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Allelic Diversity of HMW and LMW Glutenins in Indian Wheats and Their Relationship with Sedimentation Volume and Mixograph Parameters

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Two hundred and forty diverse set of wheat cultivars released in India during the last several decades were evaluated for HMW and LMW glutenin alleles, for assessing their diversity and effect on sedimentation volume and mixograph parameters. Both SDS-PAGE and PCR based markers were employed in identifying alleles encoded at *Glu-1* and *Glu-3* loci. Extensive allelic variation was observed at both the *Glu-1* and *Glu-3* loci. There was prevalence of *Glu-A1b*, *Glu-B1i*, *Glu-D1a*, *Glu-A3c*, *Glu-B3b*, *Glu-B3g* and *Glu-D3b*. The alleles *Glu-A1b*, *Glu-B1i*, *Glu-D1d*, *Glu-A3b*, *Glu-B3g/h* and *Glu-D3b* exhibited high SDS-sedimentation volume. *Glu-B1i* and *Glu-D1d* showed highly significant positive effect ($p < 0.001$) on sedimentation volume and also had additive effects. However, surprisingly overall there was decline in the frequency of *Glu-B1i* allele during last two decades in Indian wheat breeding and not a single 1B/1R translocation cultivar possessed this allele. *Glu-A1b* showed significant positive effect on mixograph peak time, peak slope and peak width. *Glu-B3g* exhibited significantly higher mixograph peak time and width at 8 and *Glu-B3h* showed higher dough stability. *Glu-B3j* (1B/1R translocation) exhibited highest peak slope indicating the negative effect on dough strength. This information can be useful in designing breeding program for the improvement of Indian bread wheat quality.

Keywords: glutenins, sedimentation volume, mixograph, allele, wheat

Abbreviations: HMW-GS – high molecular weight glutenin subunits; LMW-GS – low molecular weight glutenin subunits; PCR – polymerase chain reaction

Introduction

Gluten, the visco-elastic complex of wheat dough, determines the quality of various end-use products of wheat. Gluten is composed of glutenins and gliadins, the major storage proteins in wheat endosperm. Glutenins are divided into high (HMW) and low (LMW) molecular weight glutenins (Payne 1987; Gupta and MacRitchie 1994; Shewry et al. 2003; Maucher et al. 2009) distinguished by their differential mobility on SDS-PAGE. HMW glutenins are encoded by *Glu-A1, GluB1* and *Glu-D1* loci and LMW glutenins by

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Glu-A3, *Glu-B3* and *Glu-D3* loci (Payne and Lawrence 1983; Gupta and Shepherd 1990). Many studies have been conducted in assessing the role of HMW-GS in imparting dough strength and extensibility (Branlard and Dardevet 1985; Payne 1987; Gupta and MacRitchie 1994; Luo et al. 2001; Liu et al. 2005; Figueroa et al. 2009). Based on the composition of HMW and LMW glutenin alleles, models were developed for predicting dough strength and extensibility (Cornish et al. 2006). However, inconsistent results obtained for the nomenclature of *Glu-3* alleles across the laboratories made it more complicated the assessment of the role of individual LMW-GS alleles and their interactive effects with HMW glutenins (Ikeda et al. 2008). This has been primarily due to the complexity in band patterns of their large numbers and overlapping mobility with the gliadins on SDS-PAGE (Jackson et al. 1996; Igrejas et al. 1999).

Recently, large numbers of LMW glutenin genes have been characterized and several functional markers developed to identify LMW glutenin alleles (Zhang et al. 2004; Zhao et al. 2007a and b; Appelbee et al. 2009; Wang et al. 2009, 2010). Therefore, combining SDS-PAGE methodologies with PCR based markers can be employed in the undisputed classification of many of the LMW glutenin alleles and hence useful in identification of relationship between these alleles and several quality traits (Maucher et al. 2009; Liang et al. 2010; Ram et al. 2011**)**. In the present study, 240 diverse set of wheat cultivars representing different climatic zones in India, were evaluated for HMW and LMW glutenin alleles using both SDS-PAGE and PCR based allele specific markers. Allelic relations of both HMW and LMW glutenin subunits with sedimentation volume and mixograph characteristics individually and in combination were identified. SDS sedimentation volume and mixograph parameters are widely used for evaluating gluten strength and mixing properties, respectively, in bread wheats.

