

Variations in high-molecular-weight glutenin subunits in the main wheat growing zones in China

Jun Ji^{1,3}, Xiaoli Guo², Fa Cui^{1,3}, Dongcheng Liu³, Jiazhu Sun³, Wei Zhang^{1,3}, Aimin Zhang^{3*}, Junming Li^{1,3*}

¹Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang, 050021, P. R. China

²College of Biology, China Agricultural University, Beijing 100193, P. R. China

³The State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P. R. China

*Corresponding author: amzhang@genetics.ac.cn; ljm@sjziam.ac.cn

Abstract

Variations in high-molecular-weight glutenin subunits (HMW-GS) at the *Glu-1* loci were studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method among 1942 advanced lines and cultivars from eight wheat-growing zones in China. There were 26 alleles and 83 types of HMW-GS composition detected, including some interesting and unique alleles and allelic compositions. Among the 26 alleles, the most frequent HMW-GS alleles were null at the *Glu-A1* locus, 7+8 and 7+9 at the *Glu-B1* locus, and 2+12 at the *Glu-D1* locus. The HMW-GS compositions N, 7+8, 2+12 and N, 7+9, as well as 2+12 more frequently occurred than others among the 1942 wheat cultivars and advanced lines. The results can provide useful information for breeding programmes to improve bread-making quality and develop new cultivars.

Keywords: HMW-GS; allelic variation; Growing zone; wheat.

Abbreviations: HMW-GS - High-molecular-weight glutenin subunits; SDS-PAGE - Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Introduction

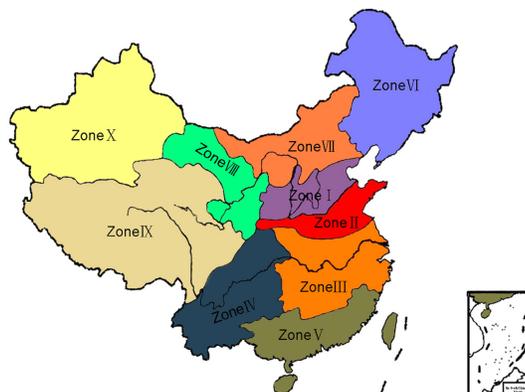
Wheat storage proteins, gliadins, and glutenins are the main components of gluten. They are the main contributors to the rheological and bread-making properties of wheat flour. Gluten proteins, which are polymeric with disulphide bonds linking the individual glutenin subunits, give dough its unique viscoelastic properties. Although high-molecular-weight glutenin subunits (HMW-GS) constitute only 10% of the storage protein of the wheat endosperm (Payne et al., 1981), genotypic variations of HMW-GS have proven to be important in determining bread-making quality in many countries (Lawrence, 1986; Randall et al., 1992; Ahmad et al., 1998; Igrejas et al., 1999; De Bustos et al., 2001; Rodriguez-Quijano et al., 2001). Studies on the genetic determinism of wheat glutenin have revealed that HMW-GS genes are located on the long arm of chromosomes 1A, 1B, and 1D at loci *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively. And each *Glu-1* locus consists of two tightly linked genes, which code for x- and y-type subunits (Payne 1987). In hexaploid wheat, the *Glu-A1* locus codes for 1Ax or null (N) subunit, whereas the *Glu-B1* locus usually codes for 1Bx and 1By. Sometimes, *Glu-B1* codes for 1Bx or 1By subunits only; and the *Glu-D1* locus codes for 1Dx and 1Dy subunits. Thus, for hexaploid wheat, each genotype usually produces three to five HMW-GS (Payne and Lawrence, 1983). For each HMW-GS-coding locus, a high degree of polymorphism has been revealed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for bread

