Greener Journal of Agricultural Sciences

ISSN: 2276-7770; ICV: 6.15

Vol. 6 (11), pp. 349-355, December 2016

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http://gjournals.org/GJAS



Research Article (DOI: http://doi.org/10.15580/GJAS.2016.11.120816211)

High Molecular Weight Glutenin Subunit Variation among Indian Wheat Varieties

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ARTICLE INFO ABSTRACT

Article No.: 120816211

DOI: 10.15580/GJAS.2016.11.120816211

Submitted: 08/12/2016 **Accepted:** 19/12/2016 **Published:** 27/12/2016

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Keywords:

Bread wheat, SDS-PAGE, quality score and chapatti

Improving wheat for end use quality is an important goal for wheat breeders. The composition of high molecular weight glutenin subunits (HMW-GS) contributes to a great extent in determining wheat quality for processing. The HMW-GS are known to be encoded by six genes located at the *Glu-1* loci on the long arms of chromosomes 1A, 1B and 1D. The endosperm storage proteins of 62 Indian bread wheat cultivars were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to determine their high molecular weight (HMW) glutenin subunit composition. In total, three alleles (Ax2*, Ax1 or Ax null) at *Glu-A1*, seven allele/allelic pairs (Bx7, Bx7+By8, Bx7+By9, Bx20, Bx13+By16, Bx13+By19 and Bx17+By18) at *Glu-B1* and two allelic pairs (Dx2+Dy12 and Dx5+Dy10) at Glu-D1 were identified in the analysed varieties. Allele or allelic pair Ax2* (59.7%) at *Glu-A1*, Bx17+By18 (27.4%) at *Glu-B1* and Dx2+Dy12 (69.4%) at *Glu-D1* were present most frequently in wheat varieties taken for study.

Quality score of varieties were ranged from 4 to 10, with an average of 7.55 indicating vast genetic diversity among the varieties. The presence of subunits combinations in different varieties with low, medium and high gluten strength indicated that breeding wheat for bread, chapatti and biscuits is possible as per end use requirement.

INTRODUCTION

High molecular weight glutenin subunit composition of a genotype heavily determines the end use quality of wheat. These high molecular weight glutenin subunits are encoded by genes at the Glu-A1, Glu-B1 and Glu-D1 loci on the long arm of homoeologuous chromosomes 1A, 1B and 1D, respectively (Payne and Lawrence, 1983). These loci encode an x-type subunit of higher molecular weight and a y-type subunit of lower molecular weight (Harberd et al., 1986). Most of the bread wheat cultivars possess from three to five active high molecular weight glutenin subunits due to silencing of some of these genes. The HMW-GS composition of a genotype is one of most important genetic factor for determining processing quality of wheat. There are differences in quality effects of glutenins coded by same locus. The alleles Ax1 and Ax2* of Glu-A1 have been found to have a better effect on bread-making quality compared to Axnull allele (Gupta et al., 1994; He et al., 2005). The High Molecular Weight subunits Dx5+ Dx10 of the Glu-D1 are correlated with higher dough strength, whereas the Dx2+ Dx12 alleles are correlated with poor bread-making quality (Branlard and Dardevet, 1985; Glanibelli et al., 2001).

Deletions at different HMW-GS loci on A,B or D genomes have shown to produce processed products with varied quality scores (Jondiko et al., 2012; Mondal et al., 2008; Uthayakumaran et al., 2003) and specialty breads with improved quality (Zhang et al., 2014). Rheological studies on deletions of different high molecular glutenin subunits loci at one genome and keeping intact HMW loci at other genome helps to investigate the role of each of these subunits on dough functionality (Jondiko et al., 2012; Mondal et al., 2008). Quality scores were assigned to individual high molecular weight glutenin subunits based on sodium dodecyl sulfate (SDS) sedimentation volume and alveographic parameter W (Payne et al., 1987; Pogna et al., 1992). Characterization of wheat genotypes for high molecular weight glutenin subunits is useful for breeders in choosing parental lines and selecting progenies with superior gluten quality.

