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Effects of Genotype and Environment on HMW-GS Expression and its Relationship with Steamed Bun and Bread-Baking Quality

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Abstract: Three winter wheat cultivars from eight different locations with 3 replications were used to study the effects of genotypes, environments and their interactions on high molecular weight of glutenin subunits (HMW-GS) expression and its relationship with steamed bread and baking quality. The results indicated that although some subunits were affected by environments, genotypes played an important role for X-type HMW-GS, Y-type HMW-GS, Glu-D1 location and Glu-B1 location. Total HMW-GS amounts were affected by the interactions between genotype and environment. Individual HMW-GS content of the same genotype was affected by environment in different degree. Yantai location was superior to others' locations for many HMW-GS expression amounts. The same kind of HMW-GS from different genotypes appeared different in the same environments. Correlation analysis appeared HMW-GS expression amount positively correlated with bread volume, and negatively with bread peak firmness. Effects of individual and total HMW-GS on steamed bun quality were different from that on bread quality. And effects of total HMW-GS on bread volume were better than that of individual HMW-GS. Bread quality was determined firstly by HMW-GS types, secondly by the HMW-GS expression amount; while steamed bread quality was mainly affected by HMW-GS expression amounts.

Key words: Chinese winter wheat, environment, genotype, HMW-GS expression, bread-baking quality, steamed bread quality

INTRODUCTION

It is well known that the variations of wheat flour quality are mostly caused by gluten protein content and compositions. Gliadins and glutenins account for 80% of wheat protein and are the principal components in gluten, the main factors determining dough viscoelasticity. Gliadins are encoded by six *Gli* loci multigenes family (*Gli-1* and *Gli-2*) mapped on the short arms of the group1 and 6 chromosomes, respectively (Payne *et al*., 1982; Wrigley and Shepherd, 1973) while glutenins are encoded by genes of group 1 chromosome. The glutenin fraction consists of high Mr polymers stabilized by inter-chain disulphide bonds. On the basis of their mobility in SDS-PAGE the monomers are usually classified into two groups, the High Molecular Weight Glutenin Subunits (HMW-GS) mapped on the long arm of 1 chromosome and the Low Molecular Weight Glutenin Subunits (LMW-GS) mapped on the short arm of 1 chromosome. LMW-GS is subdivided into B, C and D subunits (Jackson *et al*., 1983). Gliadins determine dough extensibility, while glutenins determine its elasticity (Payne *et al*., 1979; Shewry *et al*., 1995; Sun *et al*., 2001; Zhao, 2001). The wheat quality is significantly correlated with the ratio of glutenins to gliadins. With the increase of glutenins, gluten content, sedimentation volume and the stability time of Farinograph become greatly increscent. Moreover, the processing quality becomes better than before. HMW-GS and LMW-GS all affect the wheat processing quality; generally HMW-GS 5+10 is beneficial to wheat quality. However, the cultivars having HMW-GS 5+10 don't always perform well (Deng *et al*., 2004). Glutenin subunit compositions and types are controlled by genetics and have heredity stability in different wheat cultivars, but their contents are also affected by environmental conditions (Payne *et al*., 1981). Genotype (G), Environment (E) and their interactions (G×E) all affected the protein compositions, bread volume etc (Zhang *et al*., 1999; Huebner *et al*., 1997). Different researchers obtained not always the same results. Protein quality such as the distribution of glutenin polymers and the ratio of gliadin to glutenin was remarkably

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affected by genotype, environment and their interactions (Gupta *et al*., 1992; Blumenthal *et al*., 1995; Grayboshch *et al*., 2004). Zhu *et al*. (1995) research demonstrated that the wheat baking bread quality was affected by G, E and G×E. The variations of baking quality characteristics changed bigger than that of grains protein content. Panozzo and Eagles (2000) studied the effects of G, E and G×E on wheat quality by using seven cultivars grown in fifteen different environments. The results indicated the ratio of glutenin to flour protein content was highly correlated with genotypes, but the variations of E affected the gliadins greater than G. Moreover, the dough rheological properties were more influenced by E than by G. In addition, Park *et al*. (2003) indicated gliadins content was greatly affected by G and E. Effects of E were much more than that of G.

