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ALLELIC COMPOSITION AT *GLU* GENES IN A COLLECTION OF SPRING WHEAT GERMPLASM

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Abstract

For HMW-GS, a total of 16 different subunits and 30 allelic haplotypes were identified. The expression of HMW-GS ranged from 3 to 5 per genotype instead of 6 per genotype, revealing the phenomenon of gene silencing. The 2* was the most frequent subunit for Glu-A1 locus and was found in 52.45% lines. Eight subunits were identified for Glu-B1 locus. Five subunit pairs i.e. 6+8, 7+8, 7+9, 13+16 and 17+18 were present in the germplasm. Some single subunits i.e. 6, 7, 8 and 20 were also found. Subunit pair 17+18 was the most frequent with a partial frequency of 32.17%. For Glu-D1, four subunit pairs i.e. 2+12, 5+10, 2+11 and 5+11 were observed. The 5+10 was the most frequent subunit for Glu-D1 and was present in 65.03% lines. The 2+12 subunit pair was identified in 34.97% lines. For Glu3 loci 18 LMW subunits were identified. For Glu-A3, five subunits i.e. b, c, d, e and g were identified. Eight subunits i.e. b, c, d, f, g, h, i and j were identified for Glu-B3 locus. Four subunits i.e. a, b, c and l were identified for Glu-D3 locus.

Introduction

The property of wheat flour to be used for different products depends mainly on the wheat gluten proteins (Weegels *et al.* 1996). With respect to solubility, wheat proteins are grouped into four classes: albumin, globulin, prolamin and glutens. Gluten comprises 78 - 85% of total protein and is a large complex of mainly polymeric and monomeric proteins known as glutenins and gliadins, respectively (Gupta and MacRitchie 1991). They contribute largely towards the quality of wheat flour. The glutenins are polymeric proteins with disulphide bonds linking the individual glutenin subunits and are responsible for the unique viscoelastic properties of wheat dough. Wheat contains two types of glutenin subunits, one is low molecular weight glutenin subunits (LMW-GS) (10-70 kDa) and the other is high molecular weight glutenin subunits (HMW-GS) (80-130 kDa) (Payne *et al.* 1980). Both HMW and LMW glutenin subunits play a major role in determining the viscoelastic properties of wheat flour. Although HMW-GS represents only 10% of the endosperm storage proteins (Payne *et al.* 1981), genetic diversity of HMW-GS is important for end use quality of wheat (Rehman *et al.* 2014).

Genes underpinning HMW-GS have been reported on chromosomes 1AL, 1BL and 1DL at loci Glu-A1, Glu-B1 and Glu-D1, respectively. Each of these loci contains two genes coding for x- and y-type subunits (Payne 1987). A high degree of polymorphism for each locus has been observed using SDS-PAGE (Sajjad *et al.* 2012, Rehman *et al.* 2014). Many studies based on the quality scores assigned by Payne (1987) and Lukow *et al.* (1989) have revealed that the genetic variations at these loci play a critical role in determining dough properties (Rodriguez-Quijano *et al.* 2001).

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These variations contribute to 50 -70% of the genetic variation for wheat dough properties (Lukow *et al.* 1989). Wheat flour is used fo making chapatti and bread. A wheat genotype could express maximum six HMW-GS but due to gene silencing most of the cultivars express only 3-5 HMW-GS (Tariq *et al.* 2018).

LMW-GS comprise almost 70% of glutenins and 20 - 30% of the total protein (Melas *et al.* 1994) and 15 - 20 diverse LMW-GS proteins can be recognized in 1D and 2D gels of hexaploid wheat (Lew *et al.* 1992). In spite of their abundance, very little research work has been carried out on them as compared to HMW-GS, mainly because of the difficulty in their identification in SDS-PAGE gels. The major hurdle in their identification was the overlapping between LMW-GS and gliadins. The present investigation shows the composition of HMW-GS and LMW-GS in a collection of 116 spring wheat landraces, breeding lines and cultivars.

Materials and Methods

A collection of 116 spring wheat landraces, breeding lines and varieties was included in the study. Storage proteins were extracted from single seed following sequential procedure developed by Singh *et al.* (1991). The extracted proteins were run on SDS-PAGE using the protocol standardized by Sambrook and Russell (2001). Allelic variation for HMW-GS at Glu-1 loci was recorded according to Payne and Lawrence (1983). Quality scores were calculated according to Payne (1987) by adding together the scores of individual sub-units. Allelic variation for LMW-GS was determined according to Jackson *et al*. (1996). Allelic designation of each subunit was adopted from MacGenes (McIntosh *et al.* 2008). The genetic diversity at each locus was calculated using Nei's index (Nei 1973) using formula, $H = 1 - \sum Pi^2$ where H and Pi denote the genetic variation and the frequency of alleles, respectively.

