J. Wheat Res. 4 (1) : 74-78

Relationship of high and low molecular weight glutenins with chemical and rheological properties of wheat flour

Shikha Sharma, Sewa Ram* and Ranjan Gupta[1]

**Directorate of Wheat Research,
Karnal 132 001, India**

Abstract

One hundred and forty two Indian wheat cultivars were evaluated for glutenin subunits and various chemical and rheological quality traits to understand relationship among them. Flour protein content showed strong positive correlation with gluten content and gluten index ($p<0.01$). Gluten index and swelling index of glutenin (SIG) exhibited strong positive relationship with mixograph mixing time ($p<0.001$). With respect to glutenin subunits, *Glu-D1* and *Glu-B3* loci exhibited significant effect on most of the gluten strength related parameters. 5+10 subunit encoded by *Glu-D1* locus exhibited strongest effect on gluten strength. *Glu-B3* and *Glu-D3* loci exerted significant effect on gluten swelling index and mixing time, whereas *Glu-A3* influenced mixing time only. The best subunit combinations for stronger gluten related properties were identified as 2*, 17+18, 5+10, *Glu-A3d*, *Glu-B3g* and *Glu-D3b*. This information can used in designing breeding programme for the improvement of bread making quality of Indian wheat.

Keywords: Glutenin subunits, common wheat, bread making quality, gluten strength, SDS-PAGE.

Introduction

Gluten, which is composed of glutenins and gliadins, is an important determinant of the wheat flour quality. Glutenins are divided into high (HMW) and low (LMW) molecular weight glutenins (Payne *et al.,* 1987; Gupta and MacRitchie, 1994; Gianibelli *et al.,* 2001; Maucher *et al.,* 2009), distinguished by their differential mobility on SDS-PAGE. Genes encoding HMW glutenins are located on long arm of chromosome 1A, 1B and 1D at *Glu-A1, Glu-B1* and *Glu-D1* loci, respectively (Payne *et al.,* 1987). The LMW glutenins are encoded by *Glu-A3*, *Glu-B3* and *Glu-D3* loci located on the short arm of chromosomes and are tightly linked to the *Gli-1* loci (Jackson *et al.,* 1996). Allelic variation in HMW-GS and LMW-GS are both associated with difference in wheat quality (Payne *et al.,* 1987; Luo *et al.,* 2001). Role of individual HMW-GS in imparting dough strength and extensibility has been well studied (Branlard and Dardevet 1985; Gupta and MacRitchie 1994; He *et al.,* 2005; Figueroa *et al.,* 2009) because of their few numbers and easy separation on SDS-PAGE gel. Although, LMW-GS also have pronounced effect on dough rheological properties in both bread and durum wheat (Luo *et al.,* 2001), their role has received less attention, largely because of the complexity in band patterns due to their large numbers and overlapping mobility with the gliadins on SDS-PAGE (Singh and Shepherd, 1988).

[1]Current Address: Department of Biochemistry, Kurukshetra University, Kurukshetra 136 118, India
* Corresponding author email: *sewaram01@yahoo.com*

There are few reports indicating the association of LMW-GS with dough quality of wheat flour (Gupta *et al.,* 1994; Luo *et al.,* 2001; He *et al.,* 2005; Maucher *et al.,* 2009). In the present investigation, 142 diverse set of Indian wheat cultivars were evaluated for different quality parameters measured by chemical test such as swelling index of glutenins and rheological traits determined by gluten content and mixograph mixing time. We compared the rheological properties associated with different *Glu-1* and *Glu-3* genotypic groups and the information can be used in designing breeding programme for the improvement of bread making quality of Indian wheat.

Material and methods

The different abbreviations used in the text includes, HMW-GS= High-molecular-weight glutenin subunits, LMW-GS=Low molecular weight glutenin subunits, SDS-PAGE = Sodium dodecyl sulphate polyacrylamide gel electrophoresis , FPC=Flour protein content, WGC= Wet gluten content, DGC = Dry gluten content, SIG= Swelling index of glutenin.