Zhu and Khan (2001, 2002) investigated the quantitative variation of HMW-GS in relation to glutenin polymers, mixing properties and hence breadmking quality across different environments. The results demonstrated that G, E and their interactions significantly affected protein and dough mixing properties. Flour protein content and SDS-soluble glutenin polymers were influenced by E more greatly than by G. E also affected the distribution of glutenin polymers that closely correlated with HMW-GS quantities. So, the quantities of HMW-GS were affected by grown locations. The optimum ecological environment benefited for the development of the sound ratio of glutenin to gliadin, which contributed to develop the good gluten quality. Their interactions affected the grain quality, glutenin, gliadin and the ratio of them (Jing *et al*., 2003).

Furthermore, Asian noodle quality was studied under different genotypes and environments. Noodle hardness and water absorption were remarkably affected by environments. Their interactions influenced the noodle firmness (Graybosch *et al*., 2004). However, Souza *et al*. (2004) indicated the cultivars played an important role in determining bread and noodle quality characteristics. The relationship between the type of HMW-GS and bread score had been widely studied by many researchers and HMW-GS quantity affected the bread quality. But effects of HMW-GS expression amounts on steamed bread were studied very little. Zhu and Khan (2001) research indicated flour protein content significantly affected Chinese steamed bread quality, and dough stickiness was an important factor causing poor Chinese steamed bread quality. The stability time of Australian wheat cultivars significantly negatively correlated with steamed bread quality, but on the contrary for Chinese wheat cultivars, there was significantly positive correlations. Protein content, gluten strength and extension of extensograph positively correlated with steamed bread volume and springiness (He *et al*., 2003). Surprisingly, little information is available on the relative effects of genotype, environment and their interactions on HMW-GS expression from Chinese wheat, dough rheological characteristics, steamed bun quality and their relationship with steamed bread. Therefore, the goals of this investigation were to understand more about effects of genotype, environment and G×E interactions on HMW-GS expression, and to determine the relationship between HMW-GS expression and steamed bread by using three winter wheat grown in eight different locations.

MATERIALS AND METHODS

Plant materials: Jimai20, Yan278 and Wei62036 from Shandong Province (China) were used in this study. They had different characteristics of gluten strength (Table 1).

Experimental design: Three winter wheat cultivars were grown at 8 locations (Heze, Dezhou, Taian, Yantai, Weifang, Zibo, Liaocheng and Jinan) in 2002-2003. The climate and soil were different in different locations, but the same experimental design was conducted. Randomized complete block design was used with three replicates at each location. The randomized plot area was 12 m^2 and the length was 6 m. Total available nitrogen of 40 mg•Kg^{-1}, available phosphorus of 50 mg•Kg^{-1}, and available potassium of 70 mg•Kg^{-1} are contained in the 0-20 cm. Fertilizer was applied before plough containing 60,000 Kg ha^{-1} base fertilizer, 225 Kg ha^{-1} ammonium hydrogen phosphate, 150 Kg ha^{-1} urea, 375 Kg ha^{-1} potassium sulfate. Before booting, 225 Kg ha^{-1}urea was topdressed; and another 75 Kg ha^{-1}urea was topdressed at anthesis. Harvested seeds after storing 3 month were used to mill flour by Senior mill (Brabender, Germany).

HMW-GS extraction analysis: Glutenin subunits were extracted from 30 mg of flour following the sequential procedure of Gupta and MacRitchie (1991). The monomeric proteins (albumins, globulins and gliadins) were eliminated by 2 extractions with DMSO and 50% propan-1-ol. Glutenin subunits were reduced with β-mercaptoethanol and alkylated with 4-vinylpyridine. HMW-GSs were separated using a 10% acrylamide SDS-PAGE according to Gupta and MacRitchie (1991).

