



# Selection of Pakistani and CIMMYT Wheat Lines for Better Grain Yield and Quality

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## ABSTRACT

Variation in grain yield and composition of high molecular weight glutenin subunits is related to genotypic variation in wheat end-use quantity and quality. In the present study, 26 Pakistani and 47 CIMMYT wheat genotypes were studied to determine grain yield and the composition of HMW-GS alleles. Sixteen different Glu-1 alleles were identified at *Glu-A1*, *Glu-B1* and *Glu-D1* loci resulting into 28 allelic combinations. Number of unique combinations of HMW-GS in Pakistani and CIMMYT accessions was five and twelve, respectively. Analyses of yield and high molecular weight glutenin subunits from these experiments projected few genotypes possessing high protein quality and yield that was far greater than the current wheat varieties in Pakistan. Therefore, new varieties can be improved potentially and made more consistent in yield and quality if suggested genotypes in the present study are used in breeding programs. © 2012 Friends Science Publishers

Key Words: Grain yield; Germplasm; Glutenin; Quality; Wheat

## **INTRODUCTION**

Use of limited germplasm and race for breeding economically important traits has narrowed the genetic constitution of the prevailing wheat varieties in Pakistan (Sajjad *et al.*, 2011). This warrants the need for new sources of genetic diversity for developing promising cultivars that suit the changing environments (Moghaddam *et al.*, 1998). Both, local genotypes developed through a combination of natural and artificial selection (Belay *et al.*, 1995) and newly introduced CIMMYT genotypes have valuable characters that can be further exploited for the development of new cultivars with better adaptability and broader genetic base (Keller *et al.*, 1991; Tesemma *et al.*, 1998). Improving grain yield and grain quality are two major challenges for many wheat breeding programs.

The bread making quality in wheat depends on the composition of endosperm storage proteins namely, gliadins and glutenins (Branlard & Dardevet, 1985; Payne *et al.*, 1987; Dessalegn *et al.*, 2011). Gliadins are monomeric prolamins synthesized by the *Gli-1* and *Gli-2* loci located on the short arms of the homeologous chromosoms 1 and 6, respectively (Payne *et al.*, 1984; Payne, 1987). Kasarda (1999) classified glutenin into two types, low molecular weight (23-68 kDa) glutenin subunits (LMW-GS) and high molecular weight (77-160 kDa) subunits (HMW-GS). The LMW-GS are encoded by *Glu-3* genes located on the short arms of chromosomes 1A, 1B and 1D and are in close proximity of *Gli-1* loci (Singh & Shepherd, 1988; Pogna *et* 

*al.*, 1990). The HMW-GS are controlled by the *Glu-1* loci located on the long arms of 1A, 1B and 1D homeologous chromosomes. Each locus for HMW-GS encodes one x-type gene and one y-type subunit gene (Payene, 1987). Nonetheless, gene silencing in hexaploid bread wheat results in the expression of only three to five HMW-GS. The composition of HMW-GS differs between cultivars with allelic variation (Shewry *et al.*, 2003). Assessment of the contribution of each HMW-GS to bread making quality of wheat has been reported by assigning quality scores to individual HMW-GS or HMW-GS pairs (Payene, 1987; Lukow *et al.*, 1989). In addition, HMW-GS are convenient genetic markers for estimating genetic variation among wheat genotypes (Bushehri *et al.*, 2006).

The present study was carried out to determine the HMW-GS composition in 26 Pakistani varieties and 47 CIMMYT lines available to Pakistani breeders. Genetic diversity at the *Glu-1* loci, quality score and its relationship with grain yield per plant was determined. The resulting information may be used as guidelines for the selection of suitable parents to evolve high yielding wheat varieties with better end-use properties.

### MATERIALS AND METHODS

**Plant culture:** Seventy three wheat genotypes were gown in the experimental area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, during 2008-2009. Standard cultural practices

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were used and data about grain yield per plant were recorded on ten guarded plants grown in RCBD layout with three replications.

**Protein extraction and SDS-PAGE:** A single spike was harvested separately from each of 73 wheat accessions for Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Two or more individual seeds of each variety were used to extract proteins according to Singh *et al.* (1991). Marquise, Chinese Spring, Hope and Gabo were included in the analysis as standards. The SDS-PAGE was performed following the method of Laemmli (1970) using 11.5% Polyacrylamide with minor modifications.