Identification of glutenin subunits: One hundred and forty two diverse set of wheat cultivars developed and released in India were evaluated for different quality parameters. The material was grown in the crop season of 2010-2011 at DWR, Karnal, India. Allelic classification of glutenins was accomplished using both SDS-PAGE and PCR based markers (Ram *et al.,* 2011). The data was analysed by making genotypic groups within three loci of HMW-GS; *Glu-A1* (1, 2* and N), *Glu-B1* (20, 17+18, 7+8 and 7+9), *Glu-D1* (2+12 and 5+10) and three loci of LMW-GS; *Glu-A3* (*b, c, d* and *e*), *Glu-B3* (*b, g, h, i* and *j*), *Glu-D3* (*a, b, g* and *i*).

Flour quality: Flour protein content (FPC) was determined by Foss Infratec™ 1241(flour module) Near-Infrared-Reflectance (NIR), following the manufacturer's operating procedure. The wholemeal flour of the wheat cultivars was produced on a Tecator 1093 cyclotec sample mill with a 0.5 mm sieve and SIG was performed according to Wang and Kovacs (2002a). The grain samples were cleaned and tempered based on the 14% moisture content. Milling was performed in Brabender mill to flour extraction rate of around 68%. Wet gluten content (WGC), dry gluten content (DGC) and gluten index were determined using Glutomatic system (Perten Instruments Co., Ltd., Sweden). A 10-gm Mixograph was used to assess mixing time and it was computed with the Mixsmart software.

Statistical analysis: Analysis of variance and Tukey's multiple range test of the means at alpha = 0.05 level was performed using the General Linear Model procedure of SAS statistical software, to understand the loci effect and influence of individual HMW-GS and LMW-GS alleles on wheat flour quality, respectively. Correlation coefficients were calculated in MS-EXCEL software.

Results and discussion

Correlation between chemical and rheological traits: Correlation coefficients between different parameters are shown in Table 1. Flour protein content (FPC) showed strong correlation with gluten content and gluten index ($p<0.0001$). Tabiki *et al.* (2006) and Dowell *et al.* (2008) also reported the significant correlation of FPC with gluten content. Gluten strength measured by SIG was positively related only to dough rheological properties measured by mixograph mixing time. This was in accordance with that reported by Wang and Kovacs (2002a). However, it did not exhibited relationship with FPC and the gluten content. This was in contradiction to earlier reports by Xin-zhong *et al.* (2004) and Collar *et al.* (2007). The mixograph mixing time showed significant relationship with all the viscoelastic properties of wheat flour except for FPC. However, Martinant *et al.* (1998), Maghirang *et al.* (2006) and Dowell *et al.* (2008) reported the significant positive relationship between mixograph parameter and protein content. These discrepancies may be due to the difference in the genetic background of the cultivars used in different studies.

Table 1. Correlation coefficient between chemical and rheological properties of wheat flour [a]

	FPC	GI	DGC	SIG	MT
FPC	1.00				
GI	0.29**	1.00			
DGC	0.47****	ns	1.00		
SIG	ns	ns	ns	1.00	
MT	ns	0.50****	-0.21 *	0.30**	1.00

[a] FPC: Flour protein content (14 % moisture basis), DGC: Dry gluten content, GI: gluten index, SIG: Swelling index of glutenin, MT: mixing time. *, **, ***, **** significant at $p< 0.05$, 0.01, 0.001, and 0.0001, respectively; ns: not significant.

