Advances in Life Sciences 5(8), Print : ISSN 2278-3849, 3008-3015, 2016

Relationship Between HMW Glutenin Subunit and Bread Making Quality of Wheat in Indian Cultivars

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ABSTRACT

Varieties of bread wheat contain three to five high molecular weight (HMW) subunits of glutenin. The high molecular weight (HMW) subunits of glutenin are coded by genes at three genetically unlinked loci, Glu-1A, Glu-1B and Glu-1D, which occur on chromosomes 1A, 1B and 1D respectively. Glutenin is responsible for most of the viscoelastic properties of wheat flour doughs and is the main factor dictating the use of a wheat variety in bread making. The SDS-Sedimentation test determines the strength of the dough by recording the swelling of the flour. In present study, we had taken six popular bread cultivars viz; HD 2967, HD3059, PBW 373, C306, PBW 343 and NI 5439 and HMW proteins were extracted from single seed of each variety and different protein fractions were separated by SDS-PAGE. Glu-1-score, wet gluten content and the SDS-Sedimentation values in all the 6 genotypes under study were in perfect synchrony with highest Glu-1 scores and SDS-Sedimantaion value in HD2967 (10, 58) followed by HD3059(10, 55), C 306 (5, 42), NI 5439 (6, 57) PBW 343 (6, 36) and PBW 373 (6, 38). SDS sedimentation value has significant correlation with Glu-1 score, which indicates the bread making quality. We concluded that variation in bread making quality of the different varieties was due to varying Glu-1- score. 1B/1R translocation adversely affects milling quality, sedimentation value and end use quality.

Key word *HMW, SDS-PAGE, SDS-Sedimetation, Glu-1-score and 1B/1R translocation.*

Wheat gluten has two main subunits; glutenin and gliadin. High molecular weight (Glu-1) plays a major role in determining dough strength (Gupta *et al*. 1994). Wheat contains three to five diverse high molecular weight (HMW) glutenin subunits. HMW subunits are encoded by Glu-1A, Glu-1B and Glu-1D gene families, located on 1A, 1B and 1D chromosome respectively (Payne et *al*. 1983). During the wheat improvement via natural breeding, classical breeding and molecular breeding, the gene have undergone extensive allelic variation. That's why many substitution or translocation of HMW subunit of glutenin is possible in varieties. Various wheat lines have been developed by translocation to increase various genetic resources. Most common translocations of wheat breeding have been concerned with the short arm of rye (1RS) due to presence of disease resistance genes (Mettin *et al*. 1973, Zeller and Hsam 1983) and 1RS translocations are fruitful for grain yield (Dhaliwal *et al.*1990, Villareal *et al*. 1991, Carver and Rayburn 1994). Translocation results in the loss of genetic encoding low molecular weight glutenin proteins and the grain of genes encoding rye monomeric ã-scalins, which increased salt water soluble protein concentration and also reduced dough strength (Lee *et al*. 1995, Sarkar et *al.* 2015). Unfortunately, this translocation has negative effect on bread making quality, mainly owing to dough stickiness and poor maxing.

SDS-PAGE method is one of the most widely used method used in laboratories to fractionate HMW and identify alleles forms, associated with good or poor bread quality. The sedimentation test determines the strength of the dough by recording the swelling of the flour which depend on the protein content and quality. In 1947, Zeleny used isopropanol alcohol in his experiment. While Dick and Quick (1983) used SDS along with Lactic acid to determine gluten strength. More the SDS Sedimentation value and so more is gluten strength. Thus sedimentation value can be correlated to gluten strength responsible for bread quality.

In India after 60 years of green revolution, knowledge of quality of wheats' end product (viz; bread, chapatti, paratha, puri, sattu, papad, pizza, noodles, naan, matthi & biscuit etc) has not reached among users due lack of extension and

literacy. All developed wheat genotype is not suitable for bread, chapatti or biscuit. Keeping this fact in mind, objectives of the current study is to examine the relationship between HMW Glutenin subunit and bread making quality for the screening of wheat among Indian cultivars.

MATERIALS AND METHODS

Selected genotypes for assay have cultivated under the same uniform conditions, yielding the seeds for the evaluations. Phenol test have been used for genetic purity assessment of seed. In present study, we had taken six popular bread cultivars containing two 1B/1R translocation lines viz; HD 2967, HD3059, PBW 373, C 306, PBW 343 and NI 5439 to test this variation in protein subunit affects bread making quality.