and durum wheat (Payne and Lawrence, 1983; Gupta and MacRitchie 1991; Branlard et al., 1989). Based on quality scores assigned to individual HMW-GS or HMW-GS pairs by Payne (1987) and Lukow et al. (1989), the relation of allelic variations at the three *Glu-1* loci with bread-making quality has been thoroughly studied. Numerous studies have revealed that the allelic variations at the loci play a crucial role in determining dough viscoelastic properties (Lawrence 1986; Randall et al., 1992; Ahmad et al., 1998; Igrejas et al., 1999; De Bustos et al., 2001; Rodriguez-Quijano et al., 2001). The variations can account for 50%–70% of the genetic variation for dough quality in wheat from Britain, Spain, and Canada (Payne and Lawrence, 1983; Payne et al., 1987; Lukow et al., 1989). Studies show that subunit pair 5+10 at the *Glu-D1* locus has played a very important role in the bread-making quality (Rooke et al., 1999; Popineau et al., 2001). Similarly, recent studies have revealed that subunit pair 14+15 at the *Glu-B1* locus has a significant positive effect on the dough viscoelastic properties (Liu et al., 2005; Deng et al., 2011). As one of the largest wheat-growing countries, China plants about 25 million hectares of wheat a year. Owing to variations in the ecological conditions of different wheat-growing areas, China has been divided into 10 different zones based on the ecological type: the North Plain Winter Wheat Zone (Zone I), Yellow and Huai River Valley Facultative Wheat Zone (Zone II), Lower Yangtze River Valley Winter Wheat Zone (Zone III), Southwestern

Table 1. Distribution of 1942 advanced wheat lines and cultivars from eight ecological wheat zones in China.

	Zone I	Zone II	Zone III	Zone IV	Zone VI	Zone VIII	Zone IX	Zone X
No. of genotypes	322	852	74	326	59	130	23	156
Frequencies (%)	16.6	43.9	3.8	16.8	3	6.7	1.2	8

Note: See text for explanation of ecological zones.

**Fig 1.** Distribution of 10 wheat-growing ecological zones in China (Zhuang and He, 2003).

Winter Wheat Zone (Zone IV), Southern Autumn-Sown Spring Wheat Zone (Zone V), Northeastern Spring Wheat Zone (Zone VI), Northern Spring Wheat Zone (Zone VII), Northwestern Spring Wheat Zone (Zone VIII), Qinghai-Tibetan Plateau Spring-Winter Wheat Zone (Zone IX), and Xinjiang Winter-Spring Wheat Zone (Zone X) (Zhuang and He, 2003; Fig 1). Among the ten zones, Zones I, II, III, IV, and VI account for about 85% of the wheat growing area and production in China. Among these five zones, Zone II is the largest area, with an annual wheat acreage of around 12 million hectares. It accounts for about 40% of the national wheat area and 65%-70% of the total wheat production in China (Liu et al., 2005). In the present study, 1942 advanced lines and cultivars from eight of the ten Chinese wheat-growing zones were analysed (excluding Zones V and VII). The aims were to screen current allelic variations in the HMW-GS of Chinese wheat, and provide this information to wheat-breeding programmes for the selection of the best parent genotypes to improve the bread-making quality of wheat.

Results

Distribution of 1942 advanced lines and cultivars in eight different ecological zones

Based on growth habits, origins, and breeding locations of the advanced lines and cultivars, 1942 genotypes were procured from 8 of the 10 different wheat ecological zones in China (Table 1). The genotypes included 322 from Zone I, 852 from Zone II, 74 from Zone III, 326 from Zone IV, 59 from Zone VI, 130 from Zone VIII, 23 from Zone IX, and 156 from Zone X. The lines came from eight zones and nearly half was from Zone II, which is where about 40% of wheat in China is produced.

