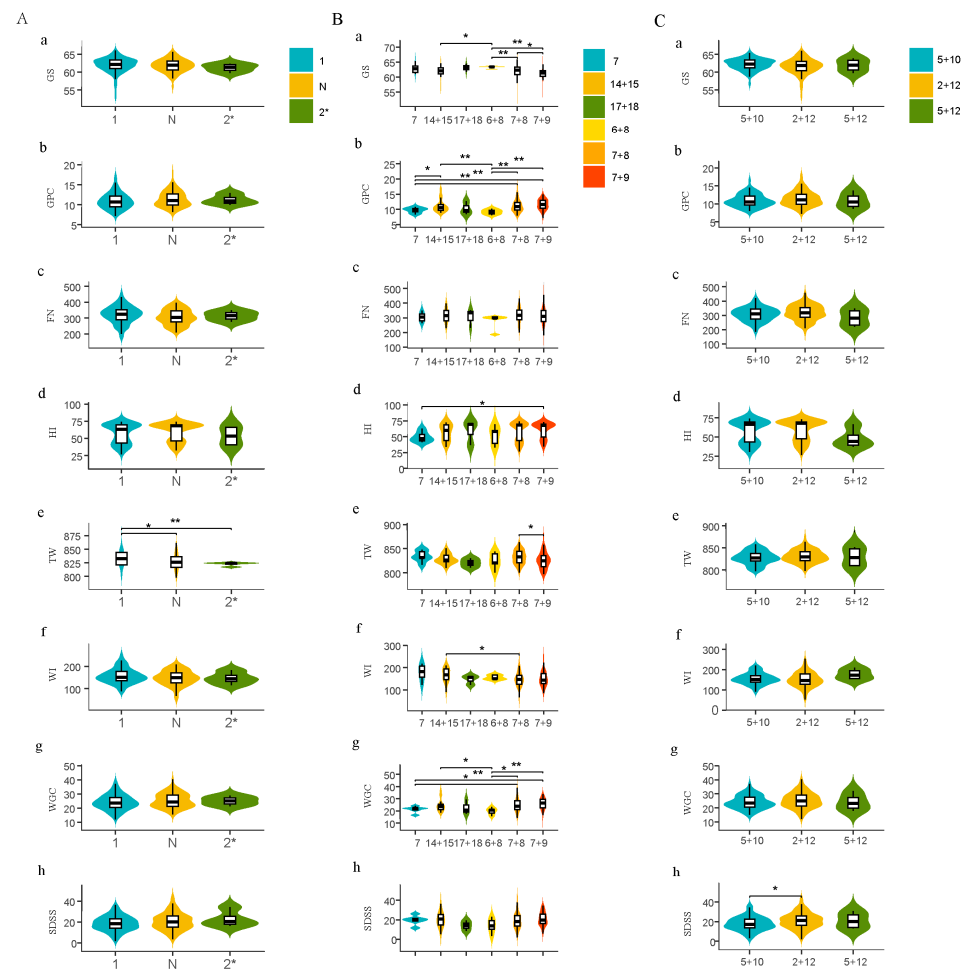


Figure 3. Comparison of alleles at *Glu-A1* (A), *Glu-B1* (B), and *Glu-D1* (C). (a): grain starch (GS); (b): grain protein content (GPC); (c): falling number (FN); (d): hardness index (HI); (e): test weight (TW); (f): weakness index (WI); (g): wet gluten content (WGC); and (h): SDS sedimentation (SDSS). * and **, significant at $p < 0.05$ and 0.01 , respectively.