S No.	Wheat cultivars	Pedigree
1	HD 2967	ALD/COC//URES/HD2160M/HD2278
2	HD3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES
3	C 306	REGENT19473*CHZ//*2C591/3/P 19/C 281
4	NI 5439	REMP 80/3*NP 710
5	PBW 373	ND/VG9144//KAL/BB/3/YACO'S' /4/ VEE#5'S'
6	PBW 343	ND/VG9144//KAL/BB/3/YCO'S' /4/ VEE#5'S'

Gluten extraction

The new procedure is based on a extraction method earlier described by payne *et al*. (1980) and provides total wheat storage protein (glutenin) preparation from single kernels of seed within 30 minutes. Using the new method, two stock solutions are required: Extraction buffer (TRIS-0.75g, SDS-2g, Mercaptoethanol-4ml, total volume is maintain up to 100ml with distilled water) and Dye (Bromophenol blue-0.4g, glycerol-50ml, distilled water-50ml). Single kernel (25-40 mg of flour) is taken in eppendrof tubes and added 400 ul of extraction buffer to eppendrof. After vortexing, tubes are kept in hot water bath (85°C) for 15 minutes. Dye (40 ul) is added to sample and centrifuged for 5 min at 10000 rpm. The supernatant is transferred into new tube and 10-20 ul of the supernatant are loaded into a sample well of the 10% w/v polyacrylamide gel of SDS-PAGE for separation of the glutenin subunits. Electrophoresis was performed with a constant current of 25-40 *mA*/gel for 120 min, and temperature (25°C) of electrode buffer was maintained by circulating water. Gels were stained with Coomassie Blue R and then destained with water and the allele of subunits to be analyzed according to Payne *et al*. 1983 (Figure1).

Table 1. Qualitative scores for HMW glutenin single subunits or pairs of subunits (payne *et al*. 1983).

Chromosome			Glu-1 score
Glu-1A	Glu-1B	Glu-1D	
-	-	5+10	4
1	-	-	3
2 ?	-	-	3
-	17+18	-	3
-	7+8	-	3
-	13+16	-	3
-	7+9	-	2
-	-	2+12	2
Null	-	-	1
-	7	-	1
-	7+8	-	1
-	20	-	1

Table 2. Rye-adjusted quality score (Payne *et al.* 1983)

Glu-1 quality score between	Point to be subtracted due to 1BL/1RS translocation	Rye-adjusted quality score
8-10	-3	5-7
5-7	-2	3-5
3-4	-1	2-3

HMW and Glu-1 score analysis

Scores of HMW has determined by presence of protein bands and its size (Figure 1). Glu-1 score of genotypes are calculated by addition of score of Glu-1A, Glu-1B & Glu-1D subunits. The scores of HMW glutenin subunits and its Glu-1 scores were followed by previously used manner of Payne *et al*. 1983 & 1987 (Table 1). Rye adjusted Glu-1 score was calculated by the presence of 1B/1R based on the hypothesis of Payne *et al*. 1987. Varieties with a Glu-1 score of between 8-10 had three points subtracted, those between 5-7 had two points subtracted, and those between 3-4 had one point removed (Table2).

Quality analysis

The seeds containing 15% moisture were mild on the Brabender Quadrumat Senior mill. After the milling, the flour was analyzed for quality tests:

1- SDS sedimentation

The SDS-Sedimentation test (Dick and quick, 1983) is used to determine the strength of dough by swelling volume of the flour in a lactic acid-SDS solution. Lactic acid-SDS solution (100ml) contained 2g SDS, 2ml lactic acid (88%) solution and distilled water.

