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Variation for Glutenin and Waxy Alleles and Their Effect on Quality Properties in Sichuan Wheat Landraces

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Abstract: Genetic variation of high-molecular-weight glutenin subunits (HMW-GS), low-molecular-weight glutenin subunit (LMW-GS) genes, waxy locus (*Wx*) and quality characters, including protein content, wet gluten content, sodium dodecyl sulfate (SDS) sedimentation value and dough rheologic properties, were investigated in sixty-seven Sichuan landraces wheat in China. The relationship between these genetic variation and quality characters were also estimated. Alleles *Glu-A1c* (98.5%), *Glu-B1b* (98.5%) and *Glu-D1a* (100%), were dominant alleles at *Glu-A1*, *Glu-B1* and *Glu-D1* locus, respectively. Five, three and five types of different LMW-GS allele compositions were also identified and *a* (frequencies 74.5%), f (61.2%) and i (55.2%) were the dominant types at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci, respectively. Significant difference ($p<0.05$) between types a and d at *Glu-A3* locus were found in protein content, sedimentation value, wet gluten content and stability time, difference ($p<0.01$) between types g and h at *Glu-B3* locus in sedimentation value, development time and breakdown time and difference ($p<0.01$) between types k and m on wet gluten content. All of the landraces carried the wild type *Wx-A1a* and *Wx-B1a* alleles. These information could be useful to marker-assisted select for different end-use quality wheat.

Key words: HMW-GS, LMW-GS, waxy gene, quality, wheat landraces

INTRODUCTION

Modern wheat (*Triticum aestivum*) breeding programs place high priority on selecting lines with favorable qualities for the intended end-use. Numerous genetic and biochemical studies have focused on the characterization of alleles of wheat quality genes and their influence on end-use quality (D'Ovidio and Masci, 2004; Gianibelli *et al*., 2001; Graybosch, 1992). Among the various quality components, glutenin subunit composition and waxy alleles are of particular interest to wheat breeders.

Glutenin proteins are the major factors responsible for the unique viscoelastic dough characteristics of wheat flour, which determine mixing and bread baking performance (Gianibelli *et al*., 2001). These proteins can be separated into two groups by sodium dodecylsulfate polyacrylamidegel

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electrophoresis (SDS-PAGE): the high molecular weight glutenin (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The genes encoding HMW-GS reside at the *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*), located on the long arms of group 1 homologous chromosomes (Lawrence and Shepherd, 1981; Payne *et al*., 1980). Each *Glu-1* locus comprises two tightly linked genes (designated x and y), which exhibit multiple alleles among different varieties. Payne (1983) and Lawrence GJ (1983) proposed a nomenclature system to facilitate the genotyping of HMW-GS. Different HMW-GS alleles and allelic frequencies have been reported for various sets of wheat germplasm since then (Branlard *et al*., 2003; Graybosch, 1992; Payne and Lawrence, 1983; Redaelli *et al*., 1997; Wei *et al*., 2000). Relationships have also been established between *Glu-1* alleles, especially at the *Glu-D1* locus and bread making quality (Gupta *et al*., 1994; Payne *et al*., 1979, 1981; Pane, 1987). Payne (1987) assigned scores to each identified *Glu-1* allele, which made it possible to predict the approximate bread making quality of wheat cultivars.

The LMW-GS are mostly encoded by the *Glu-3* loci (*Glu-A3*, *Glu-B3* and *Glu-D3*) on the short arms of group 1 homologous chromosomes (Singh and Shepherd, 1988). The estimates of gene copy number varied from 10-15 (Harberd *et al*., 1985) to 35-40 (Cassidy *et al*., 1998; Sabelli and Shewry, 1991). Over the past few years, more efforts have been focused on analysis at the molecular level. To date, more than 90 cDNA and genomic DNA clones of LMW-GS gene have been reported and the gene structure has been well established. On the basis of the sequence data, Van Campenhout *et al*. (1995) designed several primer sets specific for each of the *Glu-3* loci and successfully determined the chromosomal locations of five LMW-GS genes. D'Ovidio *et al*. (1997) and Zhang *et al*. (2004) developed primer sets specific for *Glu-B3* locus and *Glu-A3* alleles, respectively. Ikeda *et al*. (2002) isolated several LMW-GS gene clones from a soft wheat cultivar and classified them into 12 groups based on the N- and C-terminal sequences. And recently, Long *et al*. (2005) analyzed 69 known LMW-GS gene from *Triticum*, classified them into nine groups and developed group-specific primers to detected each group of LMW-GS genes.