Variations in HMW-GS

The variations in HMW-GS at the *Glu-1* loci are shown in Table 2. A total of 26 different HMW-GS individual or pairs

of subunits were detected in the 1942 advanced lines and wheat cultivars. Three subunits (1, 2^{*}, and N) were identified at the *Glu-A1* locus. In particular, subunits N and 1 were the most frequent and present in 53.3% and 40.8% of the lines, respectively. The number of cultivars with subunit 2^{*} at the *Glu-A1* was relatively small. At the *Glu-B1* locus, there were 15 alleles of subunit pairs. Among them, the frequencies of subunit pairs 7+8 and 7+9 were much higher than those of others. The frequency of subunit pairs 14+15 was also high among the remaining types. And there were some single or completely absent subunits. There were 8 alleles of subunits pairs detected at the *Glu-D1* locus. Among them, subunit pair 2+12 had the highest proportion, with a frequency of 69.3%. Next was subunit pair 5+10 (27.8%), which was not observed in wheat from Zone IX. The frequencies of the other subunit pairs were very small. Single subunits such as 2 and 12 were also detected. Therefore, the most frequent subunits and subunit pairs of wheat cultivars grown in China are N and 1 at *Glu-A1*, 7+8 and 7+9 at *Glu-B1*, as well as 2+12 and 5+10 at the *Glu-D1* locus. From the eight ecological zones studied, subunits N and 1 at the *Glu-A1* locus had the highest proportion. The frequency of subunit N was generally higher than that of subunit 1, except in Zone VIII, in which there was no difference in frequency between the two subunits. At the *Glu-B1* locus, subunit pairs 7+8 and 7+9 were prevalent. The frequency of subunit pair 7+8 was higher than that of 7+9 in seven zones (Zones I, II, III, IV, VIII, IX, and X), with the exception of Zone IX, where 7+8 was replaced by 6+8. In Zone VI, the frequency of 7+9 was higher than that of 7+8. In Zones II, III, and IV, 14+15 had the second highest proportion. Subunit pairs 6+8 in Zone I and 17+18 in Zone VIII were the third highest in frequency. At the *Glu-D1* locus, 2+12 showed the highest frequency. The good quality 5+10 showed the highest proportion in all zones except in Zone IX, where 2+12 dominated and two single alleles occurred. There was a significant allelic variation in HMW-GS at *Glu-1* locus, particularly for allelic variants at the *Glu-B1* and *Glu-D1* loci. A total of 83 types of HMW-GS compositions were found. Among them, there were 16 major types of HMW-GS composition (Table 2), explaining 86.5% of the variation.

Table 2. Frequencies (%) of HMW alleles (glutenin subunits) in 1942 wheat genotypes from eight ecological zones in China.

Locus	Allele/subunit	No. of samples	Frequency (%)								
			Total	Zone I	Zone II	Zone III	Zone IV	Zone VI	Zone VIII	Zone IX	Zone X
<i>Glu-A1</i>											
<i>a</i>	1	792	40.8	28.6	47.7	29.7	43.3	61.0	43.9	0.0	24.4
<i>b</i>	2*	115	5.9	5.3	2.7	1.4	7.7	28.8	13.1	0.0	9.6
<i>c</i>	Null	1035	53.3	66.2	49.7	68.9	49.1	10.2	43.1	100.0	66.0
<i>Glu-B1</i>											
<i>d</i>	Null	1	0.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
<i>e</i>	6	3	0.2	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0
<i>f</i>	6+8	75	3.9	7.5	2.7	0.0	2.8	0.0	3.1	21.7	6.4
<i>g</i>	7	76	3.9	0.9	0.8	4.1	14.7	0.0	0.8	0.0	9.0
<i>h</i>	8	2	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.6
<i>i</i>	7+8	871	44.9	47.2	44.7	59.5	35.9	45.8	52.3	78.3	41.0
<i>j</i>	7*+8	1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>k</i>	7+9	671	34.6	38.2	37.7	21.6	28.8	50.9	26.9	0.0	33.3
<i>l</i>	7*+9	1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>m</i>	13+16	19	1.0	1.2	1.2	0.0	0.9	0.0	0.0	0.0	1.3
<i>n</i>	13+19	2	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
<i>o</i>	14+15	117	6.0	2.8	9.9	12.2	4.0	0.0	0.8	0.0	0.6
<i>p</i>	17+18	53	2.7	2.2	1.2	0.0	2.8	4.0	10.8	0.0	7.1
<i>q</i>	20	44	2.3	0.0	1.3	2.7	8.9	0.0	0.8	0.0	0.6
<i>r</i>	22	6	0.3	0.0	0.0	0.0	0.0	0.0	4.6	0.0	0.0
<i>Glu-D1</i>											
<i>s</i>	2+12	1346	69.3	67.4	71.8	77.0	66.6	69.5	62.3	95.7	63.5
<i>t</i>	2.2+12	1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
<i>u</i>	3+12	14	0.7	0.0	1.3	0.0	0.6	0.0	0.0	0.0	0.6
<i>v</i>	4+12	27	1.4	1.2	1.4	0.0	1.8	0.0	0.8	0.0	2.6
<i>w</i>	5+12	11	0.6	0.0	1.2	0.0	0.3	0.0	0.0	0.0	0.0
<i>x</i>	5+10	540	27.8	31.4	24.2	23.0	30.7	30.5	36.2	0.0	32.7
<i>y</i>	12	2	0.1	0.0	0.1	0.0	0.0	0.0	0.8	0.0	0.0
<i>z</i>	2	1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0