Materials and Methods

Plant materials

Two hundred and forty diverse set of wheat cultivars released in India during the last several decades were evaluated for HMW and LMW glutenin alleles and different quality parameters. These cultivars represented the entire wheat production area in India comprising 6 wheat growing zones based on agroclimatic conditions. Among them 81 cultivars were from North Western Plains Zone (NWPZ), 57 from North Eastern Plains Zone (NEPZ), 41 from Central Zone (CZ; Zone III), 30 from Peninsular Zone (PZ; Zone IV) and 31 from Northern Hills Zone (NHZ; Zone V). We had only 4 cultivars from Southern Hills Zone (SHZ; Zone VI) and hence not included in analysis. The material was grown during the two crop seasons of 2008–2009 and 2009–2010 at DWR, Karnal, India.

Identification of HMW-GS and LMW-GS

HMW-GS alleles encoded at *Glu-A1*, *Glu-B1* and *Glu-D1* loci were separated on SDS-PAGE and classified as per the method of Singh et al. (1991). LMW-GS alleles were iden-

tified combining both SDS-PAGE and PCR based approach (Wang et al. 2009, 2010; Appelbee et al. 2009; Ram et al. 2011). *Glu-B3b* alleles were identified using primer combinations developed by Sharma et al. (2013). DNA was isolated as per the modified method of Benito et al. (1993). PCR amplification was performed in a total volume of 20 µl containing 50–100 ng of genomic DNA, 1X PCR buffer, 1.5 mM of $MgCl_2$, 200 µM of each deoxyribonucleotide (dNTP), 100 ng of each primer and 0.3 U of Taq DNA polymerase (Bangalore Genei). PCR amplification conditions were similar to that mentioned by Ram et al. (2011). The amplified fragments were separated on 1.5% agarose gel, stained with ethidium bromide and visualized using the Gel Documentation System (Bio-Rad). The DNA marker bands were 100, 200, 300, 600, 1000, 1500, 2500, 3000 bp in size.

Analysis of grain and flour quality

Grain protein content was measured by Foss Infratec™ 1241 Near-Infrared-Reflectance (NIR). The wholemeal flour of the wheat cultivars was produced on a cyclotec mill with a 0.5 mm sieve and SDS-sedimentation volume was measured by the modified method of Carter et al. (1999). Grain samples were cleaned and tempered based on the 14% moisture content. Milling was performed in a Brabender Senior Mill to flour extraction rate of around 65% used for Mixograph analysis. A 10-gm Mixograph (National) was used to assess dough properties and the mixograph curves were computed with the Mixsmart software.

Statistical analysis

Effect of allelic variation encoded by the *Glu-1* and *Glu-3* loci on various quality parameters was assessed through ANOVA based on the two years averaged data. Duncan multiple comparisons were employed to group individual alleles based on the effect on sedimentation volume, protein content and mixograph marameters. All computations were completed by SAS software.

Results

Allelic variation in HMW-GS and LMW-GS

Extensive allelic variation was observed at both the *Glu-1* and *Glu-3* loci (Table S1*). Allelic frequencies of both HMW-GS and LMW-GS and their distribution in different zones and periods are given in Tables 1 and 2. Among 3 alleles encoded at *Glu-A1* locus, *Glu-A1b* was the predominant allele (62.3%). Out of the 8 alleles observed at *Glu-B1* locus three alleles, namely *Glu-B1i* (34.7%), *Glu-B1b* (28.1%) and *Glu-B1c* (26.0%) were present more frequently. *Glu-D1a* (67.5%) occurred more frequently as compared to *Glu-D1d (*22.5%). 82% of the genotypes with the *Glu-D1a* allele possessed *Glu-A1b* while 43% of the genotypes with the *Glu-D1d* possessed *Glu-A1b*. There was no signifi-

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Table 1. Allelic frequencies and zone-wise distribution of HMW-GS and LMW-GS in Indian wheat cultivars (240)

