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Allelic Variation of High Molecular Weight Glutenin Subunits in the Hexaploid Wheat Landraces of Tibet, China

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Abstract: The HMW glutenin subunit (HMW-GS) allelic variations of two hundred and twenty nine Tibetan wheat landraces were analyzed by using SDS-PAGE analysis. Seventeen different allelic variations were detected in the evaluated accessions, which were three alleles at the *Glu-A1* locus, nine alleles at the *Glu-B1* locus and five alleles at the *Glu-D1* locus, respectively. Two novel HMW-GS, designated as 5** at the *Glu-D1* in As1243 and 6** at the *Glu-B1* locus in As1510, were screened out. Based on the present results and previous results, it was suggested that the HMW-GS combinations null, 7+8, 2+12, is the predominate types in all the Chinese wheat landraces. It was noteworthy that 5+10, the generally accepted HMW-GS pairs endowing wheat with good bread making quality at *Glu-D1*, could be detected in Tibetan wheat landraces, which have been found very rare in other Chinese wheat landraces. Additionally, the rare subunit combinations 2+10 and 2.1+10.1 were respectively found in Tibetan wheat landrace.

Key words: Tibetan wheat, *Triticum aestivum*, HMW-GS

INTRODUCTION

The main storage proteins in the wheat seed endosperm comprise glutenins and gliadins. The glutenins include two different components, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Lawrence and Shepherd, 1981; Wim and Delcour, 2002). The numbers and combinations of the functional HMW-GS have a profound influence on the end use quality of wheat flours (Shewry *et al.*, 1995; Shewry and Halford, 2002; Wim and Delcour, 2002). In hexaploid wheat (*Triticum aestivum* L, AABBDD, $2n = 6x = 42$), there are three different loci, designated as *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively, encoding for HMW-GS on each of the 1AL, 1BL and 1DL chromosome arms (Lawrence and Shepherd, 1981; Wim and Delcour, 2002). Each locus displays allelic variation that causes differences in protein composition and thus results in differences in baking quality processing among wheat cultivars (Branlard and Dardevet, 1985; Payne *et al.*, 1987, 1988; Luo *et al.*, 2001). Usually, each locus encodes one x and one y type HMW-GS. Owing to gene silencing, there are three to five HMW-GS expressed in hexaploid wheat in most cases, that is two by *Glu-D1*, one or two by *Glu-B1* and one or zero by *Glu-A1* loci, by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne and Lawrence, 1983).

Wheat landrace have many desirable characters and traits that are valuable for wheat breeding. Introduction of these desirable characters and traits into advanced wheat lines has been the practicable way to improve wheat yield, resistance to biotic and abiotic stress as well as processing quality. China has some endemic wheat, including the Sichuan white wheat complex (*Triticum aestivum* L.), the Tibetan semi-wild weedrace (*T. aestivum* var. *tibetanum* (Shao) Yen et J.L. Yang), Yunnan hulled wheat (*T. aestivum* conv. *Yunnanense* King ex Yen and J.L. Yang), Xinjiang rice wheat (*T. petropavlovskyi*

Udacz. and Migusch.) (Shao *et al.*, 1980; Dong *et al.*, 1981; Yen *et al.*, 1988). The HMW-GS compositions of Sichuan white wheat complex, Xinjiang rice wheat and Tibetan semi-wild weedrace wheat have been investigated, which showed a low level of diversity for HMW-GS (Wei *et al.* 2000, 2001). Besides these endemic wheats, there are many other bread wheat landraces. For example, In Tibet, besides the semi-wild weedrace wheat, which has special characters, such as the hulled glume and spike disarticulation, there are many bread wheat landraces. However, there is no information on HMW-GS composition for these wheat landraces.

The objective of this study was to detect the allelic variation of HMW-GS in 229 accession of Tibetan bread wheat landrace using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The information provided in present study will be valuable for the development of breeding strategies to improve bread-making quality.

MATERIALS AND METHODS

Two hundred and twenty nine Tibetan hexaploid wheat landraces were investigated for HMW-GS compositions (Table 1). All of these landraces were collected from Tibetan in 1988 with the financial support of IBPGR, FAO and UN and conserved in the Triticeae Research Institute of Sichuan Agricultural University, Sichuan, China.