Relatively quantitation of HMW-GS composition was made by RP-HPLC with a Hewlett Packard HP 1100 system. RP-HPLC analysis of HMW-GS was performed as described by Lei (2006) with some modification. Flour (50 mg) was extracted with 1 mL 70% (v v^{-1}) ethanol for 1 h at room temperature with shaking, then centrifuged

Table 1: Means of dough rheological characteristics in three varieties

Characteristics	JiMai20	Wei62036	Yan278	Range
Flour				
Protein (%)	12.79aA	12.35bB	11.46cC	1.33
Zeleny -SE (mL)	31.16 aA	22.74 cC	27.09 bB	8.42
Mixograph				
Peak time (min)	5.39 aA	2.07 cC	4.16 bB	3.32
Peak height	48.82 aA	43.78 cB	48.44 bA	5.04
Peak width (mm)	16.93 aA	12.93 cB	16.68 bA	4.00
Peak area (mm^2)	221.45 aA	81.58 cC	175.75 bB	139.87
8 time band width (mm)	10.30 aA	2.95 cC	9.27 bB	7.35
Extensograph				
Max. Extension-Resistance (BU)	22.10 bB	14.65 cC	22.92 aA	8.27
Extension-Area (cm^2)	979.19 aA	545.25 cC	960.37 bB	433.94
Extensibility (mm)	73.84 aA	64.79 bB	64.52 cB	9.32
Resistance/Extension	0.31 bA	0.23 cB	0.37 aA	0.14
Dough texture				
Dough stickiness	61.62 bA	69.65 aA	56.98 cB	12.67
Dough strength/cohension	5.94 bB	8.24 aA	5.83 cB	2.41
Dough adhension	14.47 cC	21.64 aA	17.38 bB	7.17

Small letters and Capital letters appeared 5% and 1% significant, respectively

at 17,000g for 5 min. The supernatant was discarded. The pellets were further extracted twice with 1 mL 50% (v v^{-1}) propan-1-ol for 30 min at 60°C with shaking, and also centrifuged at 17,000g for 5min. The supernatants were also discarded and the pellets re-suspended in 1ml solution (pH6.6) consisting of 50%(vv^{-1}) propan-1-ol, 0.1MTris, 2M urea, 1%(wv^{-1}) DTT, then placed in a water bath at 60°C for 1h, and then alkylated with 4-vinylpyridine (10 mL) at 60°C water bath for 15 min. The supernatants obtained after centrifugation at 17,000 g for 5min were filtered through PVDF syringe filters (0.45 mm) into glass tubes. Four volumes of 100% acetone were added to the supernatant, which was then stored at -20 for at least 2 days. The glutenin subunits were recovered by centrifugation and freeze-dried, then stored at -80°C. Protein content was determined by combustion analysis of 5-8 mg samples.

Freeze-dried proteins were dissolved in a solution at a concentration of 1 mg mL^{-1} in 6M guanidine Hcl adjusted to pH8.0 with TRIS, plus 50 mM DTT, and alkylated with 4-vinyl pyridine prior to HPLC. Five hundred micro-litre of protein was applied to a Jupiter (Phenomenex, Torrance CA) C18 semi-preparative RP-HPLC column or a Nucleosil (Ansys, Lake Forest, CA) C8 analytical column. Peptide bond absorbance was measured at 210 nm. Elution was achieved using a linear gradient of 76-48% of Millipore water containing 0.07% (v v^{-1}) TFA and 24-52% acetonitrile containing 0.05% (v v^{-1}) TFA for 55 min using a flow rate of 1 mL min^{-1}. HPLC peak areas were used to estimate the relative amount of protein in each peak.

Bread-baking analysis: The baking formula used 100% flour (14% moisture), 1% salt, 5% sugar, 3% compressed yeast, 2% shortening, 0.1% ammonium phosphate and 1 mL of fungal amylase (AACC, 1983). The amount of water added varied, depending on the flour water absorption obtained on the Farinograph. Bread volume was measured after cooling by the rapeseed displacement method.

Steamed bread analysis: Steamed bread was made from 100 g of wheat flour (14% moisture, fwb) by adding 1 g compressed yeast and certain amount of water that was 80% of total amount of Farinograph adding water. The procedures were as follows (Guo *et al*., 2002): Flour, dry yeast and water were mixed in a pin mixer for 1 min and the dough was kneaded till as clean as a whistle by hands. The cleaning dough was placed in the proofing oven (38±1°C, 85% relative moisture) for 1h. The dough taken out from the oven was proofed in a room temperature for 15 min again. Steamed bread was made by hands kneading, followed by steaming for 25 min. Steamed bread volume was measured after cooling for 1 h (Zhang, 2004).