Effect of HMW-GS composition on quality related traits: Each end-use product has its own specific requirement. Hard wheat's along with strong and extensible gluten as determined by chemical test SIG and rheological parameter mixing time and gluten index are used for bread making, whereas soft wheats with weak and highly extensible gluten with low protein content are preferred for biscuit making (MacRitchie *et al.,* 1991). Effect of different glutenin subunit and loci are shown in Table 2&3. There was strong effect of *Glu-D1* locus on gluten index and mixograph mixing time ($p<0.0001$) and SIG ($p<0.05$). There was no effect of *Glu-A1* loci on any of the quality parameter. No significant effects of HMW-GS were observed for protein content. Liu *et al.* (2005) reported the significant effect of *Glu-A1* and reported non significant role of both HMW-GS and LMW-GS on protein content. This demonstrated that variation at *Glu-1* and *Glu-3* loci is more likely to be associated with protein quality rather than with protein quantity. Though He *et al.* (2005) reported the influence of all the *Glu-1* loci to be significant for mixograph mixing time, however, in this investigation, there was significant effect of *Glu-D1* on mixing time. Similarly, Hernández *et al.* (2012) also reported significant effect of *Glu-D1* on mixing time. Tabiki *et al.* (2006) reported significant effect of *Glu-A1* and *Glu-D1* on wet gluten content and gluten index, while in our study we could find only *Glu-D1* to be highly significant.

Table 2. Mean square values of one way ANOVA of chemical and rheological properties exhibited by HMW-GS and LMW-GS loci [a].

Locus	DF	FPC	SIG	GI	MT
Glu-A1	2	0.54	0.23	366.59	0.75
Glu-B1	3	0.11	0.19	565.44	0.28
Glu-D1	1	0.87*	0.45*	4635***	4.5***
Glu-A3	3	0.17	0.24	480.8	1.67**
Glu-B3	4	0.13	0.53***	596.1	1.74***
Glu-D3	3	0.13	0.67***	525.8	1.1**

[a] DF: degree of freedom, FPC: flour protein content (14 % moisture basis), SIG: swelling index of glutenin, GI: gluten index, MT: mixing time. * Significant at $p< 0.05$, ** significant at $p< 0.01$, *** significant at $p < 0.001$.

When comparing individual HMW-GS alleles, it was observed that no significant difference was found for all the quality related parameters among the three *Glu-A1* allelic groups (Table 3). In our studies we could find that both the subunit 1 and 2* exerted similar effect on gluten index and other chemical and rheological properties, which was in accordance with Payne *et al.* (1987) and Takata *et al.* (2000).

Table 3. Chemical and rheological characteristics of genotypic groups representing different allelic variants of HMW-GS and LMW-GS of wheat [a].

Locus	Subunit	Number	FPC	SIG	GI	MT
Glu-A1	1	36	10.77[a]	2.3 [a]	61.8 [a]	3.1 [a]
	2*	88	10.6 [a]	2.3 [a]	56.5 [a]	3.1 [a]
	N	16	10.8 [a]	2.4 [a]	58.8 [a]	2.8 [a]
Glu-B1	20	6	10.9 [a]	2.3 [a]	61.9 [a]	3.1 [a]
	17+18	40	10.6 [a]	2.4 [a]	62.2 [a]	3.2 [a]
	7+8	50	10.6 [a]	2.2 [a]	55.4 [a]	3.1 [a]
	7+9	34	10.6 [a]	2.3 [a]	53.8 [a]	2.9 [a]
Glu-D1	2+12	104	10.6[b]	2.3 [a]	54.7 [b]	2.9 [b]
	5+10	36	10.8[a]	2.4 [a]	67.9 [a]	3.4 [a]
Glu-A3	*b*	21	10.7 [a]	2.3 [a]	58.1 [a]	3.2 [ab]
	c	88	10.7 [a]	2.2 [a]	56.2 [a]	2.9 [b]
	d	17	10.8 [a]	2.4 [a]	65.9 [a]	3.4 [a]
	e/f	13	10.7 [a]	2.4 [a]	60.9 [a]	3.3 [ab]
Glu-B3	*b*	42	10.7 [a]	2.2[b]	53.3 [a]	3.1 [ab]
	g	35	10.6 [a]	2.5[a]	64.3 [a]	3.4 [a]
	h	18	10.7 [a]	2.3[ab]	56.5 [a]	2.8 [b]
	i	19	10.7 [a]	2.2[b]	56.7 [a]	2.8 [b]
	j	19	10.8 [a]	2.2[b]	57.8 [a]	2.8 [b]
Glu-D3	*a*	34	10.7 [a]	2.1[c]	62.1 [a]	3.1 [a]
	b	35	10.6 [a]	2.4[a]	61.2 [a]	3.2 [a]
	g	46	10.6 [a]	2.3[ab]	54.9 [a]	3.1 [a]
	i	18	10.8 [a]	2.2[bc]	54.4 [a]	2.7 [b]

[a] Values followed by the similar superscript were in same group having non significant statistical difference. FPC: flour protein content (14 % moisture basis), SIG: swelling index of glutenin, GI: gluten index, MT: mixing time.