Correlations between different allelic combinations and wheat grain quality traits were analyzed (Figure 4). There were no significant differences in GS among different allelic combinations. The “null, 7+9, 2+12” allelic combination had significantly higher GPC than the “1, 7, 5+10”, “1, 7+8, 5+10”, “1, 7+8, 5+12”, “1, 6+8, 5+10”, “1, 17+18, 5+10”, “N, 7+9, 5+10” and “null, 14+15, 2+12” allelic combinations. The “1, 6+8, 5+10” allelic combination had significantly lower FN than the “1, 7+8, 2+12”, “1, 7+9, 2+12” and “1, 14+15, 2+12” allelic combinations. The “1, 7+9, 5+10” allelic combination had significantly higher HI than the “1, 6+8, 5+10”, “1, 7, 5+10”, “1, 7+8, 2+12”, “1, 7+8, 5+10”, “1, 7+8, 5+12”, “null, 7+9, 5+10” and “null, 14+15, 2+12” allelic combinations. The “1, 7+9, 2+12” allelic combination showed significantly higher TW than the “1, 6+8, 5+10”, “1, 17+18, 5+10”, “null, 7+9, 2+12” and “null, 7+9, 5+10” allelic combinations. The “1, 7, 5+10” combination had significantly higher WI than the “null, 7+8, 2+12” and “null, 7+9, 2+12” allelic combinations. The “null, 7+9, 2+12” allelic combination had significantly higher WGC than the “1, 7, 5+10”, “1, 7+8, 2+12”, “1, 7+8, 5+10”, “1, 7+8, 5+12”, “1, 7+9, 2+12”, “1, 6+8, 5+10”, “1, 17+18, 5+10”, “null, 7+8, 5+10”, “null, 7+9, 5+10” and “null, 14+15, 2+12” allelic combinations. The SDSS was significantly lower for the “1, 6+8, 5+10” and “1, 17+18, 5+10” allele combinations than for the “1, 7+9, 5+10”, “1, 14+15, 2+12”, “2*, 7+9, 5+10”, “N, 7+8, 2+12” and “N, 7+9, 2+12” allele combinations. In summary, the “1, 7, 5+10”, “1, 7+9, 5+10”, “null, 7+9, 2+12”, “1, 14+15, 2+12”, and “1, 6+8, 5+10” allelic combinations had greater effects on wheat grain quality traits.