Wheat waxy proteins are Granule-Bound, Starch Synthase I (GBSSI) enzymes responsible for amylose biosynthesis (Nakamura *et al*., 1993). Wheat cultivars that carry mutant (null) waxy alleles lack one or more waxy proteins and produce flour with special starch qualities important for white salted noodle making (McLauchlan *et al*., 2001; Zhao *et al*., 1998). Positive effects on bread making quality due to a null waxy allele have also been reported (Martin *et al*., 2004). Waxy genes are situated on chromosomes 7A (*Wx-A1*), 7D (*Wx-D1*) and 4A (*Wx-B1*) and these genes have been cloned and sequenced (Murai *et al*., 1999). Codominant markers are available for identifying the normal and null alleles from all three waxy loci by one simple Polymerase Chain Reaction (PCR) assay (McLauchlan *et al*., 2001), which provides an advantage over the relatively laborious protein analysis method for waxy alleles.

The objectives of this study for Sichuan wheat landraces in China, were to determine the HMW-GS composition, to document for the first time the LMW-GS genes composition, to determine waxy allele composition with the PCR method and to illustrate relationships between these alleles and quality characteristics.

MATERIALS AND METHODS

Plant Material

A total of 67 accessions of wheat (*Triticum aestivum* ssp. *aestivum*) landraces were used in this study (Table 1). All these landraces were collected in Sichuan province and maintained at the Triticeae Research Institute of Sichuan Agricultural University. Each landrace was tested on three lines at normal seed density, with classic nitrogen and mineral supply under full fungicide and herbicide protection from October 2006 to May 2007. Grain harvested in bulk, for each accessions, was used for quality testing in June 2007.

Table 1: HMW-GS, LMW-GS genes and waxy allele compositions of 67 accessions of Sichuan wheat landraces

		HMW-GS			LMW-GS gene			Waxy[g]		
No.	Accessions	*Glu-A1*[a]	*Glu-B1*[b]	*Glu-D1*[c]	*Glu-A3*[d]	*Glu-B3*[e]	*Glu-D3*[f]	*Wx-A1*	*Wx-B1*	*Wx-D1*
1	AS1589	c	b	a	a	h	j	a	a	a
2	AS1647	c	b	a	a	h	i	a	a	a
3	AS1657	c	b	a	a	f	i	a	a	a
4	AS1623	c	b	a	a	h	i	a	a	a
5	AS1588	c	b	a	a	f	i	a	a	a
6	AS1587	c	b	a	a	h	i	a	a	a
7	AS1648	c	b	a	a	h	i	a	a	a
8	AS1649	c	b	a	a	f	j	a	a	a
9	AS1676	c	b	a	a	f	i	a	a	a
10	AS1672	c	b	a	a	f	i	a	a	a
11	AS1652	c	b	a	a	f	i	a	a	a
12	AS1577	c	b	a	a	f	i	a	a	a
13	AS1569	c	b	a	a	f	j	a	a	a
14	AS1659	c	b	a	a	h	i	a	a	a
15	AS1579	c	b	a	a	f	i	a	a	a
16	AS1582	c	b	a	a	f	i	a	a	a
17	AS1572	c	b	a	a	h	i	a	a	a
18	AS1580	c	b	a	b	h	i	a	a	a
19	AS1625	c	b	a	a	f	j	a	a	a
20	AS1563	c	c	a	a	h	j	a	a	a
21	AS1699	c	b	a	a	f	i	a	a	a
22	AS1576	c	b	a	a	g	i	a	a	a
23	AS1675	c	b	a	e	f	j	a	a	a
24	AS1566	c	b	a	a	h	j	a	a	a
25	AS1591	c	b	a	a	h	i	a	a	a
26	AS1656	c	b	a	a	h	j	a	a	a
27	AS1586	c	b	a	a	f	l	a	a	a
28	AS1670	c	b	a	a	f	i	a	a	a
29	AS1575	c	b	a	a	f	k	a	a	a
30	AS1585	c	b	a	c	f	m	a	a	a
31	AS1643	c	b	a	d	f	i	a	a	a
32	AS1592	c	b	a	a	f	i	a	a	a
33	AS1598	c	b	a	a	f	k	a	a	a
34	AS1655	c	b	a	c	f	m	a	a	a
35	AS1590	c	b	a	c	f	m	a	a	a
36	AS1571	c	b	a	e	f	j	a	a	a
37	AS1658	c	b	a	c	f	m	a	a	a
38	AS1556	c	b	a	b	h	i	a	a	a
39	AS1573	c	b	a	a	h	l	a	a	a
40	AS1574	c	b	a	a	f	l	a	a	a
41	AS1593	c	b	a	b	h	i	a	a	a
42	AS1698	c	b	a	b	h	i	a	a	a
43	AS1646	c	b	a	a	f	l	a	a	a
44	AS1641	c	b	a	a	f	i	a	a	a
45	AS1635	c	b	a	a	f	k	a	a	a
46	AS1600	c	b	a	a	f	i	a	a	a
47	AS1702	c	b	a	a	f	l	a	a	a
48	AS1701	c	b	a	a	f	l	a	a	a
49	AS1700	c	b	a	a	f	i	a	a	a
50	AS1596	c	b	a	a	f	i	a	a	a
51	AS1660	c	b	a	a	h	i	a	a	a
52	AS1661	c	b	a	a	h	i	a	a	a
53	AS1663	c	b	a	a	h	l	a	a	a
54	AS1664	c	b	a	a	h	l	a	a	a
55	AS1666	c	b	a	e	f	j	a	a	a
56	AS1667	c	b	a	a	h	l	a	a	a
57	AS1668	c	b	a	a	h	i	a	a	a
58	AS1669	c	b	a	e	f	j	a	a	a
59	AS1679	c	b	a	d	f	i	a	a	a
60	AS1697	c	b	a	a	g	i	a	a	a