In this study, the electrophoretic patterns of HMW glutenin subunits were analysed from 62 spring wheat cultivars released between 1965 and 2001. These cultivars were developed for five wheat agroclimatic zones of India, viz, North Western Plains Zone (NWPZ), Northern Hills Zone (NHZ), North Eastern Plains Zone (NEPZ), Central Zone (CZ) and Peninsular Zone (PZ) (Table1).

Table 1. HMW glutenin subunit at Glu1 loci, year of release and zone of cultivation of Indian wheat cultivars

S.No.	variety	Year of	Zone	of	Sowing conditions	GluA1	GluB1	GluD1
1 2 3 4 5 6 7 8 9 10 11 12 13	PBW175 HD1949 HD2281 HD1982 HD2285 PBW226 HD2009 HD2270 HD2327 HD2380 HD2501 HDR77 HUW234	release 1989 1971 1983 1974 1983 1989 1975 1984 1982 1989 1990 1990 1985	cultivation NWPZ Western UP NWPZ NEPZ NWPZ NWPZ NWPZ NWPZ NWPZ NWPZ NWPZ NW		TS,RF TS,IR TS,IR LS/TS LS/VLS,IR LS,IR TS,IR LS,IR TS,IR LS,IR TS,IR LS,IR TS/LS,IR/RF TS,IR LS,RF LS,IR	2* 2* 2* 2* 2* 2* 2* 2* 2* 2* 2* 2* 2* 2	7+8 7+8 7+8 7+8 7+8 7+8 7+8 7+8 7+8 7+8	2+12 2+12 2+12 2+12 2+12 2+12 2+12 2+12
14	NW 1014	1997	NEPZ		TS,IR	2*	7+8 7+8	2+12
15 16 17 18 19 20 21	NP846 UP2338 HD2189 Kalyansona HD2329 HD2385 WL711 Hindi62	1965 1990 1979 1967 1982 1987 1977	NHZ NHZ PZ NWPZ NWPZ FEZ NWPZ NEPZ		TS,RF/LIR TS,IR TS,IR TS,IR TS,IR LS/IR TS/IR TS/IR	2* 2* 2* 2* 2* 2* 2* 2*	7+8 17+18 17+18 17+18 17+18 17+18 17+18 17+18	2+12 2+12 2+12 2+12 2+12 2+12 2+12 2+12
23 24 25 26 27 28 29	Sonalika Kanchan HD2687 NP852 Lermarojo NW1012 Kundan	1965 1994 1999 1965 1965 1997	NW/NEPZ CZ NWPZ NEPZ CZ NEPZ NWPZ		LS/IR TS,IR TS/IR TS/LS,IR TS,IR TS,IR TS,IR	2* 2* 2* 2* 2* 2* 2*	7+9 7+9 7+9 7+9 13+16 13+16 17+18	2+12 2+12 2+12 2+12 2+12 2+12 5+10
30 31	HI385 LOK1	1975 1981	CZ CZ		TS,RF TS/LS,IR	2* 2*	17+18 17+18	5+10 5+10

32	WH542	1992	NWPZ	TS,IR	2*	7+9	5+10
33	PBW299	1993	NWPZ	TS,RF	2*	7+9	5+10
34	HD2733	2001	NEPZ	TS,IR	2*	7+9	5+10
35	PBW435	2004	NWPZ	LS, IR	2*	7+9	5+10
36	NP824	1960			2*	7+9	5+10
37	Vidisha	1996	CZ	LS,IR	1	17+18	2+12
38	Raj3077	1989	NWPZ	TS/LS,IR	1	17+18	2+12
39	GW 190	1994	CZ	TS,IR	1	7+9	5+10
40	HS240	1989	NHZ	TS,RF/LIR	1	7+9	5+10
41	HUW206	1983	NEPZ	TS,IR	1	7+9	5+10
42	MACS2496	1991	PZ	TS,IR	1	7+9	5+10
43	NIAW34	1995	PZ	LS/VLS,IR	1	7+9	5+10
44	PBW373	1993	NWPZ	TS,IR	1	7	5+10
45	PBW343	1996	NWPZ	LS,IR	1	7	5+10
46	RAJ3765	1995	NW/NEPZ	LS/VLS,IR	1	7	5+10
47	NI5439	1973	PZ	TS,IR/RF	N	17+18	2+12
48	NI5643	1978	PZ	TS,IR	N	17+18	2+12
49	NI747-19	1973	PZ	TS,RF/LIR	N	17+18	2+12
50	C306	1965	NWPZ/NEPZ	TS/RF	N	20	2+12
51	HW2004	1995	CZ	TS,RF	N	20	2+12
52	N-8223	1991	PZ	TS,RF	N	20	2+12
53	C-518	-	-	-	N	20	2+12
54	C-591	-	-	-	N	20	2+12
55	WG357	1973	NWPZ	TS,IR	1	20	2+12
56	C273	-	-	-	1	20	5+10
57	PBW138	1987	NWPZ	TS,IR	N	13+19	2+12
58	NIAW301	2001	PZ	TS,IR	N	7+9	2+12
59	Sonora-64	1965	NPZ/CZ	LS/LS,IR	1	17+18	5+10
60	MP3054	-	-	-	1	17+18	5+10
61	Kharachia-65	1970	All zones	TS,IR	2*	20	2+12
62	Chinese Spring				N	7+8	2+12