In The SDS-Sedimentation test, four replication of the sample can be carried at a time and following steps are followed:

1. Takes 3.2 gram of flour to be tested in a 100 ml glass-stopped graduated cylinder.
2. Simultaneously, time starts with addition 50

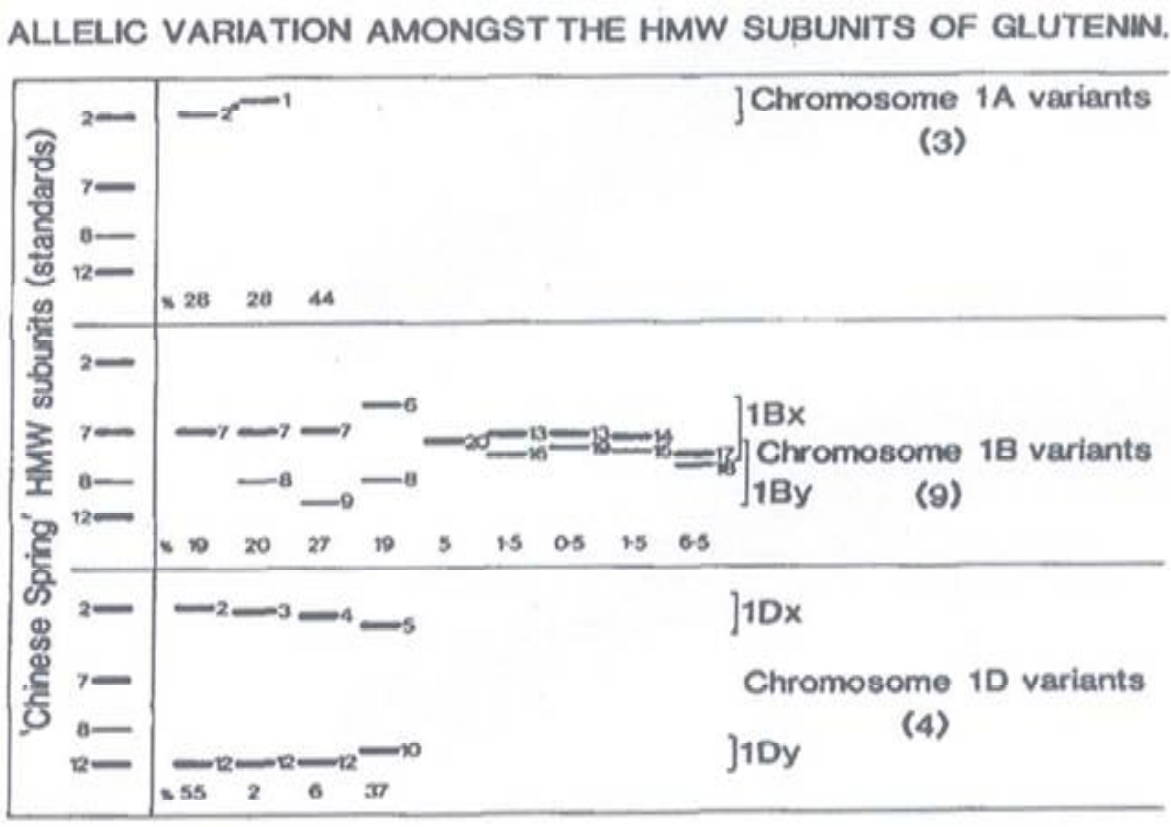

Fig. 1. Variation in the banding pattern of HMW protein subunits of glutenin. In the left hand side, Chinese spring is used as a standard (Payne *et al*. 1983)

ml of distilled water containing 0.2% bromophenol blue (addition of dye is optional). Mix thoroughly the flour and water by moving the stopper cylinder horizontally lengthwise, alternative right and left through a space of 18 cm, 12 times in each direction in 5 seconds. The flour should be completely swept into suspension during the mixing.

3. After first two minutes, mix the contents for 30 seconds in the following mode. First completely invert, then upright the cylinder. Carry out this action gently, exactly 18 times in 30 seconds. Let it stand for 1 minute and 30 seconds.
4. Add 25 ml of Lactic acid solution-SDS solution reagent and mix immediately by inverting 4 times and immediately keep the cylinder in an upright position for exactly 5 minutes.
5. After 5 minutes, records sedimentation value in ml. The sedimentation value will be equal to volume of cylinder. This is the uncorrected sedimentation value.
6. To obtain the correct sedimentation value (14% moisture basis), repeat the uncorrected sedimentation value by the appropriate factor in the following table-

Wheat moisture (%)	Factor	Wheat moisture (%)	factor	Wheat moisture (%)	factor	Wheat moisture (%)	factor
8.0	1.14	8.5	1.10	9.0	1.07	9.5	1.05
10.0	1.03	10.5	1.01	11.0	1.0	11.5	0.99
12.0	0.98	12.5	0.98	13.0	0.98	13.5	0.99
14.0	1.0	14.5	1.02	15.0	1.04	15.5	1.07

7 Sedimentation value should be measured to the nearest milliliter.

2. Gluten content

Gluten content has determined by washing of dough flour with running tap water and salt solution to remove starches and others proteins. Wet gluten formed a ball like structure called as gluten ball. Water of wet gluten has removed by oven drying at 110^0C for overnight (AACC, Method 38-12). The percentage of gluten is defined as the gluten index.

