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Characterization of high molecular weight glutenin subunits of Wheat genotypes

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Abstract
End use quality of wheat is an important goal for developing elite genotypes. Glutenins are considered key factors in improving processing quality, particularly high molecular weight glutenin subunits (HMW-GS). The HMW-GS are known to be encoded by three *Glu-1* loci located on the long arms of chromosomes 1A, 1B and 1D. The endosperm storage proteins of 90 advanced breeding lines developed for peninsular zone of India were analysed by SDS-PAGE to determine their HMW-GS compositions. A total of ten alleles were identified: three at *Glu-A1*, five at *Glu-B1*, and two at *Glu-D1*. In *Glu-A1* locus, null, 1 and 2* alleles were found with the frequency of 48%, 30% and 22%. Whereas, in *Glu-B1*, 5 alleles with frequency of 17+18 (33%), 7+9 (27%), 7 (25%), 6+8 (9%), and 20 (6%) were detected. In *Glu-D1* locus, 2+12 and 5+10 were marked with the frequency of 60% and 40%, respectively. Quality score of genotypes were ranged from 4 to 10, with an average of 6.95 indicating vast genetic diversity among the genotypes. The presence of subunits combinations in different varieties with low, medium and high gluten strength revealed that breeding wheat for bread, chapatti and biscuits is possible as per end use requirement.

Key words: Wheat quality, HMW-GS, Glutenins, SDS-PAGE.

Introduction

High-molecular-weight glutenin sub units are major parts of wheat storage proteins, and play a significant role in determining wheat processing quality. The relationship between HMW-GS composition of wheat and the gluten strength of dough has been reported in number of studies (Payne *et al*. 1979, 1984, 1987, Pogna and Mellini, 1986, Branlard *et al*., 1992). The allelic variation in HMW-GS has been reported to account for nearly 60% of the variation in bread making quality (Payne *et al*., 1987). HMW-GS are encoded by the complex *Glu-1* loci located on the long arm of chromosomes from homeologous group 1 and called *Glu-A1*, *Glu-B1* and *Glu-D1* (Shewry *et al*., 1992). In each chromosome, the locus contains two closely linked genes that encode for two polypeptides, x-type glutenin subunit and y-type glutenin subunit (Shewry *et al*., 1992). Some of these genes are silent so most cultivars showed presence of three to five different subunits. Thus, the *Glu-A1* locus encodes for null or one (x-type) glutenins (the y-type glutenin of this locus is not expressed), the locus *Glu-B1* encode for one or two and *Glu-D1* encode for two glutenins subunits (Payne *et al*., 1981). Wheat varieties possessing five high molecular weight glutenin subunits generally have strong gluten than the varieties possessing 3 or 4 subunits. Payne *et al.* (1979) observed that there are differences among glutenin subunits for the amount of gluten strength added to dough. There are also differences in quality effects among glutenin of the same locus. At *Glu-D1* locus, the subunits 5+10 is associated with better bread making quality than the subunit 2+12. The subunit 2* and 1 at *Glu-A1* locus has better effect on gluten strength than null allele at this locus. Payne *et al*. (1987) assigned *Glu 1* quality score, which is obtained by adding the score of individual or pairs of subunits present at each *Glu-1* locus. This score was associated with poor or strong gluten strength and can serve as useful selection criterion for wheat quality in breeding programmes.

The wheat production is sufficient in India. The major use of wheat is for human consumption in the form of chapatti, bread and biscuit and each use require specific dough strength. The dough strength for biscuit, chapatti and bread making needs to be weak, medium and strong, respectively. The selection of breeding lines based on high molecular weight glutenin subunits will help to reduce the cost of extensive field evaluations of large-scale materials, which can be influenced by the environmental

conditions. Few lines/varieties were selected for quality alleles at *Glu-1* locus, which can be included in the varietal development program to improve processing quality as per end use requirement.

The purpose of present investigation was to determine the high molecular weight glutenin subunit composition of advanced high yielding breeding lines developed for peninsular zone before evaluating for varietal trials. The advanced lines were selected from series of field trials and were selected for agronomic superiority.

Materials and Methods

Seeds of 90 stable bread wheat genotypes were used for this study (Table1). These genotypes includes advanced breeding lines developed at Agricultural Research Station, Niphad, genotypes from different wheat research centers and released varieties. Total proteins were extracted from fine powdered endosperm of five seeds of each genotype. Varieties with reported HMW-GS pattern viz, Kalyansona (2*, 17+18, 2+12), PBW 343 (1, 7, 5+10), NIAW917 (1, 7, 5+10), MACS6222 (2*, 7+9, 2+12) and C-306 (N, 20, 2+12) were used as standards for comparison.