MATERIALS and METHODS

Plant material: Seeds of 62 bread wheat varieties (Table 1) collected from different research and development institutions in India and maintained at Gamma field, Bhabha Atomic Research Center, Mumbai were used for this study. The grains were ground to fine powder and 10mg was weighted in 1.5mL micro tube. 250 µl of protein extraction buffer (Tris-Hcl 0.05M, pH.8, 0.02% SDS, 30.3% urea, 1% 2-mercaptoetnanol) was added to each micro tube, boiled on flame for 10 minutes, and then after cooling centrifuged at 13000rpm for 10 min. The high molecular weight glutenin subunit profile of wheat varieties was resolved by sodium dodecyl sulfate polyacrylimide gel electrophoresis (SDS-PAGE) procedure according to Laemmlii (1970) and latter modified by Bhagwat and Bhatia (1993). The high molecular weight glutenin subunit variation was identified using the numbering system of Payne and Lawrence (1983). The quality scores was assigned to each HMWGS of a subunit pair using scoring described by Payne et al. (1987) and Pogna and Mellini (1986).

RESULTS

Amongst the 62 varieties analysed for high molecular weight glutenin subunits, three alleles and their corresponding subunits Glu-A1a (1), Glu-B1b (2*) and Glu-A1c (Null) with 16.1, 24.2 and 59.7% frequency

were observed. Allelic variation at *Glu-B1* locus showed seven alleles which included *Glu-B1a* (7), *Glu-B1b* (7+8), *Glu-B1c* (7+9), *Glu-B1e* (20), *Glu-B1f* (13+16), *Glu-B1g* (13+19) and *Glu-B1i* (17+18) 4.8, 25.8, 24.2, 12.9, 3.2, 1.6 and 27.4%, respectively. The *Glu-D1* locus showed two alleles with their subunits included *Glu-D1a* (2+12) and *Glu-B1d* (5+10) with frequency of 69.4 and 30.6%. A total of 12 different alleles were identified on all three *Glu1* loci (Table 2).

The maximum frequency of subunit 2* and 1 at *Glu-A1* locus indicated that varieties were improved for higher gluten strength compared to null allele in lowest frequency. The *GluA1c* (null) allele was found to exist only in combination with *Glu-D1a* (2+12) in analysed varieties.

The coexistence of *Glu-1Ac* allele in combination with *Glu-D1a* allele was also observed earlier in Indian wheat genotypes Ram S. (2003). *Glu-B1* locus showed higher frequency of three alleles *Glu-B1b* (7+8), *Glu-B1c* (7+9) and *Glu-B1i* (17+18) and contribute better gluten strength. The subunit 20 at *Glu-B1* locus was found in eight of analysed varieties (Table 3).