Statistical Analysis

The statistical analysis for Correlation and ANOVA was done by using PAST software (Colwel and Coddington 1994). Correlation coefficient among Glu-D1, Glu-A1, Glu-B1, Glu-1 score, Wet gluten & SDS sedimentation test tells us how strongly two or more variables are related to each other. Analysis of variation (ANOVA) with single factor is used to test the null hypothesis and significant variation among populations.

RESULTS AND DISCUSSION

HMW and Glu-1 Score

Allele of HMW subunits have scored according to Payne *et al*. 1983 (Figure1). The Glu-1 quality score of a variety is simply calculated by summing the scores of the individual subunits of HMW. These results are summarized and assigned scores to each of these subunits are given in Table 3. HD2967 and HD 3059 showed the presence of 2*, 17+18 & 5+10 subunits with highest glu-1 score (10). Least glu-1 score (5) has observed in C306 with N, 20 & 2+12 subunits. Six glu-1 score value has observed in NI5439 with N, 17+18 & 2+12 Subunits. Both, PBW343 and PBW 373 have showed 1, 7+9 & 5+10 subunits with 6 rye adjusted glu-1 score due to 1B/1R translocation lines. In the collection of wheat studied here, the maximum score is 10 and the minimum is 5.

SDS Sedimentation value

The sedimentation test is conducted by holding the ground wheat or flour sample in an acid solution. During the sedimentation test gluten protein of ground wheat or flour swells and precipitate as a sediment. Highest sedimentation values (57) have observed in HD2967 and NI5439 followed by HD3059, C306 and PBW373 with 54, 40 & 39. Least sedimentation value (34) has observed in PBW343 (Table 3). The sedimentation test provided information on the protein quantity and the quality of the ground wheat and flour samples.

Wet Gluten content

The Wet gluten weight test provides information on the quality and estimates the quality of gluten in wheat or flour sample. Gluten is responsible for the elasticity and extensibility characteristics of flour dough. In present study, maximum wet gluten content (31) has observed in HD2967, followed by HD3059, NI5439, PBW 343 and PBW373 with 30.7, 29, 28.6 & 28.4% respectively. Least percentage of wet gluten is observed in C306 with 28% (Table 3). Wet gluten reflects protein content and is common flour

Table 3. Characterization of wheat genotypes evaluated for HMW glutenin subunit and Glu-1 score, Gluten content and SDS-sedimentation volumes.

Varieties	Chromosome (HMW)			Glu-1 score	Rye score	Adjusted Glu-1 score	Gluten content (%)	SDS sedimentation value
	Glu-A1	Glu-B1	Glu-D1					
HD2967	2*	17+18	5+10	10		10	31	57
HD3059	2*	17+18	5+10	10		10	30.7	54
C306	N	20	2+12	5		5	28	40
NI5439	N	17+18	2+12	6		6	29	57
PBW343	1	7+9	5+10	9	-3	6	28.6	34
PBW373	1	7+9	5+10	9	-3	6	28.4	39

specification required by end-users in the food industry.

Statistical Analysis

HMW-Subunits Glu-1A, Glu-1B, Glu-1D, Glu-1 score, wet gluten, and sedimentation values showed significant positive correlation (Table 4). In the present investigation the sedimentation value showed significant correlation among them.

In the ANOVA, if F > Fcrit, then it rejects the null hypothesis. This is the case, 104.97> 2.533. Therefore, the means of the three populations are not all equal. According to variance analysis (ANOVA) all the populations showed significant variation (Table 5) and were independent entity.