Table 1: Continued

61	AS1597	c	b	a	a	g	i	a	a	a
62	AS1594	c	b	a	c	f	m	a	a	a
63	AS1673	c	b	a	d	f	i	a	a	a
64	AS1671	c	b	a	e	f	j	a	a	a
65	AS1627	c	b	a	a	f	l	a	a	a
66	AS1558	c	b	a	a	f	i	a	a	a
67	AS1561	c	b	a	a	h	i	a	a	a

[a]*Glu-A1* allele designations are a = subunit 1, c = null, [b]*Glu-B1* allele designations are b = subunits 7+8, c = subunits 7+9, [c]*Glu-D1* allele designations are a = subunits 2+12, [d]a, included the first, the second and the eight group of LMW-GS genes; b, included the first and the second group of LMW-GS genes; c, included the second and the eight group of LMW-GS genes; d, included the first and the eight group of LMW-GS genes; e, included the second group of LMW-GS genes, [e]f, included the third and the fourth group of LMW-GS genes; g, included the third group of LMW-GS genes; h, included the fourth group of LMW-GS genes, [f]i, included the five, the sixth, the seventh and the ninth group of LMW-GS genes; j, included the fifth, the sixth and the seventh group of LMW-GS genes; k, included the sixth, the seventh and the ninth group of LMW-GS genes; l, included the fifth, the seventh and the ninth group of LMW-GS genes; m, included the fifth, the sixth and the ninth group of LMW-GS genes, [g]a, wild type; b, null allele

Table 2: Group-specific primer sets and chromosome locations of each group of LMW-GS genes

Group	Marker	Primer	Sequence (5'-3')	Product size (bp)	Chromosome location
1	*Glu3A.1*	P1F	GCCGTTGCGCAAATTTCACAG	450 and 600	1AS
		P1R	AACAGATGGATGAATAACTGGTAT		
2	*Glu3A.2*	P2F	AGTGCCATTGCGCAGATGAAT	350	1AS
		P2R	AACGGATGGTTGAACAATAGA		
3	*Glu3B.1*	P3F	GCACAAATGGAGAATAGCCAC	500	1BS
		P3R	AACAAATGGTATTTGTTGTTG		
4	*Glu3B.2*	P5F	CCTAGCTTGGAGAAACCATT	450	1BS
		P5R	CAAGATAGATGGCTGAATAG		
5	*Glu3D.1*	P6F	CCTGGCTTGGAGAAACCATC	500	1DS
		P6F	CAAGATAGATGGCTGAATAT		
6	*Glu3D.2*	P7F	ATGGAGACTAGCCGCGTCCCT	540	1DS
		P7R	TGACCTAGCAAGACGTTGCGA		
7	*Glu3D.3*	P8F	ATGGAGACTAGATGCATCCCT	600	1DS
		P8R	AGATTGGATGGAACCCTGAAC		
8	*Glu3A.3*	P10F	ATGGAGACTAGCTGCATCC	680	1AS
		P10R	CTGCAAAAAGGTACCCTTTT		
9	*Glu3D.4*	P11F	ATGGAGACTAGCTGCATCT	700	1DS
		P11R	CTGCAAAAAGGTACCCTGTA		