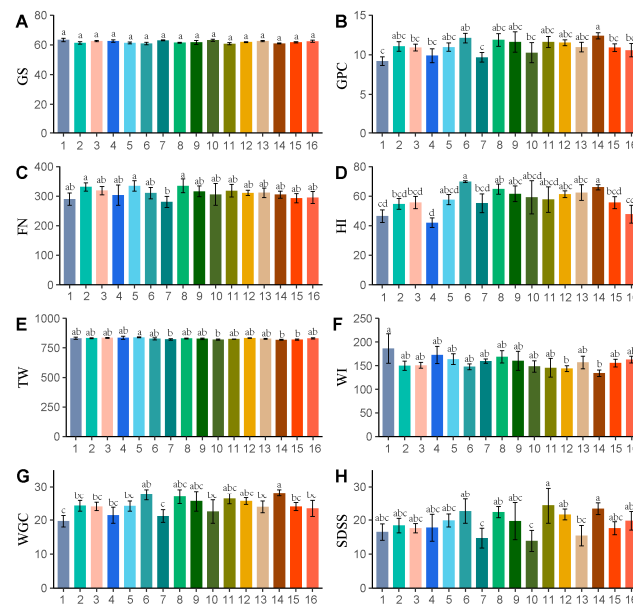


Figure 4. Performances of grain quality traits for different HMW-GS combinations. (A): grain starch (GS); (B): grain protein content (GPC); (C): falling number (FN); (D): hardness index (HI); (E): test weight (TW); (F): weakness index (WI); (G): wet gluten content (WGC) and (H): SDS sedimentation (SDSS). Horizontal axis 1: “1, 7, 5+10” (n = 3); 2: “1, 7+8, 2+12” (n = 17); 3: “1, 7+8, 5+10” (n = 17); 4: “1, 7+8, 5+12” (n = 3); 5: “1, 7+9, 2+12” (n = 14); 6: “1, 7+9, 5+10” (n = 6); 7: “1, 6+8, 5+10” (n = 5); 8: “1, 14+15, 2+12” (n = 6); 9: “1, 14+15, 5+10” (n = 5); 10: “1, 17+18, 5+10” (n = 3); 11: “2*, 7+9, 5+10” (n = 3); 12: “N, 7+8, 2+12” (n = 35); 13: “N, 7+8, 5+10” (n = 7); 14: “N, 7+9, 2+12” (n = 13); 15: “N, 7+9, 5+10” (n = 13); 16: “N, 14+15, 2+12” (n = 4). The same letters above the bars indicate insignificant differences at $p < 0.05$.

4. Discussion

Rare allelic variants on the *Glu-1* locus of HMW-GS are critical for wheat quality [4]. In the present study, HMW-GS alleles were identified in 163 wheat lines from Hubei Province, and 15 alleles were detected. In the present study, HMW-GS alleles were identified in 163 wheat lines from Hubei Province, and 15 alleles were detected. Zheng et al. [26] identified 22 alleles in wheat landraces from the Yangtze River Basin. In the study of Dai et al. [27], 26 alleles were detected in 343 Xinjiang wheat landraces and historical varieties. Moreover, 15 alleles were found at the *Glu-1* locus in commercial cultivars from China by Gao et al. [28]. Wang et al. [21] reported 14 alleles in 131 commercial varieties of wheat in the middle and lower Yangtze River region. In the study of Farahani et al. [29], 12 alleles were identified in Iranian commercial bread wheat cultivars. At the *Glu-A1* locus, alleles 1, 2*, and Null were detected, with the null allele having the highest frequency (53.33%) in commercial varieties. Consistently, previous studies have demonstrated that the null allele is the most frequent at the *Glu-A1* locus for cultivars from the middle and lower Yangtze River region [21,26,28]. In breeding lines, the frequencies of alleles 1, 2*, and Null at the *Glu-A1* locus were 58.90%, 2.74%, and 38.36%, respectively. Allele 1 was the most frequently detected at the *Glu-A1* locus. Similar results were reported by Wang et al. [21] for spring wheat in the northwestern wheat region of China. The frequency of allele 1 is higher in wheat varieties from Spain, Kazakhstan, Russia, Canada, and Europe [30–32]. Yasmeen et al. [33] showed that the 2* and null alleles were the most frequent in Pakistan wheat varieties. A comparison of the frequency of null subunits in wheat commercial varieties from all over the world revealed that the frequency of null subunits in Chinese commercial varieties was higher, which may be related to the fact that Chinese breeding mainly focuses on yield but not quality improvement. It has been demonstrated that alleles 1 and 2* at the *Glu-A1* locus have a strong positive effect on wheat flour processing quality [6,13]. In the future, we will perform the wheat flour processing quality analysis on this.

For the *Glu-B1* locus, the frequency of alleles 7+8 and 7+9 was higher in both commercial varieties and breeding lines. Liu et al. [25] found that allele 7+8 was detected at the highest frequency (78.17%) in the landraces from Hubei Province, China. *Glu-B1b* (7+8) and *Glu-B1c* (7+9) appeared to be the most frequent in 131 commercial varieties in the middle and lower Yangtze River region [21]. Similarly, previous studies have found that *Glu-B1b* (7+8) and *Glu-B1c* (7+9) are major alleles in Chinese commercial cultivars [13,28,34]. Farahani et al. [29] observed the highest frequency of alleles 7+8 (36%) and 7+9 (25%) in Iranian commercial bread wheat cultivars. Alleles of *Glu-B1b* and *Glu-B1c* are predominant in Argentine and Pakistani wheat cultivars [35,36]. Allele 13+16 is the most frequent type in Spanish varieties [30]. Wang et al. [21] identified that *Glu-B1g* (13+19) occurs at lower frequencies in the landraces. Allele 7 was identified at low frequencies in breeding lines, landraces, and commercial cultivars from China [21,28].