Pictorial representation of correlation among HMW-GS, Glu-1 score, wet gluten, and sedimentation value related with bread-making quality in six wheat cultivars have made on excel sheet (Figures 3). We have verified these conclusions by looking at the graph. As variable X increases, variable Y increases. As variable X decreases, variable Y decreases. High sedimentation values have been reported linked with stronger gluten and superior bread making quality. Positive correlation was observed between sedimentation value and gluten content.

Bread making quality is one of the essential traits of wheat associated with the genetic improvement by using HMW glutenin subunits. The HMW subunits have coded by the Glu-1A, Glu-1B and Glu-1D genes located at chromosomes 1A, 1B and 1D, respectively (Payne *et al*. 1980, 1981, 1983, 1987). In 1980, Lawrence and Shepherd have analyzed variability in wheat genotypes produced

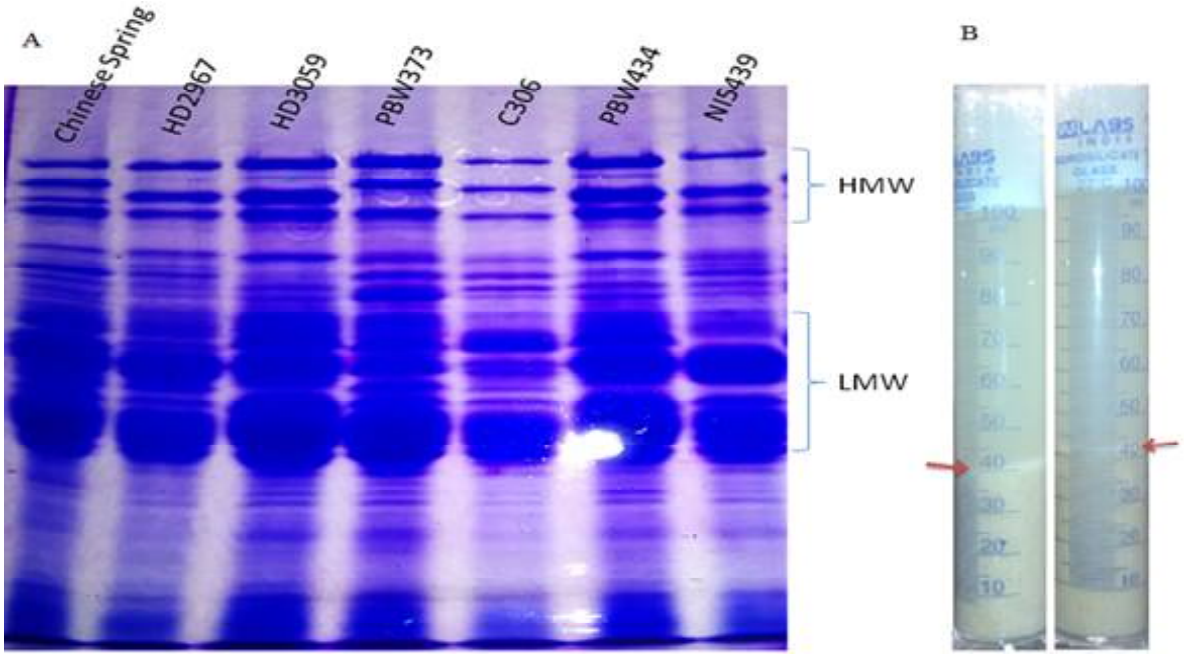

Fig. 2. (A)- shows the electrophoresis gel, Chinese spring use as a standard & (B)- SDS Sedimentation test.

Table 4. Correlation: among Glu-D1, Glu-A1, Glu-B1, Glu-1 score, Wet gluten & SDS sedimentation test.

	Glu-1D	Glu-1A	Glu-1B	Glu-1 score	Wet gluten	SDS Sedimentation
Glu-1D	1					
Glu-1A	0.99973**	1				
Glu-1B	0.98675*	0.98986*	1			
Glu-1 score	0.99788**	0.9967**	0.98215*	1		
Wet gluten	0.96351*	0.96899*	0.99384**	0.95541*	1	
Sedimentation	0.98367*	0.98703*	0.99956**	0.97849*	0.99558**	1