Locus	Allele	Total No	Frequency (%)	NWPZ (81)	NEPZ (57)	CZ (41)	PZ (30)	NHZ (31)
Glu-A1	*a*	53	22.1	19 (23.5)	10 (17.5)	7 (16.7)	6 (20)	11 (35.5)
	b	148	61.7	47 (58.0)	40 (70.2)	29 (69.0)	17 (56.7)	16 (51.6)
	c	39	16.2	15 (18.5)	7 (12.3)	6 (14.3)	7 (23.3)	4 (12.9)
Glu-B1	*a*	7	2.93	6 (7.4)	1 (1.8)	(0.0)	(0.0)	(0.0)
	i	84	35.0	30 (37.0)	21 (36.8)	12 (29.3)	15 (50.0)	6 (19.4)
	b	68	28.3	23 (28.4)	19 (33.3)	14 (34.2)	5 (16.7)	7 (22.6)
	c	63	26.3	18 (22.2)	14 (24.6)	5 (12.2)	8 (26.7)	18 (58.1)
	e	9	3.8	(0.0)	1 (1.8)	5 (12.2)	1 (3.3)	(0.0)
	others	11	4.6	4 (4.9)	1 (1.8)	5 (12.2)	1 (3.3)	(0.0)
Glu-D1	*a*	162	67.5	51 (63.0)	40 (70.2)	33 (80.5)	18 (60)	20 (64.5)
	d	78	32.5	30 (37.0)	16 (28.1)	9 (22.0)	12 (40)	11 (35.5)
Glu-A3	*b*	44	18.3	15 (18.5)	9 (15.8)	8 (19.5)	4 (13.3)	8 (25.8)
	c	156	65.0	56 (69.1)	37 (64.9)	28 (68.3)	18 (60.0)	17 (54.8)
	d	25	10.4	7 (8.6)	8 (14.0)	3 (7.3)	2 (6.7)	5 (16.1)
	e/f	15	6.3	3 (3.7)	3 (5.3)	2 (4.9)	6 (20)	1 (3.2)
Glu-B3	*b*	72	30.0	26 (35.8)	15 (29.8)	12 (34.2)	13 (50.0)	6 (22.6)
	g	60	25.0	25 (30.9)	13 (22.8)	8 (19.5)	7 (23.3)	7 (22.6)
	h	39	16.3	7 (8.6)	13 (22.8)	7 (17.1)	5 (16.7)	7 (22.6)
	i	35	14.6	9 (11.1)	10 (17.5)	9 (22.0)	3 (10)	4 (12.9)
	j	24	10.0	12 (14.8)	6 (10.5)	2 (4.9)	2 (6.7)	6 (19.4)
	others	10	4.2	2 (2.5)	2 (3.5)	3 (7.3)	3 (10)	(0.0)
Glu-D3	*a*	73	30.4	20 (24.7)	15 (26.1)	16 (39.0)	11 (36.7)	11 (35.5)
	b	76	31.7	21 (25.9)	24 (42.1)	10 (24.4)	8 (26.7)	13 (41.9)
	g	59	24.6	24 (29.6)	12 (21.1)	12 (29.3)	7 (23.3)	4 (12.9)
	i	17	7.1	10 (12.3)	1 (1.8)	2 (4.8)	2 (6.7)	2 (6.5)
	j	8	3.3	4 (4.9)	1 (1.8)	(0.0)	2 (6.7)	1 (3.2)
	others	7	2.9	2 (2.5)	4 (7.0)	1 (2.4)	(0.0)	(0.0)

Notes: Values in bracket are allelic percentage within zone. Others include rare alleles.
NWPZ: North Western Plain Zone; NEPZ: North Eastern Plain Zone; CZ: Central Zone; PZ: Peninsular Zone; NHZ; North Hill Zone.

cant variation in the distribution of HMW-GS alleles encoded at *Glu-A1* and *Glu-D1* loci in different zones. However, *Glu-B1a* allele encoded at *Glu-B1* locus was present only in cultivars representing northern zones (NEPZ and NWPZ) and *Glu-B1i* was present at greater frequency in PZ and lower in NHZ. With respect to periods, there was decrease in the frequency of the stronger alleles like *Glu-A1b, Glu-B1i and Glu-D1d* after 1960.