Textural analysis: A Texture Analyzer (TA-XTplus) equipped with a Windows version of Texture Expert software (Stable Micro System, Scarsdale, NY) was used to measure bread and steamed bread texture.

Data analysis: The DPS software was used to analyze the data.

RESULTS AND DISCUSSION

Mean square variance analysis of effects of genotype and environment on HMW-GS expression: Genotypes had extremely significant effects on X-type HMW-GS amount and total HMW-GS expression (Table 2). Moreover, effects of genotypes on Y-type HMW-GS, Glu-D1 and

Table 2: Mean squares analysis of effects of genotype and environment on HMW-GS

Source	DF	X-HMW-GS	Y-HMW-GS	Glu-1B	Glu-1D	Total HMW-GS
Environment	7	24.73*	10.51	20.74*	8.24	65.15*
Genotype	2	128.58**	30.20*	24.35*	51.25*	248.76**
G×E	14	12.97	5.31	8.39	7.22	25.68*
Error	48	0.011	0.015	0.011	0.009	0.009

* And ** is significant and extremely significant, respectively

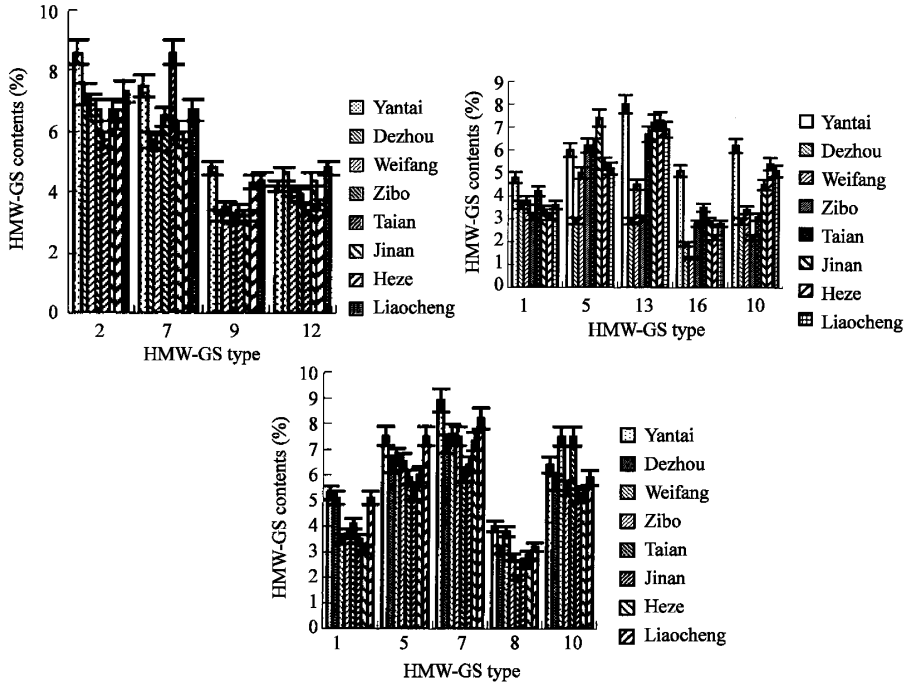

Fig. 1: Comparison of individual HMW-GS content in different locations

Glu-B1 were significant. HMW-GS expression, X-type subunits and Glu-B1 subunits were significantly affected by environments. Their interactions influenced the total HMW-GS expression. Therefore, genotypes played a major role for the expression of HMW-GS and some subunits were affected by environments. These perhaps caused the variance of wheat quality in different environments.

Behaviors in different locations of individual HMW-GS expressing amount: Individual HMW-GS expression in one genotype was more or less affected by locations (Fig. 1). For many of HMW-GS, Yantai location was superior to others'. Performance of one subunit in different genotypes appeared different. For example, Tai'an location showed best effect on Glu-B1x7 in Wei62036; but in Yan278, Yantai appeared excellent. Whether in Jimai20 or Yan278, Glu-A1x1 was affected well by Yantai location. In Jimai20, Glu-D1x5 had a best behavior in Jinan location; while in Yan278, Yantai location was better than others. In contrary, Glu-D1y10 in Jimai20 was affected by Yantai much better than others.