Significant levels:*P=0.01-0.001; **P<0.001

SS: Some of Squares

df: Degree of freedom

MS: Mean Squares

Significant levels: P<0.001

F>Fcrit (104.9781>2.53355

by allelic variation at each locus. Bread making quality of wheat depends on allelic variation at the Glu-D1 locus. Three alleles have belonged to the Glu-1A locus, 8 to Glu-1B and 2 to Glu-1D (Sewa Ram 2003). Glu-1A has contained three alleles Glu-1A (subunit 1), Glu-1A (subunit 2*) and Glu-1A (Null allele) alleles have found in the wheat genotypes. Out of the 8 alleles, four alleles have observed at Glu-1B, namely Glu-1B (17+18), (7+8), (7+9) and 20 have present in all used genotypes. Allele of Glu-1D encoding the subunit (5+10) and (2+12) subunit. The Glu-1 score have calculated by summing the scores of the individual HMW subunits it contains. Subunit 5+10 is given a score of 4 and 2+12 a score of 2, have related with higher bread-making quality potential (Sarkar *et al*. 2015). In addition, SDS sedimentation value has significant correlation with Glu-1 score, which indicates the bread making quality (Payne *et al*. 1992). The sedimentation value has been correlated with the gluten strength. More is the gluten strength of wheat more is the swelling of dough. It is clearly showed that HD2967 & NI5439 have highest Glu-1 score with maximum SDS sedimentation value followed by HD3059. C306 have least Glu-1 score with greater SDS sedimentation value than translocation lines. PBW343 and PBW373 have more wet gluten contain than others but least sedimentation value due to 1B/1R translocation.

In current study, HD2967 and DH3059 have showed the combination of subunits 2*, 17+18 and 5+10 with significant positive correlation on sedimentation value ($p<0.001$) as compared to other genotypes. Wet gluten content also has significant positive correlation with the combination of subunits 2*, 17+18 and 5+10 subunits. Thus gluten strength of wheat having 2*, 17+18 and 5+10 glutenin subunit combinations contain good bread making quality (Sarkar et al. 2015, Sewa Ram 2003, Dhaliwal et al 1990, Payne el. 1983 & 1987). The SDS-sedimentation analysis indicated the significant

Table 5. ANOVA among Glu-D1, Glu-A1, Glu-B1, Glu-1 score, Wet gluten & SDS-Sedimentation value.

ANOVA: Single Factor						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10476.15	5	2095.229	104.9781	4.49E-18	2.533555
Within Groups	598.7617	30	19.95872			
Total	11074.91	35				