Table 2. Frequency of HMW-GS and LMW-GS alleles distributed over 5 decades

Locus	Allele	Frequency	1961–1970 (18)	1971–1980 (40)	1981–1990 (74)	1991–2000 (54)	After 2001 (54)
Glu-A1	*a*	53 (22.1)	4 (22.2)	3 (7.5)	11 (14.9)	20 (37.0)	15 (27.8)
	b	148 (6.7)	10 (55.6)	27 (67.5)	53 (71.6)	25 (46.3)	33 (61.1)
	c	39 (16.3)	4 (22.2)	10 (25)	10 (13.5)	9 (16.7)	6 (11.1)
Glu-B1	*a*	7 (2.9)	1 (5.6)	(0.0)	(0.0)	3 (5.6)	3 (5.6)
	b	68 (28.3)	2 (11.1)	16 (40)	26 (35.1)	9 (16.7)	15 (27.8)
	c	63 (26.3)	4 (22.2)	6 (15)	11 (14.9)	25 (46.3)	18 (33.3)
	e	9 (3.8)	1 (5.6)	1 (2.5)	2 (2.7)	2 (3.7)	3 (5.6)
	i	84 (35.0)	9 (50.0)	15 (37.5)	33 (44.6)	13 (24.1)	14 (25.9)
	others	9 (3.8)	1 (5.6)	2 (5)	2 (2.7)	2 (3.7)	2 (3.7)
Glu-D1	*a*	162 (67.5)	9 (50.0)	30 (75)	59 (79.7)	31 (57.4)	33 (61.1)
	d	78 (32.5)	9 (50.0)	10 (25)	15 (20.3)	23 (42.6)	21 (38.4)
Glu-A3	*b*	44 (18.3)	1 (5.6)	6 (15)	15 (20.3)	9 (16.7)	13 (24.1)
	c	156 (65)	15 (83.3)	21 (52.5)	46 (62.2)	36 (66.7)	38 (70.4)
	d	25 (10.4)	2 (11.1)	6 (15)	9 (12.2)	7 (13.0)	1 (1.9)
	e/f	15 (6.3)	(0.0)	7 (17.5)	4 (5.4)	2 (3.7)	2 (3.7)
Glu-B3	*b*	70 (29.2)	6 (33.3)	12 (30)	25 (33.8)	7 (13.0)	20 (37.0)
	g	60 (25.0)	3 (16.7)	14 (35)	21 (28.4)	9 (16.7)	13 (24.1)
	h	39 (16.3)	2 (11.1)	8 (20)	12 (16.2)	10 (18.5)	7 (13.0)
	i	35 (14.6)	6 (33.3)	6 (15)	10 (13.5)	10 (18.5)	3 (5.6)
	j	24 (10.0)	(0.0)	0 (0.0)	2 (2.7)	15 (27.8)	7 (13.0)
	others	8 (3.3)	1 (5.6)	0 (0.0)	3 (4.1)	2 (3.7)	2 (3.7)
Glu-D3	*a*	73 (30.4)	12 (66.7)	12 (30)	20 (27.0)	13 (24.1)	16 (29.6)
	b	76 (31.7)	2 (11.1)	13 (32.5)	21 (28.4)	14 (25.9)	26 (48.2)
	g	59 (24.6)	2 (11.1)	9 (22.5)	23 (31.1)	18 (33.3)	7 (13.0)
	i	17 (7.1)	2 (11.1)	2 (5.0)	6 (8.1)	4 (7.4)	3 (5.6)
	j	8 (3.3)	(0.0)	2 (5.0)	3 (4.1)	3 (5.6)	(0.0)
	others	7 (2.9)	(0.0)	2 (5.0)	1 (1.4)	2 (3.7)	2 (3.7)

Both SDS-PAGE and PCR based markers were employed in identification of LMW glutenin subunit alleles. Four alleles, namely *Glu-A3b* (18.0%), *Glu-A3c* (65.6%), *Glu-A3d* (10.2%) and *Glu-A3e/f* (6.2%), were identified encoded at *Glu-A3* locus. Earlier studies (Jackson et al. 1996; Igrejas et al. 1999; Branlard et al. 2003) reported the presence of the *Glu-A3a* allele in large numbers of cultivars in Europe. This was because of difficulty in distinguishing *Glu-A1a* and *Glu-A1c* alleles on SDS-PAGE. Recently developed PCR markers could distinguish them clearly which showed the presence of the *Glu-A3c* allele in a collection of diverse wheat genotypes representing different regions (Zhang et al. 2004; Liu et al. 2010; Wang et al. 2010; Ram et al. 2011).