The other 57 subunits contributed only 13.5%. Among the 16 major types of HMW-GS composition, there were 3 types of HMW-GS composition that showed the highest frequencies and comprised nearly 50% of the total. They were N, 7+8, 2+12; N, 7+9, 2+12; and 1, 7+8, 2+12. The other subunit combinations had low and variable frequencies in the 1942 wheat cultivars and advanced lines evaluated.

The subunit composition N, 7+8, 2+12 was dominant in all zones except Zone VI, where it was lower than 1, 7+9, 2+12 and 1, 7+8, 2+12. Subunit composition N, 7+9, 2+12 had the second highest frequency in all zones except Zone VI. However, in zone VI, N, 7+9, 2+12 was similar to subunit composition N, 7+8, 2+12. The subunit composition 1, 7+8, 2+12 was the third in frequency but lower at Zones I and X. The subunit composition 1, 7+9, 2+12 had the highest frequency at Zone VI, and but varied in frequency in the other zones. The four types of subunit composition were observed the most frequently (53.4%) in the 1942 genotypes of wheat cultivars and advanced lines. On the contrary, wheat cultivars and advanced lines with subunit compositions containing subunit pair 5+10 at *Glu-D1* locus were lower in frequency in the major Chinese wheat-growing zones. Among the remaining HMW-GS composition types (Table 3), there were some high-quality HMW-GS composition types and also some single subunit composition types such as 6, 7, and 8 at *Glu-B1* locus, as well as 2 and 12 at *Glu-D1* locus, which coded for the x-type or y-type only. Interestingly, there was an advanced line with subunit composition 2*, N, 5+10. *Glu-B1* locus contained a silent subunit that did not code for Bx or By.

Distribution of cultivars with high-quality subunit pairs 5+10 and 14+15 across different periods

Based on the breeding period, the cultivars and advanced lines that had subunit pair 14+15 at *Glu-B1* loci or subunit pair 5+10 at *Glu-D1* loci were divided into five different periods (Tab. 5). The results reveal that subunit pairs 14+15 and 5+10 were lower in frequency during the 1960s and 1970s than from the 1980s to 2000s. Especially, 14+15 had the highest proportion and frequency of 50.4% during the 1990s.