HMW-GS were analysed by sodium dodecyl sulfate polyacrlamide 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 HMW-GS of a subunit pair using scoring described by Payne *et al*. (1987) and Pogna and Mellini (1986). Alveograph W values were calculated as per the score given to each subunit by Pena R. J. (2002).

Cluster analysis (UPGMA hierarchical clustering) based on the standard genetic distances was done using NTSYS-pc version 2.1.

Results and Discussion

In this study, 10 different alleles were identified representing all the three *Glu-1* Loci (Table 2). At *Glu-A1* locus, the subunit "null" was most frequent and found in 48% of breeding lines followed by subunit "1" and "2*" in 30 and 22% of breeding lines, respectively. Payne and Lawrence (1983) observed predominance of 'null' allele at *Glu-A1* locus. The presence of 'null' at *Glu-A1* locus is a characteristic of landraces in wheat (Lagudah *et al*., 1987, Cross and Guo, 1993). This indicated that these breeding lines involved extensive use of wheat landraces as progenitor in the variety development process. Several reports 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 and Luo *et al*. (2001) and Ram *et al*. (2003) showed stronger role of subunit 2* in imparting gluten strength.

Out of 10 allelic variants identified, the maximum variants were found at *Glu-B1* locus. The most frequent HMW glutenin subunit at *Glu-B1* locus was 17+18, which was found in 30 (33%) breeding lines. It was followed by subunit 7+9 (27%), 7 (25%), 6+8 (9%) and subunit 20 (6%). The frequency of various alleles found in entire set of breeding lines at three *Glu* loci (*Glu-A1, Glu-B1 and Glu-D1*) is given in Figure 1a, 1b, 1c. About 60% of breeding lines were found to possess the subunit pairs 7+9 and 17+18 at *Glu-B1* locus. Subunit 20 that is known to contribute medium gluten strength and mostly known to occur in landraces was found in six breeding lines. The subunit pair 17+18 was known for contributing strong gluten strength and desirable for bread making quality. However, 60-70% of wheat produced in India is used for making dough for chapattis and for which medium gluten strength is required. The subunit 20 at *Glu-B1* locus conferring medium dough strength is best suited for the requirement of chapatti quality (Sreeramulu *et al*., 2004). Several studies favors the use of subunit 5+10 over 2+12 at *Glu-D1* locus for strong dough required mainly for bread making quality (Kolster *et al*., 1991, Luo *et al*., 2001, Liang *et al*., 2010). The subunit 20 appears in three combinations with other subunit or subunit pair at *Glu-A1* and *Glu-D1* locus (Table 2). The subunit 2+12 is present 60% of breeding lines as compared to stronger subunit 5+10 in 40% of breeding lines at *Glu-D1* locus.

The high molecular weight glutenin subunit combinations (allele at *Glu-A1, Glu-B1* and *Glu-D1*) and their frequencies in 90 breeding lines are presented in Table 2. Among the 90 breeding lines analysed, 23 different patterns were observed based on high molecular weight combination at all three *Glu-1* loci (Table 2). The most frequent patterns were N, 17+18, 2+12 (14 breeding lines), N, 7+9, 2+12 (13 breeding lines) and 1, 7, 5+10 (12 breeding lines). In

the present study, the combination 2*, 17+18, 5+10 and 1, 17+18, 5+10 was found in eight breeding lines and predicted that the suitability of these lines for products which need high gluten strength. 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 one of the breeding line NIAW 1846**.** In three breeding lines NIAW 2349, NIAW 2248 and NIAW 2030, the subunit '20' is present with subunit 'Null' at *Glu-A1* and 2+12 at *Glu-D1* contributing weak gluten strength. In two of breeding lines 118 and 206, the subunit 20 at *Glu-B1* present in combination with weak subunit 'Null' at *Glu-A1* and strong subunit pair 5+10 at *Glu-D1*. The combinations with *Glu-1* score of 7 observed in 14 breeding lines with HMW-GS pattern of 2*, 7+9, 2+12, 1, 7+9, 2+12 and N, 7+9, 5+10 and *Glu-1* score of 8 observed in 23 breeding lines with HMW-GS pattern of 1, 6+8, 5+10, 2*, 17+18, 2+12, 1, 7, 5+10, N, 17+18, 5+10 found to confers medium gluten strength. The gel showing the allelic variation and HMW-GS patterns is presented in figure 2.