Glu-D1x5 subunit was significantly affected by Dezhou location; while other locations' effects were not significant (Table 3). Behaviors of Glu-D1y10 expression amount were same as that of Glu-D1x5. These indicated that Glu-D1 5+10 expression amount was affected little by locations that is environments, it had a wider adaptation than other subunits. All subunits except Glu-D1y12 appeared better expression in Yantai location that had a significant effect compared with other locations. However, Glu-D1y12 had an optimum expression in Liaocheng location and a lowest expression in Taian. In addition, whether Glu-A1x1, Glu-B1 7+8 or Glu-B1 7+9 or Glu-B1 13+16, their expressions were largely affected by environments, so there was a limited adaptation in production. Effects of environments on Glu-D1 location especially 5+10 expression were smaller than that of them on Glu-B1and Glu-A1.

Therefore, individual HMW-GS expression amount in the same cultivars was affected by environments; the same type of HMW-GS expression amount from different cultivars appeared different in different environments. Each HMW-GS had an optimum environment to develop

Table 3: Effects of different locations on individual HMW-GS contents (%)

Average	Yantai	Dezhou	Weifang	Zibo	Taian	Jinan	Heze	Liaocheng
*Glu-A1x*1	4.93aA	4.40abAB	3.70cBCD	3.50cdCD	3.92bcBC	3.33cdCD	3.10dD	4.47abAB
Glu-B1x 2	8.50aA	7.20bB	6.80cC	6.60dC	5.70fE	6.70cdC	6.30eD	7.30bB
*Glu-D1x*5	6.80aA	4.60bB	5.78aAB	6.33aA	6.02aAB	6.35aA	5.75aAB	6.28aA
*Glu-B1x*7	8.20aA	6.45bcB	6.58bcB	7.10bcAB	7.30abAB	6.25cB	6.55bcB	7.45abAB
*Glu-B1y*8	4.00aA	3.10cdBC	3.80bA	2.80eD	2.10gF	2.50fE	3.00dCD	3.23cB
*Glu-B1y*9	4.80aA	3.20dD	3.50cC	3.10dD	3.43cC	3.20dD	4.30bB	4.40bB
*Glu-B1x*13	8.03aA	2.90gF	4.50eE	3.10fF	6.70dD	7.20bBC	7.30bB	7.00cC
*Glu-B1y*16	5.00aA	2.00eD	1.40fE	2.80cC	3.50bB	2.90cC	2.20dD	2.80cC
*Glu-D1y*10	6.30aA	4.33bAB	5.45abAB	3.88bB	5.33abAB	4.80abAB	5.30abAB	5.50abAB
*Glu-D1y*12	4.10cC	4.50bB	4.00cdCD	3.87dD	3.30fF	4.40bB	3.60eE	4.80aA

Small letters and Capital letters appeared 5% and 1% significant, respectively

Table 4: Correlation analyses between HMW-GS expression amount and steamed bread and bread quality

Correlations		*Glu-A1x*1	*Glu-B1x*2	*Glu-D1x*5	*Glu-B1x*7	*Glu-B1y*8	*Glu-B1y*9	*Glu-B1x*13	*Glu-B1y*16	*Glu-D1y*10	*Glu-D1y*12	X-type	Y-type
	Firmness	-0.163	-0.653	0.001	-0.585	-0.706	-0.323	0.06	-0.207	-0.303	0.027	-0.172	-0.371
Steamed	Springiness	0.179	0.018	0.099	-0.093	-0.472	0.146	0.261	0.533	0.201	0.094	0.24	0.053
Bread	Cohesion	-0.691	-0.764*	-0.468	-0.622	-0.513	-0.664	-0.473	-0.853**	-0.621	-0.046	-0.826*	-0.835**
	Stickiness	0.261	0.404	0.171	0.283	0.364	-0.296	-0.429	-0.087	-0.302	0.649	0.021	-0.027
	Resilience	-0.283	-0.322	-0.122	-0.123	0.161	-0.293	-0.324	-0.643	-0.4	-0.133	-0.363	-0.268
	Volume	0.728*	0.727*	0.107	0.467	0.42	0.274	-0.047	0.55	0.221	0.3	0.387	0.442
	Score	0.101	0.783*	0.138	0.834*	0.833*	0.469	-0.027	-0.011	0.458	0.523	0.213	0.418
Firmness		-0.377	-0.093	-0.163	-0.073	-0.104	-0.53	-0.694	-0.136	-0.595	-0.227	-0.505	-0.57
Bread	Springiness	0.262	0.148	-0.121	0.502	0.473	0.351	-0.227	-0.229	-0.055	0	0.071	0.205
	Cohesion	-0.186	-0.328	0.211	-0.243	-0.324	0.083	0.415	-0.203	0.214	0.272	0.077	0.004
	Stickiness	0.389	0.252	0.364	-0.056	-0.122	0.173	0.466	0.827*	0.324	-0.236	0.534	0.403
	Resilience	-0.325	-0.352	-0.154	0.034	0.065	0.007	-0.208	-0.656	-0.202	-0.02	-0.315	-0.223
	Volume	0.629	0.305	0.3	0.105	0.156	0.53	0.763*	0.529	0.639	0.129	0.726*	0.741*