Table 1: Materials used in this study

Origin	No.	Cultivars							
Mozhugongka	88	As1010	As1243	As1255	As1267	As1531	As1544	As2056	As2068
		As1011	As1245	As1256	As1268	As1532	As1772	As2058	As2069
		As1013	As1246	As1257	As1271	As1534	As1773	As2059	As2070
		As1014	As1247	As1258	As1272	As1535	As2048	As2060	As2072
		As1015	As1248	As1259	As1273	As1536	As2049	As2061	As2073
		As1016	As1249	As1260	As1274	As1537	As2050	As2062	As2074
		As1238	As1250	As1262	As1276	As1538	As2051	As2063	As2075
		As1239	As1251	As1263	As1527	As1540	As2052	As2064	As851
		As1240	As1252	As1264	As1528	As1541	As2053	As2065	As935
		As1241	As1253	As1265	As1529	As1542	As2054	As2066	WL301
		As1242	As1254	As1266	As1530	As1542	As2055	As2067	Y1882
		As1277							
		As1280	As1284	As1288	As1291	As1442	As1446	As1449	As1520
		As1282	As1285	As1289	As1293	As1444	As1447	As1518	As1521
RikaZe-Lasha	33	As1283	As1286	As1290	As1294	As1445	As1448	As1519	As1522
		As1296	As1302	As1372	As1376	As1380	As1387	As1391	As1401
		As1297	As1366	As1373	As1377	As1381	As1388	As1392	As1402
		As1298	As1367	As1374	As1378	As1382	As1389	As1393	As1403
		As1300	As1371	As1375	As1379	As1383	As1390	As1396	As1404
		As1301							
Qushui	26	As1303	As1313	As1317	As1321	As1324	As1341	As1410	As1426
		As1305	As1314	As1319	As1322	As1339	As1342	As1411	As1428
		As1309	As1315	As1320	As1323	As1340	As1409	As1424	As1776
		As1311	As1316						
Lasha	3	As1343	As1344	As1345					
Gongga	11	As1346	As1348	As1350	As1353	As1355	As1356	As1507	As1508
		As1347	As1349	As1351					
Naidong	11	As1431	As1433	As1435	As1437	As1439	As1440	As1441	As1407
		As1432	As1434	As1436					
Naidong									
-Qiongjie	13	As1490	As1494	As1496	As1500	As1502	As1504	As1505	As1506
		As1493	As1495	As1499	As1501	As1503			
Zedang	8	As1510	As1511	As1512	As1513	As1514	As1515	As1516	As1517
Gongbujiangda	5	As1484	As1523	As1524	As1525	As1526			
Jiangzhi	5	As1405	As1408	As1450	As1452	As1779			
Qiongjie	1	As1483							
Total	229								

As is the code by Triticeae research institute, Sichuan Agriculture University

To determine the electrophoretic mobility of each HMW-GS by SDS-PAGE, standards of Chinese Spring (-, 7+8, 2+12), Chuanyu 12 (1,7+8, 5+10), Longfuma 1 (2*, 7+9, 5+10), Xiaoyan 6 (-, 14+15, 2+12) and Chuannong16 (1, 20, 5+10) that included the specific subunits in the landraces were used. These landraces were analyzed by SDS-PAGE on December 2006 at Triticeae Research Institute of Sichuan Agricultural University according to the procedure of Yan *et al.* (2002). Half of the wheat grains were ground into powder and added HMW-GS extraction buffer with 0.625 M Tris-HCl buffer (pH 6.8) containing 2% (w/v) SDS, 10% (v/v) glycerol and 2 % β -mercaptoethanol, 0.002% (w/v) bromophenol blue and were shaken four times and left at room temperature for 2 h. The suspension was heated at 95°C for 5 min. The supernatant solution was obtained by centrifugation at 6000x g for 5 min and a 10 μ L of the supernatant solution was loaded into a sample well of the SDS-PAGE gel. The electrophoresis was performed at 20 mA by constant current for about 2 h until the tracking dye (bromophenol blue) migrated off the gel. The gels were stained with 0.01% (W/V) Coomassie Blue R250 for 30 min.

During the scoring of the HMW-GS for each accession, the nomenclature system for allelic variation at *Glu-A1*, *Glu-B1* and *Glu-D1* loci by Payne and Lawrence (1983) was adopted. The HMW *Glu-1* quality scores were determined the same as that by Payne *et al.* (1987).