The overall high molecular weight subunit quality score of advanced breeding lines is given in Table 2. The lowest *Glu1* score and Alveograph 'W' values was found in 10 breeding line which have 'Null' subunit at *Glu-A1*, 2+12 pair at *Glu-D1* and weak subunits at *Glu B1* (7 and 20). The maximum Glu-1 score of 10 and Alveograph 'W' value of 15 and 17 was found in eight breeding lines. The highest quality score was due to HMW-GS combination of 1, 17+18, 5+10 and 2*, 17+18, 5+10. A large proportion of wheat in India is used for Chapatti making which need medium strong gluten with a *Glu* score of 7 and 8. Among the analysed breeding lines, a large proportion of lines (30 lines) qualify for dough strength appropriate for Chapatti making. A large proportion of analysed lines (44.44% many) with *Glu-1* score ranged from 4 to 6 led to weaker dough suitable for biscuits.

Cluster analysis (UPGMA hierarchical clustering) based on the standard genetic distances is presented in Figure 3. In total 10 groups were observed at 45% genetic diversity level. At 70% genetic diversity, two clusters were formed which have major difference at subunits coded at *Glu-D1* locus. The cluster '1'consisted of three further subunit patterns (2*, 17+18, 2+12, 2*, 6+8, 2+12 and 2*, 7+9, 2+12) which differs at *Glu-B1* locus. The cluster '2' has different banding pattern at both *Glu-A1* and *Glu-D1* locus but share similarity with cluster 4. Cluster '3' has no difference at *Glu-B1* and *Glu-D1* alleles and share similarity with cluster 5 and 6. Cluster 7, 8, 9 and 10 had all common subunit 5+10 at *GluD-1* locus. The information generated from this study based on analysis of high molecular weight glutenin subunits of advanced breeding lines developed for peninsular zone will be used for further designing quality tests to strengthen the genetic worth of these lines along with maintaining yield potential.

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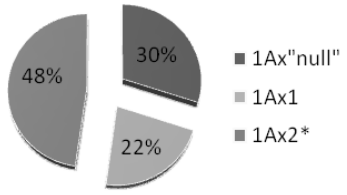