Discussion

An overall analysis reveals significant variations in HMW-GS in Chinese wheat cultivars and advanced lines. These variations can provide abundant genetic resources for quality improvement. The frequencies in HMW-GS differ across ecological zones, likely due to the end-use quality, breeding history, and regional adaptation. As the most important wheat production zone, the Yellow and Huai River Valley Facultative Wheat Zone (Zone II) has the highest proportion of subunit N at *Glu-A1*, 7+8 at *Glu-B1*, and 2+12 at *Glu-D1*. This result reveals that wheat bread-making quality in China is lower than that in other countries. For a long time, high yield has been the first priority of wheat breeders in China because of the high demand in wheat production to support the rapidly growing population. The emphasis on yield may be the main reason for the low frequencies of high-quality subunits in current Chinese wheat

Table 3. Frequencies (%) of main HMW-GS composition types in 1942 wheat genotypes from eight wheat-growing zones in China.

Genotypes	No. of	Frequency (%)									
		Total	Zone I	Zone II	Zone III	Zone IV	Zone VI	Zone VIII	Zone IX	Zone X	
Total types	1942	100	36.1	66.3	18.1	47.0	16.9	31.3	3.6	41.0	
Main types of HMW-GS composition	N, 7+8, 2+12	393	20.2	22.7	19.5	29.7	12.9	3.4	26.2	73.9	19.9
	N, 7+9, 2+12	298	15.3	20.8	18.9	9.5	10.4	1.7	5.4	0.0	13.5
	1, 7+8, 2+12	205	10.6	6.5	13.0	9.5	10.1	18.6	11.5	0.0	4.5
	1, 7+9, 2+12	141	7.3	5.6	8.9	4.1	4.3	28.8	5.4	0.0	6.4
	1, 7+8, 5+10	128	6.6	5.6	8.2	4.1	5.8	6.8	6.9	0.0	3.2
	1, 7+9, 5+10	109	5.6	5.6	5.6	1.4	6.4	5.1	9.2	0.0	3.8
	N, 7+8, 5+10	81	4.2	9.3	1.6	6.8	4.0	3.4	3.1	0.0	8.3
	N, 7+9, 5+10	66	3.4	5.3	1.4	6.8	5.5	1.7	3.1	0.0	5.8
	1, 14+15, 2+12	57	2.9	0.6	4.5	2.7	2.5	0.0	0.0	0.0	0.6
	N, 6+8, 2+12	41	2.1	4.7	1.2	0.0	0.9	0.0	0.8	21.7	4.5
	N, 7, 2+12	37	1.9	0.3	0.1	0.0	0.9	0.0	0.0	0.0	4.5
	N, 14+15, 2+12	32	1.6	0.3	2.6	2.7	2.5	0.0	0.8	0.0	0.0
	2*, 7+8, 2+12	25	1.3	1.9	0.2	1.4	1.2	5.1	3.8	0.0	2.6
	1, 20, 2+12	24	1.2	0.0	0.7	0.0	5.5	0.0	0.0	0.0	0.0
	1, 17+18, 5+10	22	1.1	1.2	0.8	0.0	1.2	1.7	4.6	0.0	0.0
	2*, 7+9, 5+10	20	1.0	0.9	1.1	0.0	1.2	1.7	0.0	0.0	1.9
Others	263	13.5	5.6	11.6	21.6	24.5	22.0	19.2	4.3	20.5	