Six allelic variants, namely *Glu-B3b* (36.7%), *Glu-B3d* (1.3%), *Glu-B3g* (21.1%), *Glu-B3h* (17.5%), *Glu-B3i* (12.6%), *Glu-B3j* (10.8%), were identified encoded at *Glu-B3* locus. There are many reports indicating different allelic frequencies representing the *Glu-B3* locus in genotypes from different countries. Branlard et al. (2003) reported *Glu-B3b* in 10.0% of cultivars in France, *Glu-B3g* in 49.0% and *Glu-B3d* in 3.5%. Other reports have also indicated the presence of *Glu-B3b* and *Glu-B3g* alleles in large numbers of cultivars (Jackson et al. 1996; Igrejas et al. 1999; Wang et al. 2009). *Glu-B3b* was present in higher percentage in CZ and PZ where warmer conditions prevail and *Glu-B3j* (1B/1R translocation) in NHZ where comparatively cooler conditions prevail. Though SDS-PAGE analyses showed three types of *Glu-D3* alleles, the combination of both SDS-PAGE and PCR based markers identified six alleles namely *Glu-D3a* (31.1%), *Glu-D3b* (32.3%), *Glu-D3c* (0.8%), *Glu-D3g* (25.1%), *Glu-D3i* (7.2%), *Glu-D3j* (3.4%) at *Glu-D3* locus. In contrast, reports by Branlard et al. (2003), Igrejas et al. (1999) and Jackson et al. (1996) showed a higher percentage of the *Glu-D3c* allele and a lower percentage of the *Glu-D3b* allele in European cultivars.

Influence of Glu-1 and Glu-3 loci and their alleles on sedimentation volume and mixograph parameters

SDS sedimentation volume and mixograph parameters reflect dough strength and mixing properties in bread wheats. High sedimentation volumes have been reported linked with stronger gluten and superior bread making quality. The effects of *Glu-1* and *Glu-3* loci on sedimentation volume and mixograph parameters are shown in Tables 3 and 4. Though, there was no significant difference among *Glu-A1* allelic variants, *Glu-A1b* exhibited higher SDS-sedimentation volume in comparison to other alleles encoded by *Glu-A1* locus. Reports by He et al. (2005), Liu et al. (2005), Tabiki et al. (2006) and Figueroa et al. (2009) indicated the stronger role of *Glu-A1* while Luo et al. (2001) and Ram et al. (2003) showed stronger role of *Glu-A1b* in imparting gluten strength. There was significant effect of *Glu-B1* ($p < 0.01$) and *Glu-D1* ($p < 0.001$) loci on SDS-sedimentation volumes (Table 4). Among *Glu-B1* alleles, *Glu-B1i* exhibited highly significant positive effect on SDS-sedimentation ($p < 0.001$). Similarly *Glu-D1d* showed significantly higher values of SDS-sedimentation ($p < 0.001$) as compared to *Glu-D1a*. There was no significant difference in sedimentation value between *Glu-B1i* and *Glu-D1d* allelic forms, however the alleles in combination exhibited highest sedimentation value ($p < 0.0001$) (Table 5) indicating their additive effect.

Glu-1 loci also influenced various mixograph parameters. The *Glu-A1* exhibited significant positive effect on mixograph mixing peak time. *Glu-A1b* showed significant positive effect on mixograph peak time, peak slope and peak width. *Glu-B1* locus showed significant effect on mixograph peak height and width at 8 ($p < 0.05$). Surprisingly, though *Glu-B1i* showed higher values of mixograph parameters, there was no statistically significant effect. *Glu-D1* locus showed significant positive effect on mixograph width at 8 ($p < 0.05$). There was significant effect of *Glu-D1* on mixing time as reported by He et al. (2005) and Hernández et al. (2012).

Table 3. Effect of allelic variation of HMW-GS and LMW-GS subunits on sedimentation volume and mixograph parameters using Duncan multiple comparisons