Allelic variation for the high molecular weight glutenin subunits (HMW-GS) at *Glu-1* loci was noted by numbering the bands at each subunit (*Glu-Al, Glu-B1 & Glu-DI*) based on nomenclature set by Payne and Lawrence (1983). Quality score was calculated according to Payne (1987) by adding together the score of individual sub-units. Statistica (V7) software was used to calculate ANOVA, correlations and scatter plot for grain yield and HMW-GS score.

#### RESULTS

Variation in HMW-GS in Pakistani wheat cultivars and **CIMMYT accessions:** The composition of HMW-GS and allele frequencies in 26 Pakistani cultivars and 47 CIMMYT wheat accessions are shown in Fig. 2 and 3. Sixteen different Glu-1 alleles were identified, three at the Glu A1, nine at the Glu B1 and four at the Glu D1 loci (Fig. 2 & 3). Those found in CIMMYT accessions but not in local cultivars included four alleles (8, 13+19, 14+15 & 20) at Glu B1 locus and two alleles (5+11, 2+11) at Glu-Dl locus. Pakistani cultivars had no allele that was absent in CIMMYT accessions (Fig. 2 & 3). These sixteen alleles at Glu-A1, Glu-B1 and Glu-D1 loci resulted into 28 allelic combinations of HMW-GS (Table I). Out of total 28 HMW-GS combinations in both groups, five were present only in Pakistani cultivars (Table I), while twelve HMW-GS combinations were present only in CIMMYT accessions (Table I).

**HMW-GS diversity in Pakistani cultivars:** For all Pakistani cultivars under study, 10 different alleles were identified, 3 corresponding to the *Glu-A1* locus, 5 to *Glu-B1* locus and 2 to *Glu-D1* locus (Fig. 2). All of the commonly found allelic variants at the *Glu-A1* locus (null, 1 & 2\*) as described by Payne and Lawrence (1983) were found in the germplasm under study. Frequency of HMW-GS (Table II) and percent partial frequency (Fig. 2) of 2\* allele were 12 and 46.15% respectively and were high among all the alleles of *Glu-A1* locus.

At Glu-*B1* locus, 5 different HMW glutenin subunits combinations (17+18, 13+16, 7+9, 7+8, 7) appeared in the Pakistani varieties. Among these subunits 7+9 and 17+18 were more frequent with frequency and partial frequency of 9 and 34.62% (Fig. 2). The subunit 7 was occurred least with frequency 2 and partial frequency of 7.69% (Fig. 2).

Fig. 1: SDS-PAGE Electrophoretic pattern showing variation in of Pakistani wheat cultivars (First 10 samples). MARQUIS, HOPE, CHINISE SPRING, and GABO were used as Standards. Location of HMW GS in standards is also shown

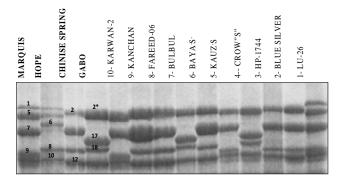


Fig. 2: Distribution of HMW-GS in the Pakistani genotypes on Glu-A1, Glu-B1 and Glu-D1

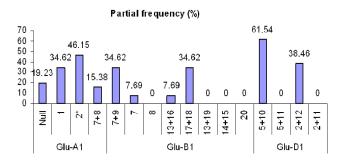
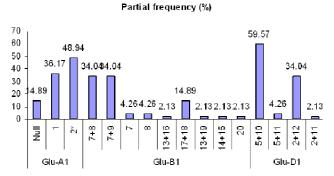


Fig. 3: Distribution of HMW-GS in the CIMMYT genotypes on Glu-A1, Glu-B1 and Glu-D1



Two alleles (5+10 & 2+12) were found at *Glu-D1* locus. Allele 5+10 had higher frequency (16) and partial frequency (61.54%).