Genotyping and Nomenclature

According to the procedure of Ng and Bushuk (1987), HMW-GS were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Using two common wheat lines, Chinese Spring (subunits: null, 7+8 and 2+12) and Chuanyu 12 (subunite: 1, 7+8 and 5+10), as references. Five random seeds were initially analyzed for each accession with an additional 15 seeds analyzed for those which were found to be heterogeneous. HMW-GS were designated according the nomenclature of Payne and Lawrence (1983) and the international nomenclature of McIntosh *et al.* (2003). LMW-GS genes were analyzed according to the PCR method using 7 specific primer sets by Long *et al.* (2005). These group-specific primer sets and chromosome location of each group of LMW-GS genes showed in Table 2. And waxy alleles were also screened using the PCR method by McLauchlan *et al.* (2001).

Quality Tests

The harvested grain was tempered to 14.0% moisture and milled on an automatic mill (Brabender, Germany). SDS sedimentation volume (SDS) was determined according to AACC approved method 56-70 by the mixer made by China Agriculture University, a 5 g sample was test per line. Protein content of flours was determined by BUCHI 321 (Kjeldahl N×5.7), according to ICC standard

No. 105. Dough rheologic properties were estimated in a 10 g Brabender Farinograph. The dry gluten, wet gluten and gluten index of the flour and semolina were determined based on ICC 155 using a Gluten Index System made by Perten. The total starch contents were measured by the method NY/T11. The amylose content was measured according to the standard methods NY 147-88 and then the amylopectin content was calculated accordingly. All of the experiments except for farinagraph were done in duplicate.

Statistical Analysis

All data analysis was performed under the software DPS (Data Proceeding System) version 6.10.

RESULTS

Variation of the HMW-GS

The HMW-GS (*Glu-1*) alleles in sixty-seven Sichuan wheat landraces are shown in k3. All landraces had the same *Glu-D1a* allele (subunits 2+12). Only two different alleles at *Glu-A1* and *Glu-B1* were detected, respectively. At the *Glu-A1* locus, one landrace, AS1673, contained allele *Glu-A1a* (subunit 1), while the other landraces had the *Glu-A1c* (null). At the *Glu-B1* locus, all landraces carried the *Glu-B1b* (subunits 7+8), except AS1563 possessed allele *Glu-B1c* (subunits 7+9) at the *Glu-B1* locus. In these wheat landraces, only three HMW-glutenin compositions were observed, where sixty-five out of sixty-seven landraces (97%) had identical HMW-GS compositions with Chinese Spring (subunits: null, 7+8, 2+12).

Variation of the LMW-GS Genes

This set of Sichuan wheat landraces displayed abundant LMW-GS genes variation (Table 1). Five types of different LMW-GS genes composition at the *Glu-A3* locus, three types of different LMW-GS genes composition at the *Glu-B3* locus and five types of different LMW-GS genes composition at the *Glu-D3* locus were identified. The most common type at *Glu-A3* locus was *a* (74.5%). The other types were found at frequencies of 6.0% (b), 7.5% (c), 4.5% (d) and 7.5% (e), respectively. f (61.2%) was the most common type at the *Glu-B3* locus, followed by *h* (34.3%) and *g* (4.5%). At the *Glu-D3* locus, the frequencies of five types were i (55.2%), j (17.9%), k (4.5%), l (14.9%) and m (7.5%), respectively.

Combined across the three loci, these types form diverse combination patterns with a total of 12 different LMW-GS gene patterns found in all materials. The most common LMW-GS patterns at *Glu-A3*, *-B3* and *-D3* were a/f/i (16 materials), a/h/i (11 materials) and a/f/l (6 materials).

Variation of the Waxy Alleles

The PCR analysis of waxy alleles revealed that all of the Sichuan wheat landraces carried the wild type *Wx-A1a* and *Wx-B1a* alleles (Table 1).