Figure 1A. Allelic frequency at the Glu-A1 locus

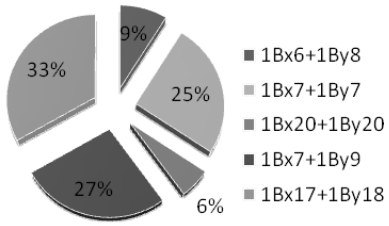

Figure 1 B. **Allelic frequency at the Glu-B1 locus**

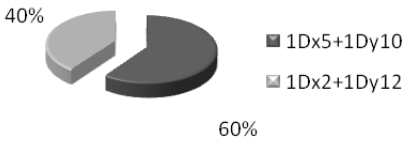

Figure 1C. **Allelic frequency at the Glu-D1 locus**

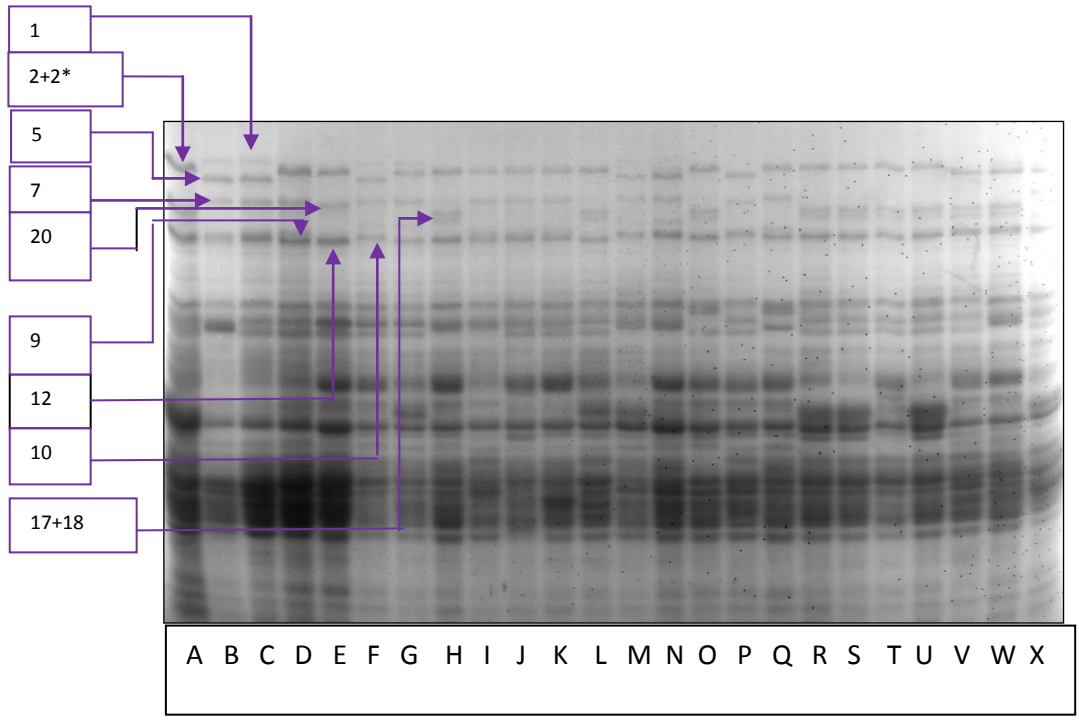

Figure 2: A:KS, B: PBW343,C: NIAW917,D: MACS6222,E: C-306, F: 178, G, 193,H: 194,I: 197, J: 198, K: 201, L: 202, M: 203, N: 204,O: 205, P: 206, Q: 207, R: 208, S: 209, T: 210, U: 211, V: 212, W: 213, X: 214