Locus	Allele	Frequency	Protein	Sed	MPT	Peak slope	Peak width	Width at 8.0	Peak ht
Glu-A1	*a*	53	11.3[a]	9.5[a]	2.6[ab]	6.2[a]	15.7[ab]	4.3[a]	56.9[a]
	b	148	11.2[a]	9.3[a]	2.8[a]	5.9[ab]	16.1[a]	4.7[a]	57.0[a]
	c	39	11.4[a]	9.1[a]	2.4[b]	5.3[b]	14.7[b]	4.5[a]	55.3[a]
Glu-B1	*a*	7	11.1[a]	9.1[b]	2.7[a]	5.8[a]	15.2[a]	4.3[a]	58.2[a]
	b	68	11.1[a]	9.1[b]	2.8[a]	5.8[a]	15.6[a]	4.7[a]	54.3[a]
	c	63	11.2[a]	9.2[b]	2.6[a]	6.3[a]	16.1[a]	4.3[a]	57.6[a]
	e	9	11.6[a]	8.9[b]	2.6[a]	5.6[a]	16.0[a]	4.3[a]	57.2[a]
	i	84	11.4[a]	9.8[a]	2.8[a]	5.6[a]	16.1[a]	4.8[a]	57.7[a]
	others	9	–	–	–	–	–	–	–
Glu-D1	*a*	162	11.2[a]	9.1[b]	2.8[a]	5.9[a]	15.9[a]	4.7[a]	56.4[a]
	d	78	11.4[a]	9.8[a]	2.7[a]	5.7[a]	15.6[a]	4.4[a]	57.4[a]
Glu-A3	*b*	44	11.2[a]	9.7[a]	2.9[a]	6.1[a]	16.2[a]	4.7[a]	56.8[a]
	c	156	11.2[a]	9.4[a]	2.7[a]	5.9[a]	15.3[a]	4.5[a]	56.8[a]
	d	25	11.5[a]	9.3[a]	2.6[a]	5.8[a]	15.4[a]	4.7[a]	56.9[a]
	e/f	135	11.2[a]	9.1[a]	2.6[a]	5.5[a]	15.1[a]	4.8[a]	54.9[a]
Glu-B3	*b*	70	11.3[a]	9.3[a]	2.7[ab]	5.7[bc]	15.8[a]	4.5[ab]	56.5[a]
	g	60	11.1[a]	9.5[a]	2. 9[a]	5.9[bc]	15.3[a]	5.1[a]	55.8[a]
	h	39	11.1[a]	9.5[a]	2.7[ab]	4.9[c]	15.4[a]	4.7[ab]	56.2[a]
	i	35	11.4[a]	8.9[a]	2.4[b]	6.5[ab]	16.0[a]	4.3[b]	56.9[a]
	j	24	11.3[a]	9.1[a]	2.7[ab]	7.3[a]	16.4[a]	4.1[b]	58.9[a]
	others	8	–	–	–	–	–	–	–
Glu-D3	*a*	72	11.3[a]	9.4[a]	2.6[ab]	5.8[a]	15.9[a]	4.4[a]	56.2[a]
	b	79	11.2[a]	9.3[a]	2.8[a]	5.9[a]	15.4[a]	4.6[a]	56.4[a]
	g	57	11.0[a]	9.0[a]	2.8[a]	5.8[a]	16.2[a]	4.8[a]	56.8[a]
	i	16	11.4[a]	9.2[a]	2.7[ab]	5.5[a]	15.7[a]	4.5[a]	57.5[a]
	j	8	11.3[a]	9.3[a]	2.3[b]	6.7[a]	16.1[a]	4.3[a]	57.9[a]
	others	7	–	–	–	–	–	–	–

Values followed by the similar superscript were in same group having non-significant statistical difference.

There was no significant difference on the effect of *Glu-3* loci on sedimentation values. *Glu-A3c* showed higher sedimentation value though not significant, as compared to *Glu-A3b, d* and null allele. The *Glu-B3* allelic group *b*, *g* and *h* exhibited higher sedimentation value than allelic group *i* and *j* (Table 4). *Glu-D3a* and *Glu-D3b* allelic groups showed higher SDS-sedimentation as compared to *Glu-D3 g, i* and *j*. *Glu-A3* loci showed no significant effect on mixograph parameters. The variation at *Glu-B3* loci significantly influenced the dough strength parameters like mixograph peak slope ($p < 0.05$) and width at

Table 4. F-values as determined by ANOVA showing the effect of *Glu-1* and *Glu-3* loci on sedimentation volume and mixograph parameters

Locus	DF	Protein (14%)	Sedimentation value	Mixing peak time	Peak slope	Peak height	Peak width	Width at 8.0
Glu-A1	2	1.4	1.4	5.7**	2.0	0.7	2.5	2.0
Glu-B1	4	1.8	3.3**	1.7	1.1	3.3*	0.3	2.8*
Glu-D1	1	2.3	17.5***	1.7	0.6	1.0	0.6	3.4*
Glu-A3	3	0.8	0.9	2.0	0.5	0.3	0.6	0.7
Glu-B3	4	1.1	1.1	2.1	5.5**	0.9	0.5	3.4**
Glu-D3	4	0.3	0.8	2.6*	0.4	0.2	0.5	1.3

DF: degree of freedom, *significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < 0.001$.