RESULTS

For the 229 Tibetan wheat accessions (Table 1), a total of 19 different combinations (Table 2) and 17 different allele variants (Table 3) (three alleles at the *Glu-A1* locus, nine alleles at the *Glu-B1* locus and five alleles at the *Glu-D1* locus, respectively) were detected in the evaluated accessions. Two types of novel x-type HMW-GS alleles, one at the *Glu-B1* locus in As1510 and the other at the *Glu-D1* locus in As1243, were screened out. We designated the novel x-type HMW-GS in *Glu-B1* of As1510, the electrophoretic mobility of this subunit situated between HMW-GS Bx6 and Bx7, as 6**. We also designated the novel x-type HMW-GS in the *Glu-D1* of As1243, the electrophoresis mobility of this subunit was faster than that of Dx5, as 5** (Fig. 1).

At the *Glu-A1* locus, two active HMW-GS, 1 and 2*, were detected. The null type gene *Glu-A1c*, encoding no HMW-GS on chromosome 1A, was observed at a very high frequency of 94.32% in the

Table 2: Frequencies for HMW glutenin subunit compositions of 229 Tibetan wheat
Locus

<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	Representatives	No. of varieties	Frequencies (%)	Quality scores
Null	7+8	2+12	As1242	175	76.42	6
Null	6+8	2+12	As1513	11	4.80	4
Null	7	2+12	As1383	11	4.80	4
1	7+8	2+12	As1342	7	3.06	8
Null	7+8	2+10	As2059	5	2.18	?
Null	21	2+12	As1428	3	1.31	?
Null	22	2+12	As2060	2	0.87	?
Null	17+18	2+12	As2066	2	0.87	6
Null	6**+8	2+12	As1511	2	0.87	?
Null	7+8	2.1+10.1	As2072	2	0.87	?
Null	7+8	5+10	As1507	1	0.44	8
Null	20	2+12	As1309	1	0.44	?
1	20	2+12	As851	1	0.44	?
1	17+18	5+10	As1531	1	0.44	10
1	7+8	5+10	As1772	1	0.44	10
Null	7+9	2+12	As1532	1	0.44	5
1	7+9	5+10	As1393	1	0.44	9
2*	7+8	2.1+10.1	As1015	1	0.44	?
2*	7+8	5**+10	As1243	1	0.44	?

?Represent the quality scores of some HMW glutenin subunit allelic locus has not assigned, so the total quality scores could not be accounted

Table 3: Frequencies for HMW glutenin subunit at *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively

Locus	Subunit	No. of varieties	Frequencies (%)
<i>Glu-A1</i>	Null	216	94.32
	1	11	4.80
	2*	2	0.88
<i>Glu-B1</i>	7+8	193	84.28
	7	11	4.80
	6+8	11	4.80
	21	3	1.31
	17+18	3	1.31
	6**+8	2	0.87
	7+9	2	0.87
	20	2	0.88
	22	2	0.88
<i>Glu-D1</i>	2+12	216	94.32
	2+10	5	2.18
	5+10	4	1.75
	2.1+10.1	3	1.31
	5**+10	1	0.43

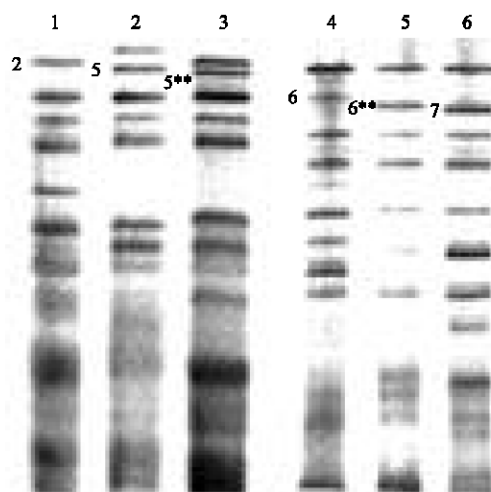


Fig. 1: Two novel high molecular weight glutenin subunits in Tibetan wheat, 1. Chinese Spring 2. Chuanyu12 3. As1243 4. As1513 5. As1510 6. Chinese Spring

229 Tibetan wheat accessions. The two types of the active HMW-GS 1 and 2* were found at a very low frequency of 4.80% and 0.88%, respectively (Table 3), indicating that the predominate HMW-GS type at this locus is the null type.