cultivars and advanced lines. Although there are low frequencies of high-quality subunit pairs such as 5+10 and 17+18 across the Chinese wheat ecological zones, a relatively higher proportion of the desired subunit combinations is identified in the Northwestern Spring Wheat Zone than other zones. Noodles are the main daily diet of residents in this zone; thus, wheat cultivars with good dough viscosity and strength are needed. This demand may be the main reason for the higher frequencies of 5+10 and 17+18 in this zone. The overexpression of the *Glu-B1*-encoded subunit Bx7 (Bx7^{OE}) can improve dough strength in wheat cultivars (Lukow et al., 1992; D'Ovidio et al., 1997) as demonstrated for a range of Australian and North American cultivars and lines (Butow et al., 2002; Radovanovic et al., 2002), as well as old Hungarian cultivars (Juhász et al., 2003). In the present study, subunit Bx7^{OE} was present in some cultivars and advanced lines. According to Zhuang and He (2003), there are ten different wheat ecological zones in China. In the present study, 1942 wheat cultivars and advanced lines from 8 out of the 10 different zones were analysed, excluding Zone V and Zone VII. Given the current economic developmental trends in China, farmers in these zones have shifted to livestock and rice cultivation. Some interesting subunits are found in this study, such as the singly expressed subunits at the *Glu-B1* and *Glu-D1* loci, as well as completely silent subunits at the *Glu-B1* locus. All materials benefit the understanding of the genetic mechanisms of subunit gene differentiation. Allelic variations at *Glu-1* loci have significant effects on the flour and bread-making quality of wheat cultivars. According to the quality scores assigned to individual HMW-GS or HMW-GS pairs by Payne (1987) and Lukow et al. (1989), subunits N at *Glu-A1* and subunit pair 2+12 at *Glu-D1* occur at higher frequencies in Chinese wheat than in wheat from other countries (Ng et al., 1989; Tahir et al., 1995; Igrejas et al., 1999). Therefore, investigating the subunit variations in different ecological zones aid the selection of the best genotypes and improve wheat quality for making breads in China.

Materials and methods

Materials

A total of 1942 advanced lines and cultivars were collected from different Chinese wheat-growing zones. They represented the major quality and end-use types grown in China.

Method of HMW-GS extraction

About 1/3 to 1/4 of de-embryoed mature grains were crushed, and HMW-GS extraction was performed as described by Ji et al. (2008). At least three different single kernels per line or cultivar were analysed individually. SDS-PAGE analysis was performed using 8% polyacrylamide gels ($C = 2.67$) to separate HMW-G subunits. The wheat cultivars Chinese Spring, Cheney, and Gao8901 with known HMW-GS bands were used in the electrophoresis as controls. HMW-GS were identified on the gel patterns based on the nomenclature of Payne and Lawrence (1983).

Conclusion

After analysing 1942 advanced lines and cultivars of wheat procured from eight ecological zones in China, twenty-six alleles and 83 types of HMW-GS composition were detected. Among them, the frequencies of subunit N at the *Glu-A1* locus and subunit pair 2+12 at the *Glu-D1* locus were higher than other high-quality subunits and subunit pairs. This finding is the main reason for the inferior quality of bread wheat in China. Some singly expressed subunits at the *Glu-B1* and *Glu-D1* loci, as well as completely silent subunits at the *Glu-B1* locus were found. This information benefits the understanding of the genetic mechanisms of subunit gene differentiation. These findings allelic variations in HMW-GS in Chinese wheat can be used in wheat breeding programmes to improve bread-making quality by selecting the best parent or donor genotypes.

Table 4. Frequencies of HMW-GS composition types in 1942 wheat genotypes from eight wheat zones in China.