Variation of the Quality Parameters

The 67 Sichuan wheat landraces exhibited a large variation for each of the 14 quality parameters recorded. Grain Protein content varied from 9.54 up to 14.76%. Amylopectin content varied from 30.43 to 43.68%. The large variation also was revealed by the Farinograph parameters (Table 3). For example, Farinograph development time had the maximum values of the coefficient of variation (CV = 59.47%) and the stability time comprised between 0.7 up to 6 min. As the Farinograph parameters, the determined values of the dry gluten, wet gluten and gluten index of the flour and semolina also showed large variation (Table 3).

Table 3: Value of mean standard deviation, minimum, maximum value and coefficient of variation calculated on the 14 quality parameters

Variables	Mean	SD	Minimum	Maximum	CV (%)
Protein content (%)	11.86	1.05	9.54	14.76	9.03
Wet gluten content (%)	35.37	6.88	24.53	44.68	19.45
Dry gluten content (%)	12.58	1.88	8.62	17.08	14.96
Gluten index	63.09	9.90	45.88	83.06	15.70
Sedimentation value (ml)	36.93	5.34	17.00	58.40	25.26
Starch (%)	63.03	1.91	56.82	67.32	3.04
Amylose (%)	24.32	1.20	21.88	28.03	49.43
Amylopectin (%)	38.71	2.25	30.43	43.68	58.02
Development time (min)	1.57	0.93	0.20	3.50	59.47
Stability time (min)	2.74	1.18	0.70	6.00	43.07
Breakdown time (min)	2.93	1.55	0.20	7.40	52.88
Farinogram quality number	29.30	15.49	2.00	74.00	52.88
Mechanical tolerance index (bu)	78.75	24.03	42.00	127.00	30.51
Water absorption ratio (%)	60.58	1.05	57.20	62.60	1.74

Table 4: Differences in protein content, sedimentation value, wet gluten content, development time, stability time and breakdown time among types at *Glu-A3*, *Glu-B3* and *Glu-D3*, respectively

	Glu-A3			Glu-B3			Glu-D3								
Quality parameters	a	b	p[a]	b	d	p	a	b	p	b	c	p	c	e	p
Protein content (%)	11.5	11.6	*	11.6	12.5	ns	11.5	11.5	ns	11.5	11.9	ns	11.9	12.4	ns
Wet gluten content (%)	33.8	34.2	*	34.2	38.4	ns	33.9	34.7	ns	34.7	35.1	ns	31.2	37.2	**
Sedimentation value (mL)	35.8	35.8	*	35.8	47.7	**	37.2	29.5	ns	29.5	37.2	**	31.4	37.9	ns
Development time (min)	1.6	1.8	ns	1.8	1.8	ns	1.6	1.0	ns	1.0	1.6	**	1.8	1.3	ns
Stability time (min)	2.6	2.9	*	2.9	3.8	ns	2.9	1.5	**	1.5	2.5	ns	3.4	3.1	ns
Breakdown time (min)	2.9	3.0	ns	3.0	3.8	ns	3.0	1.5	ns	1.5	3.0	**	3.6	2.9	ns

[a]Significance level: *0.01<p<0.05; **0.001<p<0.01, ns: Not significance

Association Between LMW-GS Genes and Quality Parameters

Variance analysis was first employed to determine the effect of different LMW-GS gene composition at *Glu-A3*, *Glu-B3* and *Glu-D3* loci. Six quality parameters (Protein content, Sedimentation value, Wet gluten content, Development time, Stability time, Breakdown time) have been analyzed (Table 4). All types of LWM-GS genes composition were not significantly different in development time and breakdown time at *Glu-A3* locus. But significant difference ($p<0.05$) between types a and d were found in protein content, sedimentation value, wet gluten content and stability time, whereas the significant difference ($p<0.01$) between b and d in sedimentation value. At *Glu-B3* locus, the three types were nonsignificant difference with each other in protein and wet gluten content. However, the significant difference ($p<0.01$) between f and g was detected in stability time and between g and h in sedimentation value, development time and breakdown time. At the *Glu-D3* locus, significant difference among the five types in all the 6 quality parameters were not detected, except wet gluten content between k and m ($p<0.01$).