At the *Glu-D1* locus, alleles 2+12 and 5+10 were detected at higher frequencies in commercial varieties and breeding lines. It has been demonstrated that allele 2+12 is common in Chinese wheat varieties [21,25–27]. We determined that the frequency of allele 5+10 was higher in breeding lines than in commercial varieties from Hubei province. Allele 5+10 was shown to be the most frequent in commercial wheat varieties from Pakistan and Iran [29,33]. The rare allele 5+12 occurred at lower frequencies in commercial varieties and breeding lines in this study. Allele 2+10 occurs uniquely in commercial varieties and less frequently in Chinese landraces [37]. The “1, 7+8, 2+12”, “1, 7+8, 5+10”, and “null, 7+8, 2+12” allelic combinations were the most frequently occurred type for commercial varieties and breeding lines, which is consistent with the results of other studies [21,25,28]. Moreover, “null, 14+15, 2+12” and “null, 14+15, 5+10” were detected in breeding lines. Allele 14+15 was found to be specific to Chinese wheat cultivars and was able to improve dough quality parameters and processing quality [25,38].

The genetic diversity index (*Hb*) of HMW-GS in commercial varieties and breeding lines was 0.537 and 0.581, respectively, which is close to that reported for other Chinese wheat commercial varieties [21,28,39]. The genetic diversity index of wheat landraces is lower than that of commercial wheat varieties in China [25,39]. The genetic diversity index of Chinese wheat varieties was higher than that of Canadian, British, and Argentinean wheat varieties, but lower than that of French, Italian, and Australian wheat varieties [34,35]. The effective allele number (*Ae*) of commercial varieties and breeding lines was 2.180 and 2.492, respectively, which is similar to the results of Chinese wheat reported by Wang et al. [21]. The *Hb* and *Ae* of breeding lines were higher than those of commercial varieties, indicating that breeding lines have a broader genetic background.

The *Glu-A1* locus showed less significant effects on wheat grain quality traits than loci *Glu-B1* and *Glu-D1*. At the *Glu-B1* locus, alleles 7+8, 7+9, and 14+15 had greater effects on HI, GPC, WGC, and SDSS. Peng et al. [40] elucidated the effects of alleles 7+8 and 7+9 at the *Glu-B1* locus on dough processing quality. At the *Glu-D1* locus, allele 5+10 had a greater effect on SDSS. Allele 5+10 is superior to other alleles in enhancing wheat bread quality [41–44]. The “1, 7+9, 5+10” and “1, 14+15, 2+12” allelic combinations had higher GPC, HI, and WGC than other combinations in the current panel of wheat accessions, which is consistent with the results of a previous study [45]. These results demonstrated the correlation between HMW-GS and wheat grain quality traits for wheat grain quality improvement.

5. Conclusions

In conclusion, we analyzed the relationship among HMW-GS and grain quality traits. The strongest effect on wheat quality was observed at the *Glu-B1* locus. Alleles 7+9, 14+15, and 5+10 were identified as the alleles related to high wheat quality. The “1, 7, 5+10”, “1, 7+9, 5+10”, “null, 7+9, 2+12”, “1, 14+15, 2+12” and “1, 6+8, 5+10” allelic combinations had greater effects on wheat grain quality traits. These alleles and allelic combinations related to high wheat quality can be used in the breeding of high-quality wheat varieties in Hubei province and other parts of China.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14061158/s1>, Table S1. HMW-GS composition and grain quality traits of 163 wheat cultivars in Hubei province. Table S2. Grain quality traits of commercial varieties in Huazhong Agricultural University Experimental farm. Table S3. Grain quality traits of breeding lines in Huazhong Agricultural University Experimental farm. Table S4. Grain quality traits of commercial varieties in Ezhou Experimental farm. Table S5. Grain quality traits of breeding lines in Ezhou Experimental farm.

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