Table 5. Effect of Glu-B1i and Glu-D1d alleles on sedimentation volume

	Glu-B1i		Non *Glu-B1i*		*Glu-B1i* + *Glu-D1d*	Non *Glu-B1i* + *Glu-D1a*	*Glu-B1i* + *Glu-D1a*	Non *Glu-B1i* + *Glu-D1d*
	Glu-D1d	*Glu-D1a*	*Glu-D1d*	*Glu-D1a*				
Mean	10.5	9.4	9.5	8.8	10.5	8.8	9.4	9.6
Observation	35	50	51	118	35	118	50	51
t-Test values	3.66**		3.01**		6.84***		0.7	

There was highly significant effect of both the alleles on sedimentation volume and also exhibited strong additive effect. **, *** indicate values that are significant at $p < 0.001$ and $p < 0.0001$ level of significance.

8.0 ($p < 0.01$). *Glu-B3g* exhibited significantly higher mixograph peak time and width at 8 when compared to other *Glu-B3* alleles. *Glu-B3h* exhibited higher dough stability by showing significantly lower mixograph peak slope as compared to other alleles. *Glu-B3j* (1B/1R translocation) exhibited highest peak slope indicating the negative effect on dough strength. *Glu-D3* locus showed significant effect on mixograph peak time ($p < 0.05$). *Glu-D3b* and *Glu-D3g* exhibited significantly higher mixograph peak time as compared to other alleles encoded at *Glu-D3* loci.

Discussion

Allelic diversity of HMW and LMW glutenins

In the present investigation, allelic diversity of HMW and LMW glutenin subunits were studied in a set of 240 diverse Indian wheat cultivars representing different zones having varied climatic conditions. Though large numbers of studies have been conducted with regard to HMW glutenin composition and their effect on dough strength (Payne, 1987; Gupta and MacRitchie 1994), few reports are available on the combined effect of both HMW and LMW glutenins (Cornish et al. 2006). Therefore, both HMW glutenins and LMW glutenins were studied with respect to their allelic distribution and in relation to their effect on gluten strength as determined by sedimentation volume and mixograph

parameters. There was reduction in the frequency of *Glu-A1c* during last 6 decades of wheat breeding in India while stronger *Glu-A1a* and *Glu-A1b* alleles increased. However, concomitant with this, there was decrease in the frequency of strong allele *(Glu-B1i)* encoded at *Glu-B1* locus. The strong allele *Glu-D1d* decreased between 1971 and 1990 and subsequently increased with the introduction of 1B/1R translocation. The data further demonstrated that the 75% of genotypes possessing *Glu-D1a* allele had *Glu-B3g* and 90% had *Glu-B3b* allele. The occurrence of strong alleles such as *Glu-B3g* and *Glu-B3b* in 60% of the cultivars in India, in combination with a weak allele at the *Glu-D1* locus, has indicated the technological value of these alleles. 15% of the total Indian cultivars carried *Glu-B1i* in combination with *Glu-D1d* and 40% of these cultivars possessed *Glu-B3b* allele.

The data also indicated that the increased use of the 1B/1R translocation from the 1980s onwards by wheat breeding programs in India resulted in the presence of the *Glu-B3j* allele in many of the cultivars released in recent years that led to reduction in gluten strength. Zone-wise analysis of the data indicated the prevalence of 1B/1R translocation in cultivars representing Northern Hills, North West and North East plains where cold conditions prevail during winter period as compared to CZ where warm and dry conditions exist. Earlier studies have also shown the presence of genes showing resistance to cold conditions in 1B/1R translocation lines. Although most of the 1B/1R translocation lines carried the *Glu-D1b* allele, 95% of them also carried either the *Glu-B1a* or the *Glu-B1c* alleles which are associated with weak gluten. Surprisingly not a single genotype with 1B/1R translocation had *Glu-B1i* allele. These data suggest that recently released varieties in India can be improved either by combining stronger alleles encoded by the *Glu-B1* locus, such as *Glu-B1i* encoding HMW-GSs *17+18*, or by incorporating the *Glu-B3* locus through recombinant breeding aimed at reducing the size of the translocated 1B/1R fragment. This is possible thanks to the availability of dense molecular maps and linked markers. Our unpublished results show that a combination of 2*, 5+10, and 17+18 HMW-GSs can significantly enhance the gluten strength of 1B/1R translocation lines and, consequently, their bread making quality. There was significant increase in the gluten strength of backcross lines (BC3F5) developed using a marker for the *Glu-B1i* allele in a 1B/1R background.