Among the 12 different combination patterns of LMW-GS gene type at *Glu-3* locus, variance analysis was also used to determine the difference (Table 5). The pattern (d/f/i) had significantly higher sedimentation value ($p<0.01$), stability time ($p<0.01$) and breakdown time ($p<0.01$) than the pattern (a/g/i). And it also had significantly higher sedimentation value ($p<0.01$) than the other three patterns (a/f/j, a/f/k and a/f/l). Furthermore, it had significantly higher value ($p<0.01$) than the pattern (a/f/j) in protein and wet gluten content. The other two patterns (c/f/m and a/h/l) had significantly higher protein content ($p<0.01$) and wet gluten content ($p<0.05$), respectively than the pattern (a/f/j). No significant differences were found in development time among 12 different combination patterns.

Table 5: Differences in protein content, sedimentation value, wet gluten content, development time, stability time and breakdown time among different LMW-GS gene combined patterns at *Glu-3* loci

Combination patterns	Protein content (%)	Wet gluten content (%)	Sedimentation value (mL)	Development time (min)	Stability time (min)	Breakdown time (min)
d; f; i	12.5	38.4	47.7	1.8	3.8	3.8
a; g; i	11.5	34.7	29.5	1.0	1.5	1.5
p[a]	ns	ns	**	ns	**	**
d; f; i	12.5	38.4	47.7	1.8	3.8	3.8
a; f; j,	10.3	28.5	32.1	1.1	2.8	2.2
p	**	**	**	ns	ns	ns
d; f; i	12.5	38.4	47.7	1.8	3.8	3.8
a; f; k	11.9	31.2	31.4	1.8	3.4	3.6
p	ns	ns	**	ns	ns	ns
d; f; i	12.5	38.4	47.7	1.8	3.8	3.8
a; f; l	11.4	33.1	34.0	1.8	2.7	3.2
p	ns	ns	**	ns	ns	ns
a; f; j	10.3	28.5	32.1	1.1	2.8	2.2
c; f; m	12.4	37.2	37.8	1.3	3.1	2.9
p	**	**	ns	ns	ns	ns
a; f; j	10.3	28.5	32.1	1.1	2.8	2.2
a; h; l	11.9	38.2	39.0	1.6	2.3	2.9
p	**	**	ns	ns	ns	ns

[a]Significance level: *0.01<p<0.05; **0.001<p<0.01, ns: Not significance

DISCUSSION

Present results indicated a very small variation of the HMW-GS alleles (with 5 alleles) in Sichuan wheat landraces. Sixty six out of sixty-seven landraces (98.5%) carried *Glu-D1a* (subunits 2+12) and *Glu-A1c* allele (null), which were of poor quality for bread making (Payne, 1987). However, at the *Glu-B1* locus, all landraces carried the *Glu-B1b* (subunits 7+8), which were considered by Payne (1987) to have a positive effect on bread making quality, except AS1563. Furthermore, we observed that there was a narrow genetic variation of HMW-GS in Sichuan wheat landraces. This was consistent with the report by Wei *et al*. (2000). Previously, Zhang *et al*. (2002) only detected 28 HMW-GS alleles in a core collection of 3,459 Chinese landraces that represented 26.26% of the 13,171 accessions obtained from the National Crop Gene Bank. However, Liu *et al*. (2007) detected 16 HMW-GS alleles when only analyzing a collection of 111 landraces from Hubei province of China recently. So, the variation frequencies of the HMW-GS alleles may be quite different among populations in china.

According to earlier reports the dominant alleles at the three *Glu-1* loci in Chinese wheat landraces were the *Glu-A1c* (subunit null), *Glu-B1b* (subunits 7+8) and *Glu-D1a* (subunits 2+12) (Wei *et al*., 2000; Zhang *et al*., 2002; Liu *et al*., 2007). Present analysis of landraces from Sichuan are consistent with these reports, but the frequencies of the dominant alleles differed in the three populations. In addition, significant differences also occurred in the frequencies of minor alleles between these Chinese landrace collections. For example, whereas low frequencies of the *Glu-A1a* allele (subunit 1) were observed in the accessions from Sichuan and in the Chinese core collection (Zhang *et al*., 2002), relatively a high frequency of this allele was found in lines from Hubei (Liu *et al*., 2007). The frequency of the *Glu-B1c* (subunits 7+9) alleles at the *Glu-B1* locus was also lower in our study than Hubei wheat landraces (Liu *et al*., 2007). It can also be noted that all sixty-seven landraces from Sichuan had the *Glu-D1a* (subunits 2+12) alleles compared with 108/111 (97.64%) in the Hubei accessions (Liu *et al*., 2007).