HMW-GS composition types	No. of Samples	Frequency (%)	HMW-GS composition types	No. of Samples	Frequency (%)	HMW-GS composition types	No. of Samples	Frequency (%)
2*, 7+9, 2+12	18	0.93	N, 7+8, 4+12	4	0.21	2*, 17+18, 2+12	1	0.05
1, 7, 2+12	16	0.82	1, 22, 5+10	3	0.15	2*, 20, 2+12	1	0.05
2*, 7+8, 5+10	14	0.72	2*, 6+8, 2+12	3	0.15	2*, 22, 2+12	1	0.05
N, 14+15, 5+10	14	0.72	2*, 7, 2+12	3	0.15	2*, 22, 5+10	1	0.05
N, 7, 5+10	12	0.62	1, 13+16, 2+12	2	0.10	2*, 6+8, 4+12	1	0.05
1, 14+15, 5+10	10	0.51	1, 13+19, 5+10	2	0.10	2*, 6+8, 5+10	1	0.05
1, 6+8, 2+12	10	0.51	1, 6, 5+10	2	0.10	2*, 7*+8, 2+12	1	0.05
1, 6+8, 5+10	10	0.51	1, 6+8, 5+12	2	0.10	2*, 7+8, 12	1	0.05
N, 17+18, 2+12	10	0.51	1, 7+9, 3+12	2	0.10	N, 13+16, 5+10	1	0.05
N, 20, 4+12	10	0.51	1, 7+9, 5+12	2	0.10	N, 14+15, 5+12	1	0.05
N, 13+16, 2+12	8	0.41	1, 8, 5+10	2	0.10	N, 17+18, 3+12	1	0.05
N, 17+18, 5+10	8	0.41	2*, 7+8, 3+12	2	0.10	N, 20, 5+10	1	0.05
N, 20, 5+10	7	0.36	N, 7+9, 4+12	2	0.10	N, 6, 2+12	1	0.05
1, 7, 5+10	7	0.36	1, 13+16, 3+12	1	0.05	N, 6+8, 12	1	0.05
2*, 7+9, 4+12	7	0.36	1, 17+18, 3+12	1	0.05	N, 7*+9, 2+12	1	0.05
1, 13+16, 5+10	6	0.31	1, 20, 4+12	1	0.05	N, 7, 3+12	1	0.05
2*, 17+18, 5+10	6	0.31	1, 22, 2+12	1	0.05	N, 7+8, 2	1	0.05
N, 6+8, 5+10	6	0.31	1, 7+8, 4+12	1	0.05	N, 7+8, 2.2+12	1	0.05
1, 7+8, 5+12	5	0.26	2*, N, 5+10	1	0.05	N, 7+8, 3+12	1	0.05
1, 7+9, 4+12	5	0.26	2*, 13+16, 4+12	1	0.05	N, 7+8, 5+12	1	0.05
1, 17+18, 2+12	4	0.21	2*, 14+15, 2+12	1	0.05	N, 7+9, 3+12	1	0.05
1, 7+8, 3+12	4	0.21	2*, 14+15, 4+12	1	0.05			
2*, 7+8, 4+12	4	0.21	2*, 14+15, 5+10	1	0.05			

Table 5. Distribution of cultivars with the high-quality subunit pairs 5+10 and 14+15 across different periods.

Subunit pairs	1960–1969		1970–1979		1980–1989		1990–1999		2000–present	
	No. of Samples	Frequency (%)								
5+10	24	4.4	67	12.4	151	28.0	163	30.2	135	25.0
14+15	2	1.7	3	2.6	28	23.9	59	50.4	25	21.4

Acknowledgments

This research was supported by grants from the National Key Basic Research Development Program of China (973 Program) (No. 2009CB118305), the National High-tech Research Development Program of China (863 Program) (No. 2011AA100103) and the earmarked fund for Modern Agro-industry Technology Research System of China (No. CARS-03-03B).