However, several other reports as by He *et al.* (2005); Liu *et al.* (2005); Tabiki *et al.* (2006) and Figueroa *et al.* (2009) indicated the stronger role of subunit 1 while, Luo *et al.* (2001) and Ram *et al.* (2003) showed stronger role of subunit 2* in imparting gluten strength. In *Glu-B1* allelic group, subunit 17+18 exhibited highest mean value for SIG, gluten index and mixing time while subunit 7+9 gave the lowest values. The *Glu-D1* group possessing 5+10 subunits gave significantly higher values for all the quality traits. Earlier report by Tabiki *et al.* (2006), also suggested the significant effect of subunit 5+10 on gluten index and mixograph peak time. Overall, subunit 17+18 and 5+10 presented superior gluten strength related properties.

LMW-GS composition and quality characteristics: Because of difficulty in an unambiguous classification of LMW glutenins by SDS-PAGE (Ikeda *et al.,* 2008) only few studies have been in reported in understanding the relationship between LMW glutenin subunits and quality traits. However, recently with development of allele specific markers for *Glu-A3* and *Glu-B3* (Wang *et al.,* 2009, 2010; Ram *et al.,* 2011), it's now possible to have undisputed classification of LMW-GS alleles. One way ANOVA and multiple comparisons were employed to understand the role of individual *Glu-3* loci and their alleles on flour quality. The results demonstrated the significant effect of *Glu-A3*, *Glu-B3* and *Glu-D3* on mixograph mixing time at $p<0.01$. The variation at *Glu-B3* and *Glu-D3* was highly significant for SIG value at $p<0.0001$ (Table 2). *Glu-B3* gave the higher gluten index and mixograph mixing time as compared to *Glu-A1* and *Glu-D1 loci*, indicating its important role in imparting gluten strength. Earlier studies (Liu *et al.,* 2005; Tabiki *et al.*, 2006; Hernández *et al.* 2012) also suggested the stronger role of *Glu-B3* on flour quality. Gupta *et al.* (1994), Eagles *et al.* (2002) and Branlard *et al.* (2003) reported less important role of *Glu-D3* as compared to other loci. In the present investigation, *Glu-D3* loci showed significant correlation with SIG values and mixograph mixing time.

Glu-A3d showed higher SIG and gluten index as compared to *Glu-A3b, c* and null allele (Table 3). This is in accordance with earlier reports of Liu *et al.* (2005) and Maucher *et al.* (2009). Oury *et al.* (2010) showed the strong influence of *Glu-A3d* in imparting gluten strength measured by SDS-sedimentation and gluten extensibility determined by alveograph. In *Glu-B3* allelic group, *Glu-B3g* presented significantly higher SIG value and mixograph mixing time as compared to allelic group *b, h, i* and *j. Glu-B3g* also exhibited higher values of wet gluten content and gluten index though not significant than its allelic counterparts. At *Glu-D3* locus, *Glu-D3b* allelic group showed the significantly higher gluten related properties as measured by SIG and mixograph mixing time. *Glu-D3a* showed lower gluten strength while *Glu-D3i* exhibited lower value of mixing time. In brief, results demonstrated the positive effect of *Glu-A3d*, *Glu-B3g* and *Glu-D3b* alleles on gluten strength.

In conclusion, this study demonstrated the role of different glutenin subunits in imparting gluten strength. *Glu-D1d* showed strongest effect on all flour quality traits. At *Glu-3* loci, *Glu-B3* and *Glu-D3* exerted significant effect on gluten strength as measured by SIG and mixograph mixing time. The subunit combinations of 2*, 17+18, 5+10, *Glu-A3d*, *Glu-B3g* and *Glu-D3b* exhibited strongest correlation with gluten strength. This information could be useful in designing breeding programme for the improvement of bread making quality of the Indian wheat.