Results and Discussion

Considerable diversity of HMW-GS was found in the germplasm. A total of 16 different subunits and 30 allelic haplotypes were identified. All the common HMW glutenin subunits were found in the germplasm. The expression of HMW-GS ranged from 3 to 5 per genotype instead of 6 per genotype, revealing the phenomenon of gene silencing. Three subunits i.e. 1, 2* and Null were identified for Glu-A1 locus. The 2* was the most frequent subunit for Glu-A1 locus and was found in 52.45% of the lines (Fig. 1). The subunit 1 was found in 41.26% genotypes while null was present in 6.99% genotypes. Eight subunits were identified for Glu-B1 locus. Five subunit pairs i.e. 6+8, 7+8, 7+9, 13+16 and 17+18 were present in the germplasm. Some single subunits i.e. 6, 7, 8 and 20 were also found. Subunit pair 17+18 was the most frequent with a partial frequency of 32.17. It was followed by subunit pair 7+9 with a partial frequency of 28.67. Subunit pair 7+8 also had relatively high proportion with a partial frequency of 15.38%. Other subunits were not as frequent as the ones explained above (Fig. 1). For Glu-D1, four subunit pairs i.e. 2 + 12, 5+10, 2+11 and 5+11 were observed. The 5+10 was the most frequent subunit for Glu-D1 and was present in 65.03% lines. 2+12 was identified in 34.97% lines. The other two subunit pairs had very low frequencies (Fig. 1). A marked difference was observed for the frequencies of different subunits between the modern cultivars (Pakistani and CIMMYT) and pre-green revolution germplasm (LLR). The subunits Null and 1 for Glu-A1, 13+16 and 20 for Glu-B1 and 2+12 for Glu-D1 were more frequent among the LLR group genotypes but these subunits were less frequent in modern cultivars.

According to the number of alleles expressed at Glu-A, Glu-B and Glu-D loci a total of 108 haplotypes were expected but anthropogenic selection reduced the observed number of haplotype to about 30 (Table 1). Deviation from Hardy-Weinberg's equilibrium was also evident from

unequal frequencies of haplotype in selected germplasm. The most frequent haplotypes were "1, 17+18, 5+10" and "2*, 17+18, 5+10". However, 25 haplotypes had frequency less than 5 (Table 1)

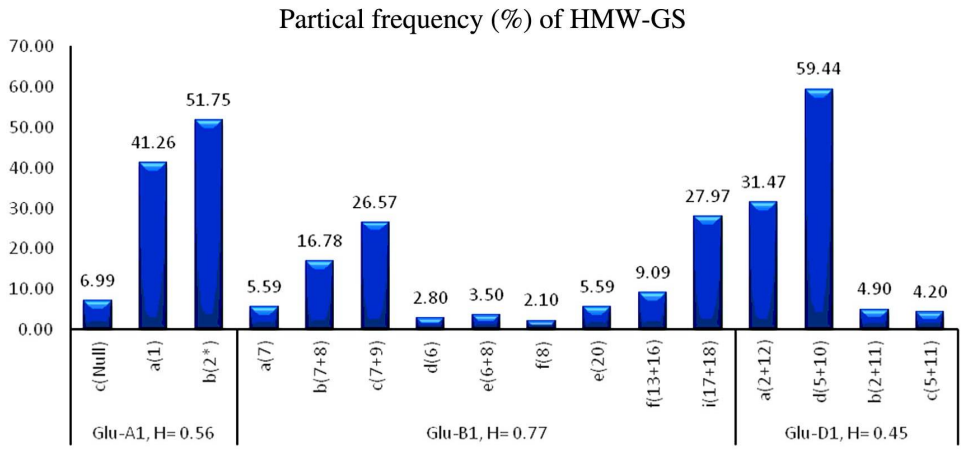

Fig. 1. Partial frequencies of HMW-glutenin subunits.