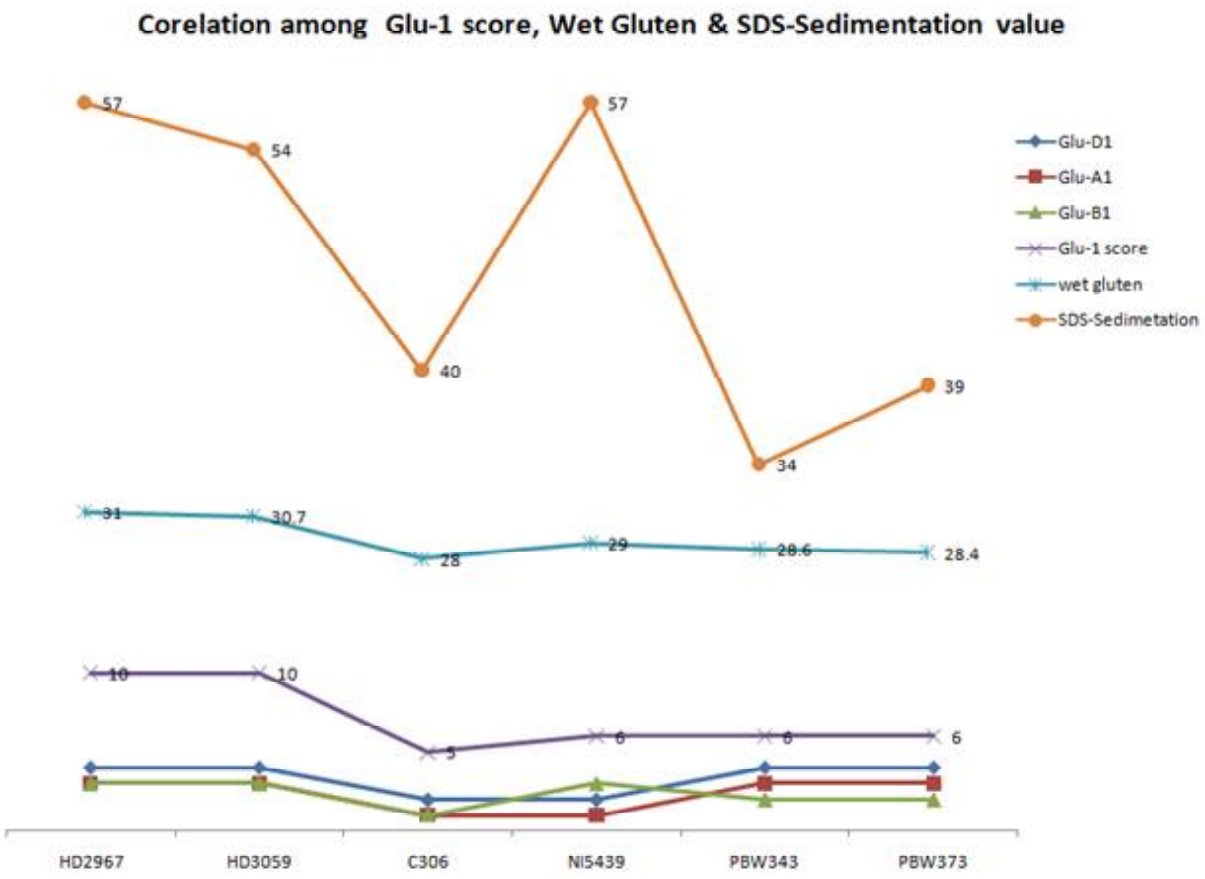

Fig. 3. Pictorial chart representation of correlation among HMW subunit Glu-1A, Glu-1B, Glu-1D, Glu-1 score, Wet Gluten & SDS-Sedimentation value with bread-making quality in six wheat cultivars.

negative correlation of subunit 20 on sedimentation value when compared with 17+18 and 7+8 subunits (Table 2). C306 genotypes have 20 subunit showed negative correlation with sedimentation value and wet gluten content. Liu and Rathjen (1996) also reported the similar negative correlation of subunit 20 with the dough strength. Sewa Ram (2003) also suggested that the null allele at the Glu-A1 locus was found associated with lower sedimentation value. Surprising result has observed that NI5439 has higher sedimentation value (58) with lower Glu-1 score.

In SDS-sedimentation analysis, PBW 343 and PBW373 have significantly negative correlation with sedimentation value due to 1B/1R translocation. Similar result have also supported by Dhaliwal *et al*. (1990) and Sewa Ram (2003). In 1990, Dhaliwal *et al*. also reported that two factors are responsible for sticky dough. First, there is a decrease in the strength contributing LMW glutenin subunits encoded by the Glu-1B. Second, there is a gain of monomeric ã-secalin subunits encoded by the Rye-1R chromosome. That's why increased stickiness of the water soluble fractions from the translocation lines. Stickiness of dough will be directly associated with interaction of water with the ratio of polymorphic/monomorphic subunits. Water ions provided the oxygen that plays a key role in the swelling of dough volume. Data clearly indicate that Glu-1B can be substituted by Rye-1R locus for improving the dough strength, is measured by sedimentation value in comparison to other (Sewa Ram 2003, Payne et *al*. 1987).

The production of a new wheat cultivar takes more time, labor and cost. Physical and chemical tests are more useful for detection of bread making quality of wheat. Wheat breeder and product users both feel the requirement of simple test for identification of bread making properties in wheat. The results of present study demonstrate that combination of HMW subunits 2*, 17+18 and 5+10 have highest Glu-1 score, sedimentation value and wet gluten content. Glu-1 score and sedimentation test will be useful as a screening tool for the selection of genotypes with high yield and good bread-making quality for wheat breeding. With

result of present work, we expect to contribute more and more to wheat breeders for selection of genotypes for wheat breeding programs to develop a new genotype with good flour quality for users and to be more competitive in the market.

ACKNOWLEDGMENTS

Rajesh Kumar Gupta acknowledges the senior research fellowship granted by ICMR for carrying out this part of his Ph D work at NRC on Plant Biotechnology, IARI, New Delhi 110012.

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Received on 07-04-2016 *Accepted on 12-04-2016*