Among the three loci of *Glu-B1*, *Glu-A1* and *Glu-D1*, *Glu-B1* showed highest variations. There were nine alleles at the *Glu-B1* locus. i.e., 7+8, 7, 6+8, 21, 17+18, 6**+8, 7+9, 20 and 22. The predominate HMW-GS type at *Glu-B1* locus was 7+8 (at a frequency of 84.28%).

In the *Glu-D1* locus, the predominate HMW-GS type was 2+12 (at a frequency of 94.32%). The remaining other four HMW-GS were 2+10, 5+10, 2.1+10.1 and 5**+10, at low frequencies of 2.18, 1.75, 1.31 and 0.43%, respectively. Though HMW-GS combination 2+12 was the predominant HMW-GS type at this locus; the frequency of the other four HMW-GS type were different from the other investigation. It is interesting that, HMW-GS 5+10, the generally accepted subunit pair with good baking quality in wheat flour, is very rare in other Chinese wheat landraces but can be detected

in Tibetan common wheat landraces. Similarly, The HMW-GS 2+10 were found to be very rich in *Aegilops tauschii*, D genome progenitor of the bread wheat, but very rare in bread wheat. However, this study indicated that 2+10 existed in Tibetan wheat landraces. Similarly, subunits 2.1+10.1, mainly occur in *Aegilops tauschii* and are very rare in common wheat (Lagudah and Halloran, 1988), but are also found in Tibetan wheat landraces.

From the HMW-GS composition at the three loci *Glu-A1*, *Glu-B1* and *Glu-D1*, the predominate HMW-GS composition was null, 7+8, 2+12. In one hundred and seventy-five accessions the subunit composition had a frequency of 76.42%. The quality score for most Tibetan wheat lines was 6, but two accessions have the quality score of 10. Some accessions could not assigned a total quality score, because some HMW-GS, such as 6**+8, 2+10. 21, 22, 2.1+10.1, 20, 5**+10, do not have a generally accepted quality score.

DISCUSSION

Based on the investigation of the HMW-GS composition in 229 accession of Tibetan wheat landraces and previous studies (Wei *et al.*, 2000, 2001; Zhang *et al.*, 2002; Wang *et al.*, 2005), it is suggested that HMW-GS combination null, 7+8, 2+12 is the predominate type in all of the Chinese wheat landraces. However, there were differences in Sichuan bread wheat, showing a percentage up to 97.8% (87 out of 89 accessions), which was much higher than that of Tibetan landrace wheat, in which the percentage is 76.42% (175 out of 229 accessions).

In the three different loci encoding for HMW-GS in the hexaploid wheat, the number of variations showed in all of the Chinese wheat landraces differently from each other. The common is that *Glu-B1* detected most variations than both *Glu-A1* and *Glu-D1* did. However, the types detected at the same HMW-GS locus in different Chinese endemic wheat are not the same. In *Glu-A1*, the Tibetan wheat landraces showed all the three types (null, 1, 2*), while Sichuan white wheat complex and Tibetan semi-wild weedrace wheat detected HMW-GS null and 1 and Yunnan hulled wheat and Xinjiang rice wheat showed only the null type at this locus (Wang *et al.*, 2005; Wei *et al.*, 2000, 2001). In *Glu-B1*, the Tibetan wheat landrace detected nine variations, while Yunnan hulled wheat, Xinjiang rice wheat and Tibetan weedrace wheat detected three, four and three types, respectively (Wei *et al.*, 2000, 2001).

In *Glu-D1*, Tibetan wheat showed five variations, while other Chinese endemic wheat showed only 2+12 at this locus except for Xinjiang rice wheat. It is very interesting that both Xijiang rice wheat and Tibetan wheat can showed the HMW-GS 5+10, the one with good baking quality but this is rare in wheat landraces (Wei *et al.*, 2000, 2001; Zhang *et al.*, 2002) and 2.1+10.1, the one very rare in common wheat and absent in other Chinese endemic wheat is rich in *Aegilops tauschii* (Lagudah and Halloran, 1988).

As a whole, it can be conclude that Tibetan wheat has the richest polymorphism in HMW-GS composition among all of the Chinese endemic wheat, including Sichuan white complex, Xijiang Rice wheat, Yunnan hulled wheat and Tibetan weedrace wheat.