Acknowledgements

Authors are thankful to Dr. (Mrs.) Indu Sharma, Project Director, DWR, Karnal for providing the facilities to conduct research work. Authors also acknowledge the help rendered by Dr. R. Sendhil, Scientist, DWR, Karnal in statistical analysis of data.

References

1. Branlard G, Dardevet M (1985). Diversity of grain protein and bread wheat quality. II. Correlation between high- molecular- weight subunits of glutenin and flour quality characteristics. *Journal of Cereal Science* **3**: 345-354.

2. Branlard G, Dardevet M, Amiour N, Igerajas G (2003). Allelic diversity of HMW and LMW glutenin subunits and omega gliadins in French bread wheat (*Triticum aestivum* L). *Genetics & Crop Evolution* **50**: 669-679.

3. Collar C, Bollain C, Rosell CM (2007). Rheological behaviour of formulated bread dough during mixing and heating. *Food and Science Technology* **13**: 99-107.

4. Dowell FE, Maghirang EB, Pierce RO, Lockhart GL, Bean SR, Xie F, Caley MS, Wilson JD, Seabourn BW, Ram MS, Park SH, Chung OK (2008). Relationship of bread quality to kernel, flour, and dough properties**.** *Cereal Chemistry* **85**: 82-91.

5. Eagles HA, Hollamby GJ, Gororo NN, Eastwood RF (2002). Estimation and utilization of glutenin gene effects from the analysis of unbalanced data from wheat breeding programs. *Australian Journal of Agriculture Research* **53**: 367-377.

6. Figueroa JDC, Maucher T, Reule W, Peña RJ (2009). Influence of high molecular weight glutenins on viscoelastic properties of intact wheat kernel and relation to functional properties of wheat dough. *Cereal Chemistry* **86**: 139-144.

7. Gianibelli MC, Larroque OR, MacRitchie F, Wrigley CW (2001). Biochemical, genetic and molecular characterization of wheat endosperm proteins. *Cereal Chemistry* **78**:635-646

8. Gupta RB, MacRitchie F (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1* of common wheats. II. Biochemical basis of the allelic effects on dough properties. *Journal of Cereal Science* **19**: 19-29

9. Gupta RB, Paul JG, Cornish GB, Palmer GA, Bekes F, Rathjan AJ (1994). Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1* of common wheats. Its additive and interaction effects on dough properties. *Journal of Cereal Science* **19**: 9-17

10. Hernández ZJE, Figueroa JDC, Rayas-Duarte P, Martínez-Flores HE, Arámbula GV, Luna GB, Peña RJ (2012). Influence of high and low molecular weight glutenins on stress relaxation of wheat kernels and the relation to sedimentation and rheological properties. *Journal of Cereal Science* **55**: 344-350.

11. He ZH, Liu L, Xia XC, Liu JJ, Peña RJ (2005). Composition of HMW and LMW glutenin subunits and their effects on dough properties, pan bread, and noodle quality of Chinese bread wheats. *Cereal Chemistry* **82**: 345-350.

12. Ikeda TM, Branlard G, Peña RJ, Takata K, Liu L, He Z, Lerner SE, Kolman MA, Yoshida H, Rogers WJ (2008). International collaboration for unifying *Glu-3* nomenclature system in common wheats. International Wheat Genetics Symposium, Brisbane, Australia, August 2008.