Effect of HMW and LMW glutenins on sedimentation volume and mixograph parameters

There was significant effect of *Glu-B1* and *Glu-D1* loci on SDS-sedimentation volume as reported by several other workers (Payne 1987; He et al. 2005; Liu et al. 2005). However, Hernández et al. (2012) showed no significant effect of *Glu-1* loci on SDS-sedimentation. Among *Glu-B1* and *Glu-D1* alleles, *Glu-B1i* and *Glu-D1d* showed strongest effects and negated the influence of alleles encoded at *Glu-3* loci. *Glu-A1b* showed stronger effect on sedimentation volume and mixograph parameters as compared to *Glu-A1a* and *c*. Luo et al. (2001) also reported stronger relationship of *Glu-A1b* allele, however, He et al. (2005), Liu et al. (2005), Figueroa et al. (2009) and Tabiki et al. (2006) suggested the stronger

role of *Glu-A1a* in quality parameters. Surprisingly, there was no significant effect of *Glu-B1* and *Glu-D1* loci on mixograph mixing time, but variation at *Glu-A1* significantly influenced mixing time. He et al. (2005) showed strong influence of all the *Glu-1* loci while Hernández et al. (2012) reported significant effect on mixograph peak time by only *Glu-D1*.

LMW-GS alleles like *Glu-A3c*, *Glu-B3b*, *Glu-B3g* and *Glu-D3b* were the predominant alleles present in Indian cultivars. *Glu-A3b* showed the stronger dough properties as reported earlier by Liang et al. (2010). However, He et al. (2005), Liu et al. (2005) and Maucher et al. (2009) reported stronger effect of *Glu-A3d* on dough strength. There was no significant effect of any of the *Glu-3* loci on SDS-sedimentation values. However, earlier reports of He et al. (2005) and Liu et al. (2005) indicated the significant effect of *Glu-3* loci on SDS-sedimentation value. This may be due to absence of strong allele *Glu-B1i* and lesser frequency of *Glu-D1d* in Chinese wheat cultivars. Hernández et al. (2012) reported significant effect of only *Glu-B3* loci on SDS-sedimentation values. These discrepancies may be due to the difference in the genetic background of the cultivars used. There are very few reports of the effect of *Glu-D3* locus and its allelic variant on quality parameters (He et al. 2005; Tabiki et al. 2006; Maucher et al. 2009). In this study, we included the new *Glu-D3* alleles identified through PCR based markers: *Glu-D3 g*, *i* and *j*. *Glu-D3b* and *Glu-D3g* exhibited stronger mixograph characteristics.

Overall, there was similarity in the order of the effect of *Glu-1* and *Glu-3* alleles on dough strength as reported by Cornish et al. (2006) and thus have greater utility in wheat quality improvement programs. Since *Glu-B1i* and *Glu-D1b* had very strong positive effect on dough strength, the effect of *Glu-3* loci was assessed under non-*Glu-B1i* and *Glu-D1d* backgrounds. At *Glu-A3* loci, there was no significant difference among *Glu-A3* alleles on SDS-sedimentation volume when present in combination in non-*Glu-B1i* and *Glu-D1a* background, but greater mean sedimentation value was shown by *Glu-A3b* followed by *Glu-A3c*. *Glu-B3h* exhibited significant effect ($p < .05$) on sedimentation values followed by *Glu-B3g* and *Glu-B3b* in the background of *Glu-D1a* and non-*Glu-B1i* allelic combinations. *Glu-B3j* showed lowest sedimentation values. *Glu-D3a* and *Glu-D3b* showed similar SDS-sedimentation value when present in combination with non-*Glu-B1i* and *Glu-D1a* alleles. Overall, *Glu-A3b*, *Glu-B3h* and *Glu-D3b* alleles encoded at *Glu-3* loci showed stronger gluten characteristics.

The study demonstrated the prevalence of *Glu-A1b*, *Glu-B1i*, *Glu-D1a*, *Glu-A3c*, *Glu-B3b*, *Glu-B3g* and *Glu-D3b* alleles in Indian wheat cultivars. Recently developed cultivars exhibited decline in frequency of strong alleles *Glu-B1i* and *Glu-D1d*. The allelic combination that exhibited highest dough strength as measured by SDS-sedimentation value and mixograph parameters was *Glu-A1b*, *Glu-B1i*, *Glu-D1d*, *Glu-A3b*, *Glu-B3g/h* and *Glu-D3b.* This information can be useful in designing breeding program for the improvement of Indian bread wheat quality.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. Allelic composition of both HMW and LMW glutenin subunits from 240 Indian wheat cultivars