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REFERENCES

- Branlard, G. and D. Dardevet, 1985. Diversity of grain protein and bread wheat quality II. correlation between high molecular weight subunit of glutenin and flour quality characteristics. J. Cereal Sci., 3: 345-354.
- Dong, Y.S., D.S. Zheng, D.Y. Qiao, X.Q. Zeng, Z.C. En and X.R. Chen, 1981. Investigation and study on Yunnan wheat (*Triticum aestivum* ssp. *yunnanense* King). Acta Agron. Sin., 7: 145-151.
- Lagudah, E.S. and G.M. Halloran, 1988. Phylogenetics relationships of *Triticum tauschii*, the D genome donor to hexaploid wheat. I. variation in HMW glutenin subunit of glutenin and gliadins. Theor. Applied Genet., 75: 592-598.
- Lawrence, G.J. and K.W. Shepherd, 1981. Chromosomal location of genes controlling seed protein in species related to wheat. Theor. Applied Genet., 59: 25-31.
- Luo, C., W.B. Griffin, G. Branlard and D.L. McNeil, 2001. Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality. Theor Applied Genet., 102: 1088-1098.
- Payne, P.I. and G.J. Lawrence, 1983. Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which code for high molecular-weight subunits of glutenin in hexaploid wheat. Cereal. Res. Commun., 11: 29-35.
- Payne, P.I., M.A. Nightingale, A.F. Krattiger and L.M. Holt, 1987. The relationship between HMW glutenin subunit composition and the bread making quality of British grown wheat varieties. J. Sci. Food Agric., 40: 51-65.
- Payne, P.I., L.M. Holt, A.F. Krattiger and J.M. Carrillo, 1988. Relationship between seed quality characteristics and HMW glutenin composition determined using wheats grown in Spain. J. Cereal Sci., 7: 229-235.
- Shao, Q.Q., C.S. Li and C.R. Basang, 1980. Semi-wild wheat from Xizang (Tibet). Acta Genet Sinica, 7: 150-156.
- Shewry, P.R., A.S. Tatham, P. Barro, P. Barcelo and P. Lazzeri, 1995. Biotechnology of breadmaking: Unraveling and manipulating the multi-protein gluten complex. Biotechnology, 13: 1185-1190.
- Shewry, P.R. and N.G. Halford, 2002. Cereal seed storage proteins: structures, properties and role in grain utilization. J. Cereal Sci., 53: 947-958.
- Wang, H.Y., X.E. Wang, P.D. Chen and D.J. Liu, 2005. Allelic variation and genetic diversity at HMW glutenin subunits loci in Yunnan, Tibetan and Xinjiang wheat. Sci. Agric. Sin., 38: 228-233.
- Wei, Y.M., Y.L. Zheng, D.C. Liu, Y.H. Zhou and X.J. Lan, 2000. Genetic diversity of *Gli-1*, *Gli-2* and *Glu-1* Alleles in Sichuan wheat landraces. Acta Bot. Sin., 42: 496-501.
- Wei, Y.M., Y.L. Zheng, Y.H. Zhou, D.C. Liu, X.J. Lan, Z.H. Yan and Z.Q. Zhang, 2001. Genetic diversity of *Gli-1*, *Gli-2* and *Glu-1* Alleles among Chinese endemic wheats. Acta Bot. Sin., 43: 834-839.
- Wim, S.V. and J.A. Delcour, 2002. Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. Criti Rev. Food Sci. Nutr., 42: 179-208.
- Yan, Z.H., Y.F. Wan, K.F. Liu, Y.L. Zheng and D.W. Wang, 2002. Identification of a novel HMW glutenin subunit and comparison of its amino acid sequence with those of homologous subunits. Chinese Sci. Bull., 47: 220-225.
- Yen, C., M.C. Luo and J.L. Yang, 1988. The Origin of the Tibetan Weedrace of Hexaploid Wheat, Chinese Spring, Chengdu-Guangtou and Other Landrace of White Wheat Complex from China. In Proc 7th Inter Wheat Genet Symp 1988, Miller T.E. and R.M.D. Koebner (Eds.), Bath Press, Bath, UK., pp: 175-179.
- Zhang, X.Y., B.Sh. Pang, G.X. You, L.F. Wang, J.Z. Jia and Y.C. Dong, 2002. Allelic variation and genetic diversity at *Glu-1* loci in Chinese wheat (*Triticum aestivum* L.) Germplasms. Sci. Agric. Sin., 35: 1302-1310.