Sixteen HMW-GS combinations were observed in Pakistani varieties (Table I). The subunit compositions of 2\*, 7+9, 5+10 and 2\*, 17+18, 2+12 were most frequent and each was observed in 4 Pakistani cultivars out of 26 included in the study. Second most frequent combination was 1, 7+9, 5+10 found in 3 of 26 cultivars. Each of 2\*, 7+8, 2+12 and N, 17+18, 5+10 HMW-GS combinations was present in 2 cultivars. Other 11 subunit combinations

GluA1	GluB1	GluD1	Frequency	Pakistani cultivars	Quality scores
1	7+8	5+10	1	KOHSAR-95,	10
1	13+16	5+10	1	SA-75	10
1	17+18	5+10	1	ZAMINDAR-80	10
2*	17+18	5+10	1	ZARDANA-89	10
1	7+9	5+10	3	LU-26, PUNAB-76, PUNAB-85	9
2*	7+9	5+10	4	KARWAN, MANTHAR, PASINA-90, SATLUJ-86	9
Null	17+18	5+10	2	NAEEM-82, PAVON-76	8
2*	7+8,	2+12	2	FAREED-6, SHAHKAR-95	8
2*	17+18	2+12	4	BULBUL, PARWAZ-94, PUNAB-81, PUNAB-96	8
2*	7	5+10	1	MH-97	8
1	13+16	2+12	1	UFAQ	8
1	7	5+10	1	PARI-73	8
1	7+9	2+12	1	KOHINOOR-83	7
Null	17+18	2+12	1	SA-42	6
Null	13+16	2+12	1	SHALIMAR-88	6
Null	7+9	2+12	1	BLUE SILVER,	5

#### Table I: Combination of Glu-1 alleles and quality score in Pakistani Varieties

Table II: Combination of Glu-1 alleles and quality score in CIMMYT Varieties

GluA1	GluB1	GluD1	Frequency	CIMMYT accessions	Quality scores
1	7+8	5+10	5	HOOSAM-3, KAUZ/STAR, PBW65/ROEK, SW89-5277/BOR95, MILAN	10
1	17 + 18	5 + 10	2	SNI/PBW65, TE173	
2*	17 + 18	5 + 10	3	TOBA97/BAV-92, PASTOR//2*MILAN/KAUZ, HUW234/LR 34	10
2*	7+8	5 + 10	3	PVN/YACO, F60314.76, VEE/PVN	10
1	7+9	5 + 10	6	NG 8201/KAUZ/4, RL6043/4*NAC, JUP/ZP//COC, SW91-4903/CIRCUS, OPTA//SOR, JOHARA-14	9
1	7+9	5+11	1	AMSEL/2*BAU	9
2*	7+9	5+11	1	SKAUZ	9
2*	7+9	5 + 10	1	MANGO,	9
Null	17 + 18	5 + 10	1	CROW''S''	8
2*	7+8	2+12	6	SHA7/VEE5, GIRWILL-7, GAMDOW-6, LOCAL WHITE, BAYA'S', KANCHAN	8
2*	17 + 18	2+12	1	MYNA'S'	8
2*	7	5 + 10	1	ATTILA/PASTOR	8
2*	20	5 + 10	1	WEAVER/TSC	8
2*	13+19	2+12	1	WEAVER//VEEP	8
2*	7+8	2+11	1	HD29/2*WEAVER	8
1	7	5 + 10	1	PBW343*2/KURUKU	8
Null	7+8	5 + 10	1	KAUZ/TSC	8
Null	7+9	5 + 10	0	KAUZ'S'	7
Null	14 + 15	5 + 10	1	ESDA/SHWA	7
2*	7+9	2+12	4	SLVS/PASTOR, NG8201/KAUZ/PASTOR, PBW343*2/CHAPIO, V763.2312	7
1	8	5 + 10	2	KAUZ2*/MNV, KASYON	6
Null	7+8	2+12	1	HXL8246	6
Null	7+9	2+12	2	OPATA/RAYON/KAUZ, HP-1744	5

exhibited lowest frequency (1) and only one variety was present in each combination.

The HMW glutenin subunit quality scores for the cultivars, included in this study, calculated according to Payne *et al.* (1987). The overall quality score for individual cultivars ranged from 5 to 10 (Table I). According to quality score KOHSAR-95, SA-75, ZAMINDAR-80 and ZARDANA-89 had highest score of 10. Seven cultivars (LU 26, KARWAN, MANTHAR, PASINA 90, PUNJAB-85, SATLUJ-86 & PUNJAB-76) had quality score of 9. BLUE SILVER had the lowest score of 5.