Total 16 Glu-1 alleles were observed (Fig. 1). A similar extent of allelic diversity at Glu-1 loci has been reported previously (Liu *et al.* 2007 and Sajjad *et al.* 2012). Jun *et al.* (2012) identified 26 HMW glutenin subunits with 83 different combinations in a population of 1942 advanced lines and cultivars from eight different wheat-growing zones of China. The genotypes studied had a high frequency of high quality subunits i.e. 5+10, 17+18 and 2*. This is probably because the material mainly consisted of approved Pakistani varieties and advanced lines. In Pakistan, chapatti quality is an important criterion for selection of the wheat lines. The frequency of these high quality subunits is higher than that reported by Jun *et al.* (2012) in 1942 Chinese cultivars and Sajjad *et al.* (2012) in Pakistani and CIMMYT lines. A considerable variation in the frequency of subunits was observed between the pre-green revolution and post green-revolution germplasm. The landraces had a high frequency of null and 1 subunits for Glu-A1, subunit 20 for Glu-B1 and subunit pair 2+12 for Glu-D1. This distribution was very much like the one found by Jun *et al.* (2012) in Chinese cultivars and by Rehman *et al.* (2014) in Pakistani landraces. The phenomenon of higher allelic diversity in landraces than those in modern varieties has also been observed at 44 SSR loci in Pakistani wheat collection (Sajjad *et al.* 2018). Besides landraces, D-genome synthetic wheat lines also exhibited higher allelic diversity Glu-1 loci than common Pakistani wheat varieties (Tariq *et al.* 2018). The accessions with high quality HMW glutenin subunits also performed better in terms of chapatti quality and NIR gluten. The varieties Lasani, Sehar, LU26s, Shafaq and several others had the best combination of HMW-GS. These varieties also had very good scores of gluten, chapatti and bread quality (Rehman *et al.* 2014).

Eighteen LMW subunits were identified for Glu3 loci (Fig. 2). All the common LMW glutenin subunits were found in the germplasm. Every line had 3 - 5 subunits. For Glu-A3, five subunits i.e. b, c, d, e and g were identified. Glu-A3c had the highest frequency of 52.45%, followed by Glu-A3b (20.98%) and Glu-A3d (14.75%). Other subunits had very low frequencies. Eight subunits i.e. b, c, d, f, g, h, i and j were identified for Glu-B3 locus. Glu-B3j (26.57%), Glu-B3h (22.38%), Glu-B3b (15.38%), Glu-B3i (13.29%) and Glu-B3g (11.89%) had the higher

Table 1. Observed haplotypes at Glu1 genes in selected germplasm.

Sr. no.	GluA1	GluB1	GluD1	Frequency		Glu score
1	1	7+8	5+10	1	Faisalabad-08	10
2	1	13+16	5+10	7	T11, T12, ZA-77, PASBAN 90, SA-42, SA-75, WH542	10
3	1	17+18	5+10	18	T25, 8A, 9D, YECORA, KIRAN-95, MEHRAN-89, SULEMAN-96, ZARGOON-79, CHAKWAL-97, CHENAB-79, FAISALABAD-85, LYP-73, MANTHAR, PARI-73, PASINA-90, BOBWHITE'S', G109, G110,	10
4	2*	7+8	5+10	8	T24, SEHER-06, CHAKWAL-50, JAUHAR-78, SH-2002, OASIS, SAAR, G113	10
5	2*	13+16	5+10	3	SINDH-81, Naeem-82, PRL'S'/PVN	10
6	2*	17+18	5+10	17	PARWAZ-94, SHAFAQ-06, AARI-10, K|HIRMAN, SOGHAT-90, ZARDANA 89, ZARLASHTA 99 PUNJAB-76, PUNJAB-85, PUNJAB-96, V-90A332, PBW 450, FRET-2, KARIEGA, G107, G112, ZAMINDAR-80	10
7	1	7+9	5+10	3	ABADGAR-93, KOHSAR95. PAK81	9
8	2*	7+9	5+10	8	T19, LASANI-08, ANMOLE91, SALEEM2000, PIRSABAK-04, LU26, WATAN, NACOZARI76	9
9	2*	7+8	2+12	3	C-258, PIRSABAK-05, ZARDANA89	8
10	2*	17+18	2+12	4	INQLAB-91, SARSABZ, BLUE SILVER, MEXIPAK65	8
11	2*	7	5+10	3	BAYA'S', FRET-1, G116	8
12	1	13+16	2+12	1	T16	8
13	1	17+18	2+12	4	T23, C-250, MIRAJ-08, CHENAB-70	8
14	1	7	5+10	2	BACANORA, BYRSA-87	8
15	1	20	5+10	2	C273, C271	8
16	1	20	2+12	2	BHITTAI, KAKATSI	6
17	1	7+8	2+12	7	T13, T14, T17, CHAKWAL-86, KOHISTAN97, CHAM-6, HARTOG	8
18	1	7+9	2+12	2	Fsd-83, IQBAL-2000	7
19	1	6+8	5+10	1	T.J-83	8
20	2*	7+9	2+12	3	GA-2002, BHAKKAR2000, G107	7
21	2*	13+16	2+12	4	T20, FAREED-6, MARVI2000, V-03007	8
22	2*	20	2+12	1	C-518	6
23	2*	6+8	2+12	3	SASSI, CHAM-4, PFAU/WEAVER	6
24	0	13+16	5+10	1	T22	8
25	0	6+8	5+10	1	T15	6
26	0	17+18	2+12	1	PUNJAB-96	6
27	0	7+8	5+10	1	T18	8
28	0	7+8	2+12	1	C-245	6
29	0	20	2+12	3	C-217, C-228, C-591	4
30	0	7	2+12	1	LOCAL TALL	4