13. Jackson EA, Morel MH, Sontag-Strohm T, Branlard G, Metakovsky EV, Redaelli R (1996). Proposal for combining the classification systems of alleles of *Gli-1* and *Glu-3* loci in bread wheat (*Triticum aestivum* L.). *Journal of Genetics and Breeding* **50**: 321-336

14. Liu L, He ZH, Yan J, Zhang Y, Xia X, Peña RJ (2005). Allelic variation at the *Glu-1* and *Glu-3* loci, presence of the 1B.1R translocation, and their mixographic properties in Chinese bread wheats. *Euphytica* **142**: 197-204

15. Luo C, Griffin WB, Branlard G, McNeil DL (2001). Comparison of low and high molecular wheat glutenin alleles effect on flour quality. *Theoretical and Applied Genetics* **102**:1088-1098

16. Maghirang EB, Lookhart GL, Bean SR, Pierce R0, Xie F, Caley MS, Wilson JD, Seabourn BW, Ram MS, Park SH, Chung OK, Dowell FE (2006). Comparison of quality characteristics and bread making functionality of hard red winter and hard red spring wheat. *Cereal Chemistry* **83**: 520-528.

17. MacRitchie F, Kasarda DD, Kuzmicky DD (1991). Characterisation of wheat protein fractions differing in contributions to bread making quality. *Cereal Chemistry* **68:** 122–130.

18. Martinant JP, Nicolas Y, Bouguennec A, Popineau Y, Saulnier L, Branlard G (1998). Relationships between mixograph parameters and indices of wheat grain quality. *Journal of Cereal Science* **27**: 179-189.

19. Maucher T, Figueroa JDC, Reule W, Peña RJ (2009). Influence of low molecular weight glutenins on viscoelastic properties of intact wheat kernels and their relation to functional properties of wheat dough. *Cereal Chemistry* **86**: 372-375.

20. Oury FX, Chiron H, Faye A, Gardet O, Giraud A, Heumez E, Rolland B, Rousset M, Trottet M, Charmet G, Branlard G (2010). The prediction of bread wheat quality: joint use of the phenotypic information brought by technological tests and the genetic information brought by HMW and LMW glutenin subunits. *Euphytica* **171**: 87-109.

21. Payne PI, Seekings JA, Worland JA, Jarvis MG, Holt LM (1987). Allelic variation of glutenin subunits and gliadins and its effect on bread-making quality in wheat: analysis of F5 progeny from Chinese Spring x Chinese Spring (Hope 1A). *Journal of Cereal Science* **6**: 103-118.

22. Ram S (2003). High molecular weight glutenin subunit composition of Indian wheats and their relationships with dough strength. *Journal of Plant Biochemistry and Biotechnology* **12**: 151-155.

23. Ram S, Sharma S, Verma A, Tyagi BS, Peña RJ (2011). Comparative analysis of LMW glutenin alleles in bread wheat using allele-specific PCR and SDS-PAGE. *Journal of Cereal Science* **54**:488-493

24. Singh NK, Shepherd KW (1988). Linkage mapping of genes controlling of endosperm storage proteins in wheat. I. Genes on the short arm of group 1 chromosome. *Theoretical and Applied Genetics* **75**:628-641

25. Tabiki T, Ikeguchi I, Ikeda M (2006). Effects of high molecular weight and low molecular weight glutenin subunit alleles in common wheat flour quality. *Breeding Science* **56**: 131-136.

26. Takata K, Yamauchi H, Nishio Z, Kuwabara T (2000). Effect of high molecular weight glutenin subuits on bread-making quality using near isogenic lines. *Breeding Science* **50**: 303-308.

27. Wang C, Kovacs MIP (2002a). Swelling index of glutenin test. I. Method and comparison with sedimentation, gel-protein, and insoluble glutenin tests. *Cereal Chemistry* **79**: 183-189.

28. Wang LH, Zhao XL, He ZH, Ma W, Appels R, Peña RJ, Xia XC (2009). Characterization of low-molecular-weight glutenin subunit *Glu-B3* genes and development of STS markers in common wheat (*Triticum aestivum* L.). *Theoretical Applied Genetics* **118**:525-539.

29. Wang L, Li G, Peña RJ, Xia X, He Z (2010). Development of STS markers and establishment of multiplex PCR for *Glu-A3* alleles in common wheat (*Triticum aestivum* L.). *Journal of Cereal Science* **51**:305-312.

30. Xin-zhong HU, Yi-min WEI, Kovacs MIP, Chun W (2004). Swelling index of gluten related to dough characters and noodle quality. *Scientia Agricultura Sinica* **18**: 1-10.