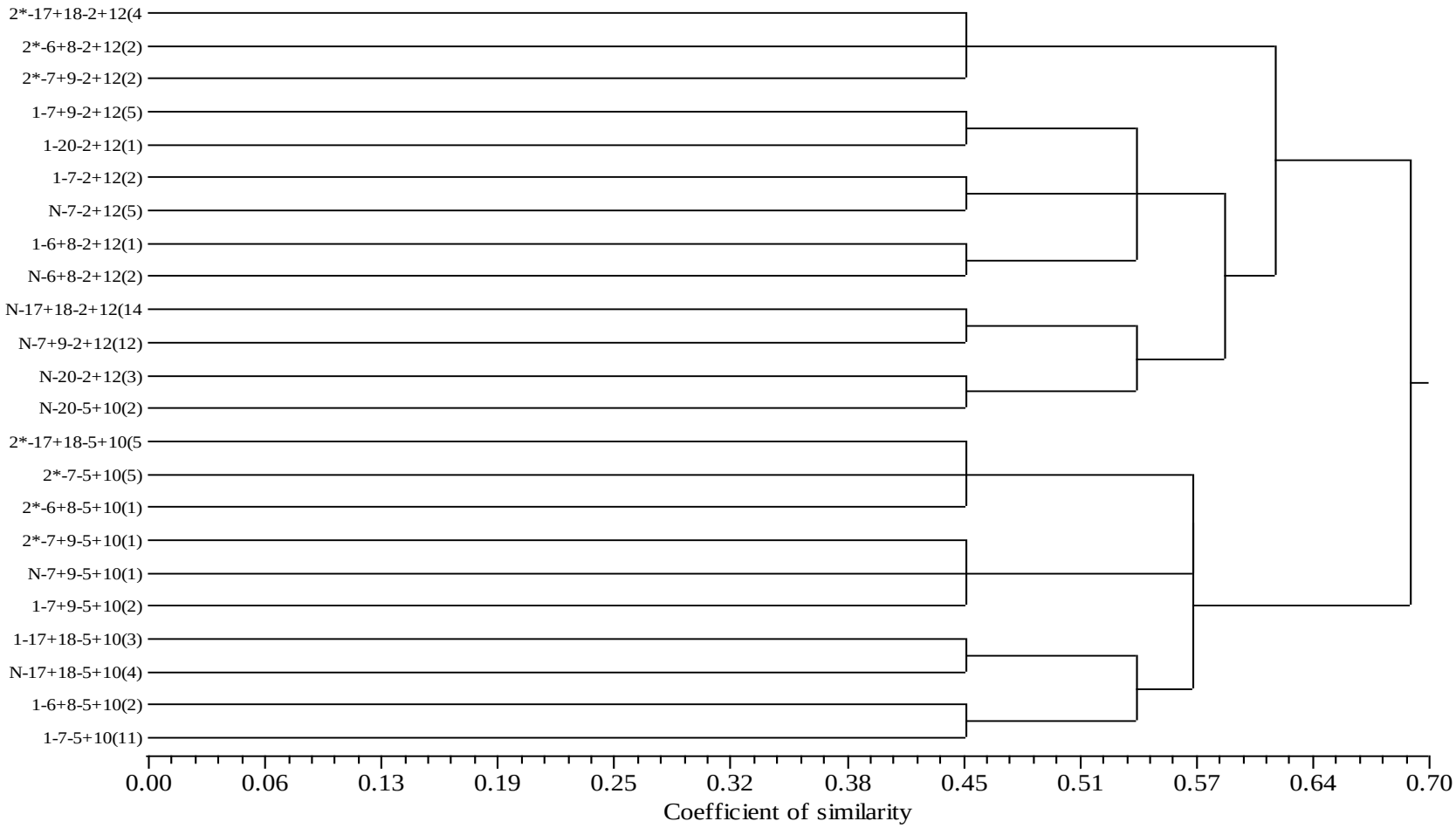

Figure 3: Clustering of wheat genotypes based HMW-GS pattern

Table 1: List of wheat genotypes used in the study

S. N.	Genotype	S. N.	Genotype	S. N.	Genotype
103	HD3040	135	NIAW 2351	166	MP3097
104	GW2008-161	136	NIAW 2059	167	JS-6-31
106	GW 2010-288	137	NIAW 1045	170	DBW 51
107	GWL 331	138	NIAW 1395	171	DBW 14
108	GW 2010-285	139	NIAW 2400	172	DBW 17
109	NIAW 2304	140	NIAW 1447	173	VW 915
110	NIAW 2064	141	NIAW 1415	175	VW 913
111	NIAW1047	142	NIAW 612	176	VW 912
112	NIAW 1088	143	NIAW 2349	177	PBW 452
113	NIAW 583	144	NIAW 2346	178	PHS 0622
114	NIAW 1343	145	NIAW 2255	193	RAJ 4037
115	NIAW 2268	146	NIAW 2248	194	RAJ 4266
116	NIAW 345	147	NIAW 2303	197	RAJ 4268
117	NIAW 2300	148	NIAW 789	198	FLW 3
118	NIAW 2313	149	NIAW 2310	201	NIAW 34
119	NIAW 1049	150	NIAW 2345	202	NIAW 2247
120	NIAW 2273	151	NIAW 1342	203	NIAW 2075
121	NIAW 560	152	NIAW 1275	204	NIAW 421
122	NIAW 2302	153	NIAW 1994	205	HD2998
123	NIAW 2065	154	NIAW 1846	206	HD2932
124	NIAW 1537	155	NIAW 2275	207	NIPHAD 4
125	NIAW 1689	156	NIAW 8223	208	NI343
126	NIAW 1594	157	NIAW 2348	209	NI 747-19
127	NIAW 1121	158	NIAW 9406	210	NI 5643
128	NIAW 514	159	NIAW 2030	211	NI 9947
129	NIAW 179	160	NIAW 2279	212	NI 5439
130	NIAW 1258	161	NIAW 1331	213	NI 345
131	NIAW 1412	162	NIAW 2325	214	JK ADTY
132	NIAW 2073	163	NI 179	216	HALNA
133	NIAW 1161	164	MP3075		
134	NIAW 1044	165	MP 3336		

Table 2: High molecular weight glutenin subunit composition of wheat genotypes under study

Glu-A1	Glu-D1	Glu-B1	Fr	Wheat genotypes	Glu-1 score	Alveograph 'W'
2*	2+12	17+18	4	109,119,131,161	8	13
		6+8	2	173,175	6	8
		7+9	2	113,166	7	12
2*	5+10	17+18	5	103,108,126,130,132	10	17
		7	5	127,128,133,171,176	8	13
		7+9	1	178	9	16
		6+8	1	117	8	12
1	2+12	7+9	5	104,114,170,177,193	7	10
		6+8	1	120	6	6
		20	1	154	6	6
		7	2	135,157	6	7
1	5+10	17+18	3	106,107,213	10	15
		6+8	2	160,165	8	10
		7+9	2	151,156	9	14
		7	11	112,125,132,134,136,141, 155,167,203,204,214	8	11
N	2+12	17+18	14	115,116,121,122,124,139,158,194,202,205,208,209,210,211	6	10
		6+8	2	110,164	4	5
		7+9	12	111,140,144,145,147,148,149, 150,152,,197,198,201	5	9
		7	5	123,137,138,142,207	4	6
		20	3	143,146,159	4	5
N	5+10	7+9	1	153	7	13
		17+18	4	129,162,163,212	8	14
		20	2	118,206	6	9