frequencies for the locus, while, others had very low frequencies (Fig. 2). Four subunits i.e. a, b, c and l were identified for Glu-D3 locus. The allele *b* was the most frequent subunit for Glu-D3 locus and was found in 51.75% of the lines, followed by Glu-D3a which was present in 36.36% lines. The other two subunits had very low frequencies (Fig. 2).

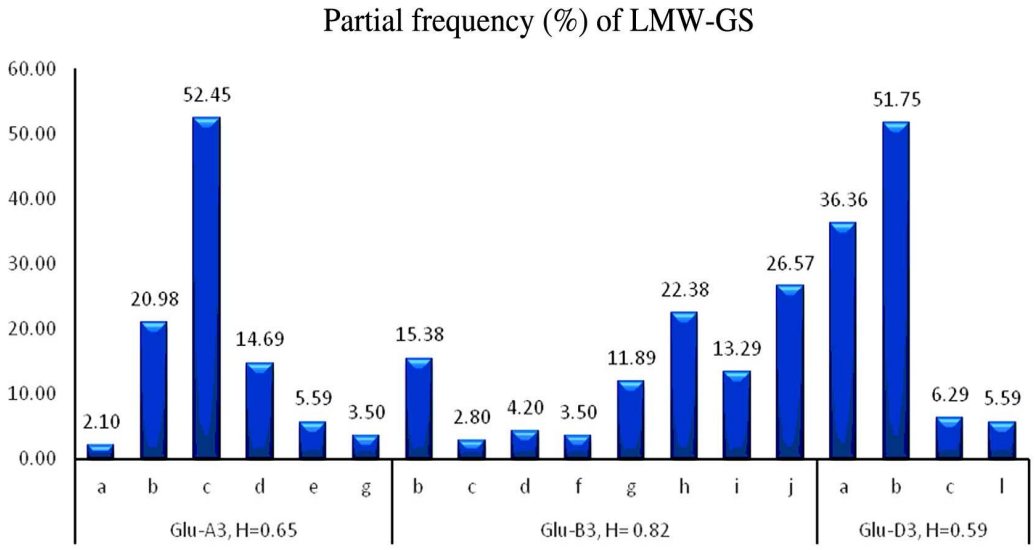

Fig. 2. Partial frequencies of LMW-glutenin subunits in 92 spring wheat genotypes.

HMW-GS are important in determining wheat dough elasticity, and LMW-GS are related to dough extensibility and gluten strength (Cornish *et al.* 2001, Ma *et al.* 2005). Si *et al.* (2012) found 10 Glu-B3 subunits using molecular marker approach which is the best suited approach for the identification of LMW glutenin subunits. The frequency pattern reported was 'i'>'a'>'d'> 'g'>'f'>'b'>'e'>'c'>'j'>'h'. Subunit b had a more pronounced effect on gluten strength. Rehman *et al.* (2014) also reported the presence of superior quality subunits for both high and low molecular weight glutenin subunits. The most frequent LMW-GS were Glu-A3c, Glu-B3j, and Glu-D3b. These results are in line with the ones reported in the present study. The identification of LMW-GS using SDS-PAGE is a difficult job because of high number of candidate subunits and their similar molecular weight. Some better approaches e.g. MLDi-TOF MS, 2D-electrophoresis and molecular markers are recommended for this purpose. The use of allele specific molecular markers is the best approach for this purpose. It is possible to improve the results of the present study regarding LMW-GS by using any of the above stated techniques.

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