References

- Ahmad M, Griffin WB, Sutton KH (1998) Quantification of glutenin and gliadin as a measure of bread baking quality by size exclusion and reverse phase HPLC. In: A.E. Slinkard (Ed), Proc 9th Int Wheat Genetics Symp Vol 4. p 124-126
- Butow BJ, Gras PW, Haraszi R, Békés F (2002) The effects of different salts on mixing and extension parameters on a diverse group of wheat cultivars using 2-g mixograph and extensigraph methods. Cereal Chem 79: 823-826
- Branlard G, Autran JC, Monneveux P (1989) High molecular weight glutenin subunit in durum wheat (*T. durum*). Theor Appl Genet 78: 353-358
- De Bustos A, Rubio P, Soler C, Garcia P, Jouve N (2001) Marker assisted selection to improve HMW-glutenins in wheat. Euphytica 119: 69-73
- D'Ovidio R, Masci S, Porceddu E, Kasarda DD (1997) Duplication of the Bx7 high-molecular-weight glutenin subunit gene in bread wheat (*Triticum aestivum* L.) cultivar Red River 68. Plant Breed 116: 525-531
- Gupta RB, MacRitchie F (1991) A rapid one-step one dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. J Cereal Sci 14: 105
- Igrejas G, Guedes-Pinto H, Carnide V, Branlard G (1999) The high and low molecular weight glutenin subunits and ω -gliadins composition of bread and durum wheats commonly grown in Portugal. Plant Breed 118: 297-302
- Juhász A, Gardonyi M, Tamás L, Z. Bedő Z (2003) Characterisation of the promoter region of *Glu-1* Bx7 gene from overexpressing lines of an old Hungarian wheat variety, Grain Qual, 3 Proceedings of 10th International Wheat Genetics Symposium, Paestum, Italy, p 1348-1350
- Lawrence GJ (1986) The high molecular weight glutenin composition of Australian cultivars. Aust J Agr Res 37: 125-133
- Liu L, He ZH, Yan J, Zhang Y, Pena RJ (2005) Allelic variation at the Glu-1 and Glu-3 loci, presence of 1B/1R translocation, and their effect on mixographic properties in Chinese bread wheats. Euphytica 142: 197-204.
- Lukow OM, Payne PI, Tkachuk R (1989) The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. J Sci Agric 46: 451-460

- Lukow OM, Forsyth SA, Payne PI (1992) Over-production of HMW glutenin subunits coded on chromosome 1B in common wheat, *Triticum aestivum*. *J Genet Breed* 46: 187-192
- Ng PKW, Pogna NE, Mellini F, Bushuk W (1989) *Glu-1* allele compositions of the wheat cultivars registered in Canada. *J Genet Breed* 43: 53-59
- Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex loci, *Glu-A1*, *Glu-D1* which code for HMW subunits of glutenin hexaploid wheat. *Cereal Res Commun* 11: 29-35
- Payne PI (1987) Genetics of wheat storage proteins and the effects of allelic variation on bread making quality. *Annu Rev Plant Physiol* 38: 141-153
- Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relation between HMW glutenin subunits composition and bread making quality of british grown wheat varieties. *J Sci Food Agri* 40: 51-65
- Popineau Y, Deshayes G, Lefebvre J, Fido R, Tatham AS, Shewry PR (2001) Prolamin aggregation, gluten viscoelasticity, and mixing properties of transgenic wheat lines expressing 1Ax and 1Dx high molecular weight glutenin subunit transgenes. *J Agric Food Chem* 49: 395-401
- Radovanovic N, Cloutier S, Brown D, Humphreys DG, Lukow OM (2002) Genetic variance for gluten strength contributed by high molecular-weight glutenin proteins. *Cereal Chem* 79: 843-849
- Randall PG, Manley M, Meiring L, McGill AEJ (1992) The high molecular weight glutenin subunits of South African wheats. *J Cereal Sci* 16: 211-218
- Rodriguez-Quijano M, Nieto-Taladriz MT, Gomez M, Vazquez JF, Carrillo JM (2001) Quality influence comparison of some x-and y-type HMW-glutenin subunits coded by *Glu-D1* locus. In: Bedo Z, Lang L (eds), *Wheat in a Global Environment*, Kluwer, Netherlands, p189-194
- Rooke L, Bekes F, Fido R, Barro F, Gras P, Tatham A, Barcelo P, Lazzeri P, Shewry P (1999) Overexpression of a gluten protein in transgenic wheat results in greatly increased dough strength. *J Cereal Sci* 30: 115-120
- Tahir M, Hussain SA, Turchetta T, Lafiandra D (1995) The HMW glutenin subunits of bread wheat varieties bred in Pakistan. *Plant Breed* 114: 442-444
- Zhuang QS, He ZH (ed) (2003) *Wheat improvement and pedigree analysis in Chinese wheat cultivars*. China Agriculture Press, Beijing, p 2-5