Screening for LMW-GS was difficult, not only because of the large number of bands, some of which overlapped, but also because of different patterns resolved under different gel conditions and reported in different publications (Branlard *et al*., 2003; Gupta and Shepherd, 1990). So, we directly identified LMW-GS gene groups by PCR method (Long *et al*., 2005), which was more accurate and efficient. The method would be very useful for establishing allele identification standards.

Similar to the efforts to establish associations between HMW-GS alleles and dough quality, studies have also been conducted to link LMW-GS variants to quality traits. Both positive and negative effects of specific LMW-GS on wheat flour quality have been reported by Branlard *et al*. (2001) and Eagles *et al*. (2002). In present study, we directly identified LMW-GS gene groups by PCR method and nine groups (Long *et al*. 2005) have been determined at the Sixty-seven Sichuan wheat landraces *Glu-3* loci. But it was not that all of the groups had been found at every landrace *Glu-3* loci. Five, three and five types of different LMW-GS gene group composition have been found at *Glu-A3*, *Glu-B3* and *Glu-D3* locus respectively. Significant difference ($p<0.05$) had been found in protein content, sedimentation value, wet gluten content and stability time between a and d at *Glu-A3*. From the Table 1, type a contained the first, the second and the eighth groups of LMW-GS genes, type d contained the first, the eighth groups of LMW-GS genes. And type a always associated with low protein content, sedimentation value, wet gluten content and stability time when compared with type d (date not shown). So, the second group of LMW-GS genes might have a negative effect on bread making quality. Long *et al*. (2005) clarified that the LMW-GS genes of group 2 contained less than 90 amino acid residues and included only 13 repeat in its repetitive domain, which was the shortest among the nine LMW-GS groups. The number of repeats present in the repetitive domain of LMW-GS is mainly responsible for the general hydrophilic character (D'Ovidio *et al*., 1997; D'Ovidio and Masci, 2004).

At *Glu-B3* locus, stability time is significant difference ($p<0.01$) between f (include the third and fourth groups) and g (only include the third group). It is indicated the fourth group of LMW-GS genes might play an important role in determining stability tine. Four LMW-GS gene groups were assigned to the short arm of chromosome 1D at *Glu-D3* locus, while three and two groups were located on the short arms of chromosome 1A and 1B at *Glu-A3* and *Glu-B3* loci, respectively (Long *et al*., 2005). It was more complicated than *Glu-A3* and *Glu-B3* loci for analyzing. But significant difference ($p<0.01$) was also found in wet gluten content between types k and m. From the Table 1, type k contained the sixth, the seventh and the ninth groups of LMW-GS genes, type m contained the fifth, the sixth and the ninth groups of LMW-GS genes. The difference between the fifth group and the seventh group might cause significant difference ($p<0.01$) in wet gluten content between types k and m. In marker-assisted breeding, we can choose the fifth group or the seventh group for high or low wet gluten content wheat according to our intention.

Sixty seven Sichuan wheat landraces didn't have null alleles at *Wx-A1*, *Wx-B1* and *Wx-D1* locus. Du *et al*. (2007) determined 1739 China wheat landraces and found only 31 waxy mutation types (mutation frequency was 1.8%). So, the natural mutation frequency of waxy allele was very low.

CONCLUSION

In present study, only 5 alleles were detected at the three HMW-GS *Glu-1* loci in sixty-seven Sichuan wheat landraces of China. The lower variation of HMW-GS alleles in these materials was found compared with Hubei wheat landraces. A total of 13 types of different LMW-GS genes composition at *Glu-3* loci were observed. Materials containing the type d had higher protein content, sedimentation value, wet gluten content and stability time than materials with type a. And significant differences were found in sedimentation value, development time and breakdown time between type g and h. Among the 12 different combination patterns of LMW-GS gene type at *Glu-3* loci, the pattern (d/f/i) had significantly higher sedimentation value, stability time and breakdown time than the pattern (a/g/i). All of the landraces carried the wild type *Wx-A1a* and *Wx-B1a* alleles. This study documents the variability at important quality loci and illustrated the relationships between the types of LMW-GS gene composition and quality characteristics in Sichuan wheat germplasm. These results will be useful to plan crossing and selection strategies to improve end-use quality and to breed high-quality variety.

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