The high molecular weight glutenin subunit combinations (allele at *Glu-A1*, *Glu-B1* and *Glu-D1*) and their frequencies in 62 cultivars are given in table 3. Among the 62 wheat varieties analysed, 19 different patterns were observed based on high molecular weight glutenin subunit combination at all three *Glu-1* loci. The most frequent patterns were 2*, 7+8, 2+12 (15 varieties), 2*, 17+18, 2+12 (7 varieties) and 1, 7+9, 5+10 and 2*,

7+9, 5+10 and N, 20, 2+12 (5 varieties each). Three of the analysed varieties namely Kundan, HI385 and LOK1 carried strong subunits at all loci 2* at *Glu-A1*, 17+18 at *Glu-B1* and 5+10 at *Glu-D1*. The major requirement for Indian wheat is for chapatti making and subunit 20 has great role to play for contributing medium gluten strength needed for dough for chapattis. Subunit 20 at *Glu-B1* is present in combination with strong Subunit '1' at *Glu-A1* and weak subunit 2+12 at *Glu-D1* locus in the cultivar Kharchia65. In five varieties C-306, HW2004, C-518, C-591 and N8223, the subunit '20' is present with subunit 'Null' at *Glu-A1* and 2+12 at *Glu-D1* contributing weak

gluten strength. The gel showing the allelic variation and HMW-GS patterns is presented in figure 1a and 1b.

The high molecular weight quality score calculated by adding individual score assigned to each subunit or subunit pair by Payne and Lawrence (1983) and Pogna et al., (1989). The most of varieties (~90%) analysed has higher gluten strength with *Glu-1* score ranging from 8-10 and only 10% of varieties has *Glu-1* score of 5-7 (Table 3). This indicated that the wheat varieties bred in most zones are suitable for bread rather than chapattis and there is need to breed for varieties with medium *Glu-1* score suitable for chapatti making quality.

Table 2. Allele frequencies of HMW Glutenin subunits at *Glu-1*

loci ili 62 varieties di bread wheat							
Locus	Allele	No. of	varieties	Frequency			
carrying							
Glu-A1	N	11		17.7			
	1	14		22.6			
	2*	37		59.7			
Glu-B1	7	3		4.8			
	7+8	16		25.8			
	7+9	15		24.2			
	20	8		12.9			
	13+16	2		3.2			
	13+19	1		1.6			
	17+18	17		27.4			
Glu-D1	5+10	19		30.6			
	2+12	43		69.4			

Table 3. Combinations of HMW-GS alleles (*Glu-A1*, *GluB1* and *GluD1*) and their frequencies in 62 bread wheat varieties from India

Glu-A1	Glu-D1	Glu-B1	Genotype	Frequency	Glu1 score	Alveograph' W'
2* 2+12		7+8	PBW175,HD1949,HD2281,HD1982,HD2285,P BW226,HD2009,HD2270,HD2327,HD2380,HD 2501,HDR77,HUW234,NW1014,NP846	15	8	8
		17+18	UP2338, HD2189, Kalyansona, HD2329, HD2385,WL711,Hindi62	7	8	13
		7+9	Sonalika, Kanchan, HD2687, NP852	4	7	12
		20	Kharachia-65	1	6	8
		13+16	Lermarojo,NW1012	2	8	12
	5+10	17+18	Kundan, HI385, LOK1	3	10	17
		7+9	WH542,PBW299,HD2733,PBW435, NP824	5	9	16
1	2+12	7+8	HD2428	1	8	6
		17+18	Vidisha, Raj3077	2	8	11
		20	WG357	1	6	6
	5+10	17+18	Sonora-64, MP3054	2	10	15
		7+9	GW190, HS240, HUW206, MACS2496, NIAW34	5	9	14
		7	PBW343, PBW373, Raj3765	3	8	11
		20	C273	1	8	10
N	2+12	17+18	NI5439, NI5643, NI747-19	3	6	10
		7+9	NIAW301	1	5	9
		20	C-306,N8223, HW2004,C-518,C-591	5	4	5
		13+19	PBW138	1	-	4
					7.45	10.45