**HMW-GS diversity in CIMMYT lines:** Sixteen different alleles at Glu-1 loci were identified in 47 CIMMYT accessions, 3 corresponding to the *Glu-A1* locus, 9 to *Glu-B1* locus and 4 to *Glu-D1* locus (Table II). At *Glu-A1* locus three HMW-GS (Null, 1, 2\*) were present. Frequency and partial frequency of 2\* allele i.e., 23 and 48.94% respectively were high among all the alleles of *Glu-A1* locus

(Fig. 3). The sub-unit 1 was second most frequent with frequency and partial frequency 17 and 36.17% respectively (Fig. 3).

At Glu-*B1* locus, 9 different HMW glutenin subunit combinations (17+18, 13+16, 7+9, 7+8, 7, 8, 13+19, 14+15, & 20) appeared in the CIMMYT accessions under study. Among these subunits 7+8 and 7+9 were more frequent each with frequency and partial frequency of 16 and 34.04%, respectively (Fig. 3). Subunits 13+16, 13+19, 14+15 and 20 occurred least each with frequency of 1 and partial frequency of 2.13% (Fig. 3). At *Glu-D1* locus 4 subunits were found (5+10, 5+11, 2+12 & 2+11). Among them 5+10 had more frequency (28) and partial frequency (59.57%). The subunit combination 2+11 was least frequent with frequency 1 and partial frequency 2.13% (Fig. 3).

Twenty different allelic combinations of *Glu-A1*, *Glu-B1* and *Glu-D1* were found (Table I). Each of the combinations 1, 7+9, 5+10 and 2\*, 7+8, 5+10 was highest

in frequency of 6 (Table I). The combinations 1, 7+8, 5+10 was second with frequency of 5. Fourteen different subunit combinations exhibited in lowest frequency that was only 1 and each combination had only one accession.

Thirteen accessions SW89-5277/BOR95, MILAN.TOBA97/BAV-92. ASTOR//2\*MILAN/KAUZ, HUW234/LR34, PVN/YACO, SNI/PBW65, VEE/PVN, HOOSAM-3, KAUZ/STAR, TE173, F60314.76 and PBW65/ROEK had the maximum score of 10 indicating that these could be utilized to improve the bread making quality. The second highest score (9) for HMW-GS was observed in NG-8201/KAUZ, SW91-4903/CIRCUS, JUP/ZP//COC/3, RL6043/4\*NAC, OPTA//SOR AMSEL/2\*BAU, SKAUZ, MANGO and JOHARA-14. Thirteen accessions including CROW"S", SHA7/VEE5, GIRWILL-7, GAMDOW-6, LOCAL WHITE, BAYAS', KANCHAN. MYNA'S', ATTILA/PASTOR, WEAVER/TSC, WEAVER//VEEP, HD29/2\*WEAVER, PBW343\*2/KURUKU, KAUZ/TSC had quality score of 8. Only two CIMMYT accession, OPATA/RAYON/KAUZ and HP-1744 had lowest quality score of 5 (Table I).

**Grain yield:** Wheat germplasm used in present study exhibited highly significant variation for grain yield per plant (Table III). With a mean grain yield of 35.5 g per plant, Pakistani varieties showed a narrow range of grain yield (24.5-48.4 g per plant). SA-75 showed the lowest yield per plant while PAVON-76 produced the highest (48.4 g per plant). On the other hand, CIMMYT germplasm exhibited a higher range of grain yield (20.5-64.6 g per plant) and mean grain yield (37.7 g per plant) as compared to Pakistani germplasm (Table IV).

**Relationship of grain yield and HMW-GS score:** Correlation between grain yield and HMW-GS was found non-significant (0.08, P>0.01). The scatter plot of grain yield against quality score highlighted CIMMYT lines with respect to grain yield and HMW-GS score including TOBA-97/BAV-92 and KAUZ/STAR quality score of 10 and per plant yield of 64.6 g and 57.2 g respectively(Fig. 4).