* And ** are significant at 0.05 and 0.01 level, respectively

and express its amount, which was one of the important reasons to cause different quality in different environments.

Correlation analysis between HMW-GS expression amount and texture characteristics of steamed bread and baking bread: Effects of different HMW-GS expression amount on steamed bread had different coefficients (Table 4). Glu-A1x1 and Glu-D1x2 had significantly positive correlation with steamed bread volume; and Glu-D1x2, Glu-B1x7 and Glu-B1y8 only positively correlated with steamed bread score. Glu-D1 5+10, Glu-B1y9, Glu-D1y12, X-type subunits and Y-type subunits positively correlated with steamed bread volume and score. All subunits except Glu-B1 7+8 positively correlated with steamed bread springiness, but not significantly. Negative correlations appeared significant between steamed bread cohesion and Glu-D1x2, Glu-B1y16, X-type subunits and Y-type subunits.

For bread quality, all HMW-GS expression amounts showed positive correlations with bread volume, and there was significant for Glu-B1x13. X-type subunits and Y-type subunits significantly positively correlated with bread volume. Negative correlations appeared not significant between all HMW-GS expression amount and bread firmness. Moreover, Glu-D1 5+10 and Glu-B1 13+16 negatively correlated with bread springiness, but not significantly, while other subunits appeared positive.

Consequently, whether individual HMW-GS expression amount or total HMW-GS amount, their contributions were different to steamed bread quality and bread quality. Large number of individual HMW-GS expression amount especially Glu-A1x1, Glu-D1x2 and Glu-B1 7+8 positively contributed to steamed bread volume and score. Although, individual HMW-GS appeared positive for bread volume, there was not significant. While, X-type subunits and Y-types subunits showed significant effects on bread volume, this indicated that effects of total HMW-GS amount on bread volume were larger than that of individual HMW-GS, which was possibly caused by their cooperation among HMW-GS subunits. Softness of steamed bread and making bread was positively affected by large number of individual HMW-GS, X-type and Y-type HMW-GS. The quality of Glu-D1 5+10 contributed greater to bread quality than other subunits, but their expression amounts negatively affected bread springiness but positively affected steamed bread. These demonstrated that bread quality was firstly determined by the quality of HMW-GS, secondly by the expression amount of HMW-GS. However, the steamed bread quality was mainly affected by individual HMW-GS expression amounts.

CONCLUSION

Genotypes mainly affected the expression of X-type HMW-GS, Y-type HMW-GS, Glu-D1 location and Glu-B1 location. Total HMW-GS amounts were influenced by the interactions between genotype and environment. Individual HMW-GS expression of the same genotype

was differently affected by environment. Yantai location was superior to other locations for many HMW-GS subunits. The same kind of HMW-GS from different genotypes was different in the same environments. HMW-GS expression amount positively correlated with bread volume and negatively with bread peak firmness. Effects of individual and total HMW-GS on steamed bread quality were different from that on bread quality. Influences of total HMW-GS on bread volume were better than that of individual HMW-GS. Bread quality was firstly determined by the quality of HMW-GS types, while steamed bread quality was mainly affected by the HMW-GS amounts.

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