1 2 3 4 5 6 7 8 9 10 11 12 13 14 1 2 3 4 5 6 7 8 9 10 11 12 13 14

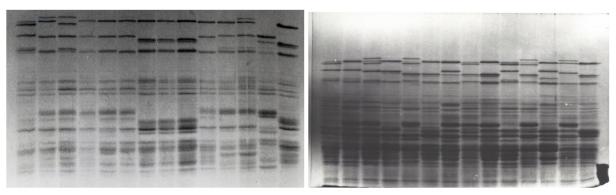


Figure 1a: HMW-GS profile of varieties: 1-NP824 (2*, 7+9, 5+10), 2- WG357 (1, 20, 2+12), 3-C273 (1, 20, 5+10), 4-C306(N, 20, 2+12), 5-C518 (N, 20, 2+12), 6-C591(N, 20, 2+12), 7-NI5439 (N, 17+18, 2+12), 8-NI5643 (N,17+18, 2+12), 9-NI747-19 (N, 17+18, 2+12), 10-N8223 (N, 20, 2+12), 11-NIAW30 1(N, 7+9, 2+12), 12-NIAW34 (1, 7+9, 5+10), 13-NI5749(N, 13+16, 2+12), 14-HD2329 (2*,17+18,2+12)

Figure 1b: HMW-GS profile of varieties 1-WH42(2*, 7+9, 5+10), 2-Sonalika (2*, 7+9,2+12), 3- C273 (1, 20, 5+10), 4-Kharchia-65 (2*, 20, 2+12), 5-Sonora 64 (1, 17+18, 5+10), 6-NP846 (2*,17+18,2+12), 7-Lermarojo 64 (2*,13+16,5+10), 8-HI-385 (2*,17+18, 5+10), 9- Hindi62 (2*, 17+18, 2+12), 10-PBW435 (2*, 7+9, 5+10), 11-MP3054 (1,17+18,5+10), 12-Chinese spring (N, 7+8, 5+10), 13-WG357 (1, 20, 2+12), 14-Kalyansona (N,

DISCUSSION

The analysis of glutenin subunits has proved useful for diversity studies and to optimize the variation in wheat germplasm for quality improvement of new wheat selections. The results of present study revealed a broad range of variation for allelic variants at *Glu-1* loci and combination of these alleles at three loci led to 19 different high molecular weight glutenin subunits patterns. Since the plant material for study was varieties released for different cultivation zones, however, there was no association of presence of varieties with specific subunit or subunit combination in the zones of cultivation.

Each zone has varieties differing for gluten strength based on HMW-GS constitution at all three Glu loci. It has been established in number of studies that glutenin protein of wheat grain plays an important role in processing quality of wheat particularly for bread and pastas (Branlard and Dardevet, 1985; Ng and Bushuk, 1988). Wheat varieties originated in East Asia countries possess predominantly allele Glu-D1a coding for subunits 2 + 12 (Fang et al., 2009; Liu et al., 2007) and contribute weak gluten strength. The results of present study also confirm the same and Glu-D1a coded subunit 2+12 is present in 69% of varieties compared to Glu-D1d, which is in 30% of varieties. This correspond that the HMW-GS compositions of wheat varieties originated in different countries of the world reveal the purpose for which they were commonly used. It has been observed in studies that alleles known for superior bread-making quality of hexaploid wheat are found in considerably lower frequency in countries such as China or Japan (Li et al., 2009; Tanaka et al., 2003). The presence of subunits1/2* at Glu-A1, 7+8, 17+18 at Glu-B1 and 5+10 at Glu-D1 resulted in better rheological properties for bread making. Whereas for chapatti making qualities which is indigenous requirement of India and other Asian countries require medium gluten strength and can be achieved by addition of subunit 20 with strong subunit at other Glu loci. Sreeramulu et al., (2004) mentioned the importance glutenin subunits for chapatti making quality. The subunit 20 was more prevalent in old varieties, which are no more in cultivation. However, there is need to bring subunit 20 in combination with other subunits at Glu-A1 and Glu-D1 locus in breeding lines for selection of varieties with medium gluten strength for chapatti making quality.

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Cite this Article: Bakshi S and Bhagwat SG (2016). High Molecular Weight Glutenin Subunit Variation among Indian Wheat Varieties. Greener Journal of Agricultural Sciences, 6(11): 349-355, http://doi.org/10.15580/GJAS.2016.11.120816211.