#### DISCUSSION

Sixteen Glu-1 alleles were found in the collection of wheat genotypes studied in present work (Fig. 2 & 3). A similar extent of allelic diversity at Glu-1 loci has been reported previously (Tahir *et al.*, 1996; Zhang *et al.*, 2002; Liu *et al.*, 2007). The CIMMYT wheat accessions exhibited maximum diversity by possessing all of the sixteen alleles as compared with Pakistani genotypes with only 10 HMW-GS alleles. Dessalegn *et al.* (2011) also observed 10 HMW-GS alleles in Ethiopian wheat genotypes. This indicates an under-utilization of the CIMMYT germplasm in wheat breeding programs in Pakistan and Ethiopia. As the allelic variation for *Glu-A1* locus in Pakistani and CIMMYT was similar, the efforts should be made to exploit more diverse loci including *Glu-B1* and *Glu-D1*. Similar results were reported by Dotlacil *et al.* (2002) and Liang *et al.* 

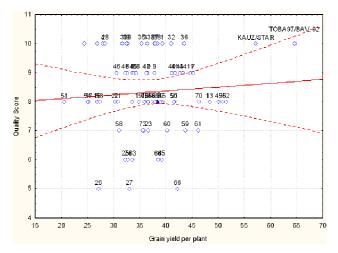
Table III: Analysis of variance (ANOVA) for grain yield

SoV	df	SS	MS	F	р
Replications	2	31.7	15.9	3.95	0.021280
Genotypes	72	12822.7	178.1	44.37**	0.000000
Error	144	578.0	4.0		
Total	218	13432.4			

Table IV: Mean, minimum and maximum values for grain yield

	Mean	Min	Max	S.E
Pakistani Varieties	35.35	24.5	48.4	<u>+</u> 0.73
CIMMYT lines	37.7	20.5	64.6	<u>+0.72</u>

Fig. 4: Scatter plot between grain yield per plant and HMW-GS quality score showing the distribution of genotypes



(2010). Bibi et al. (2011) determined 7 alleles at Glu-D1 locus in synthetic hexaploid wheat lines. The variation of HMW-GS at *Glu-D1* in synthetic hexaploid is three times higher than the variation found in Pakistani genotypes and about two times higher than that of CIMMYT lines. This comparison highlights the importance of synthetic hexaploid wheats for their utilization in wheat quality improvement and germplasm richness. However, Chaparzadeh et al. (2008) and Fang et al. (2009) reported only two alleles (null & 1) at Glu-A1 locus. The most frequent allele in both Pakistani cultivars and CIMMYT accessions at this locus was 2\* (46.15% & 48.94%, respectively) also reported as most frequent (63.4%) by Liang et al. (2010) in 142 CIMMYT lines. Conversely, Dotlacil et al. (2002) reported rare presence of sub-unit 2\* (8%) in 123 European landraces and cultivars.

The composition of KANCHAN variety  $(2^*, 7+8, 2+12)$  was the same as determined by Liang *et al.* (2010). This agreement of results on the same genotype is an evidence of the reproducibility of HMW-GS.

High scores of the Pakistani cultivars and CIMMYT accessions were due to the presence of subunits 5+10,  $2^*$ , 1, 17+18, 7+8 and 13+16 having the quality points of 4, 3, 3,

3, 3 and 3 respectively (Table I & II). Subunit 2\* imparts better quality to the wheat flour (Tahir et al., 1996). Both 7+8 and 17+18 subunit combinations have significant effects on dough extensibility (Branlard & Dardovet, 1985). The 5+10 subunit combination is correlated with high bread making quality potential, while subunit 2+12 is correlated with poor bread making quality potential (Schuster et al., 1997). All the cultivars, having quality score of 10 had subunits 5+10 which had positive correlation for baking making quality (Tahir et al., 1996). The extent of variation for grain yield per plant was higher in CIMMYT germplasm as compared to Pakistani germpalsm (Table IV). It further necessitates the use of CIMMYT germpalsm for varietal development in Pakistan. The scatter plot projected two CIMMYT lines TOBA-97/BAV-92 and KAUZ/STAR with best combination of grain yield and HMW-GS score (Fig. 4). It is therefore, recommended that exploring the exotic germplasm would render Pakistani breeders with desirable variation suitable for evolving better varieties.

#### CONCLUSION

This study revealed that CIMMYT wheat accessions have potential value in wheat breeding programs. Two CIMMYT lines TOBA-97/BAV-92 and KAUZ/STAR best in grain yield and HMW-GS score have the potential to breed wheat varieties with higher grain yield and protein quality. Further diversity studies of CIMMYT wheat lines in comparison with local cultivars are useful.

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