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CHARACTERIZATION OF WHEAT GERMPLASM WITH SEED QUALITY PARAMETERS AND GLUTENIN SUBUNITS

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Abstract

Wheat as the most important staple cereal crop of Pakistan cultivated for daily consumption and industrial uses is mostly associated with the composition of gluten proteins, which is the quality of its end-use. This paper reports the results of our research endeavor where 40 wheat genotypes collected from the core germplasm collection of CCRI (The Cereal Crops Research Institute, Pirsabak), were evaluated for seed quality parameters, variation in one high molecular weight (HMW) and two low molecular weight (LMW) glutenin subunits (GS) and association between the seed quality and HMW-GluB1 locus. Our results revealed that the average Total Grain Protein (TGP) content of all the genotypes was 16.5% (ranging from 11.3 to 20.8%), the Glutenin and Gliadins constituents were 23.6% (ranging from 13.30-31.63%) and 17.5% (ranging from 14.95-19.16%) of the TGP. The variable concentration of the gluten protein resulted in Gli/Glu ratio in a range of 0.5 to 1.39. Furthermore, the *By9* allele of HMW-GluB1 locus occurred at a frequency of 0.5 and the LMW-GS alleles *GluA3e* and *GluB3i* at 0.3 and 0.48. The absence of *By9* allele of HMW-GluB1 locus resulted in a significant decrease in the TGP, Glutenin and gliadins content and an increase in the Gli/Glu ratio. TGP was found to be statistically and most strongly associated with the By9 allele of HMW-GluB1 locus. These findings provide a baseline for molecular markers assisted breeding and gene pyramiding for improving wheat varieties.

Key words: *Triticum aestivum*, Seed quality, Glutenin, Allelic variation.

Introduction

Bread Wheat, *Triticum aestivum* L. (Cope, 1982), is the most important among major cereals of Pakistan. It is extensively cultivated among all the farming systems of all the agro climatic regimes, for diverse local and industrial uses generally determined by glutens, the wheat grain proteins, which are responsible for conferring the visco elastic properties; enabling the processing of wheat flour into suitable desired products (Ali *et al*., 2016). Glutenins are the polymeric fraction of the gluten proteins, which, along with the monomeric Gliadins are mainly responsible for the visco elastic properties of wheat dough. The Glutenins are further classified into the high molecular weight, Glutenin sub-units (HMW-GS) and the low molecular weight, Glutenin sub-units (LMW-GS). The HMW and LMW Glutenin sub-units are linked by di-sulfide bonds. The HMW-GS generally determines the dough elasticity of wheat, whereas the LMW-GS contributes both the dough extensibility and gluten strength (Dhaka & Khatkar, 2015; Maruyama-Funatsuki *et al*., 2005; Cornish *et al*., 2001). Furthermore the quality of bread, noodles, and other foods is mainly determined by Glutenins.

The specific LMW-GS alleles encoding different Glutenin subunits are generally characterized for improvement of the baking quality of wheat. Five gene families identified on Glu-A3, Glu-B3 and Glu-D3 are located on short arm of chromosomes of different wheat genome i.e., 1A, 1B, and 1D; encodes for the LMW-GS genes. Furthermore, these three loci are also tightly linked to the Gli-A1, Gli-B1 and Gli-D1 alleles, respectively, which include multigene families encoding the Gliadin subunits (D'Ovidio & Masci, 2004; Singh & Shepherd, 1988). The LMW-GSs are further divided into three subclasses: LMW-m, LMW-s, and LMW-i, named for the first amino acid residue of their mature proteins viz. methionine, serine and isoleucine, respectively (D'Ovidio & Masci, 2004), whereas some of the authorities divided it into six classes, based upon the location of cysteine residues (Ikeda *et al*., 2002), still the exact copy numbers of the LMW-GS genes are unknown which hinders the efficient ways for distinguishing the members of this complex quantitatively operating, heterogenic, co-migrating multi-gene family (Appelbee *et al*., 2009), which result into a complex SDS-PAGE pattern that complicates the identification of individual allele and its correlations with bread-making quality. To overcome this complexity, DNA markers were used to allow quantification of the effects of individual Glutenin loci on gluten strength and other traits associated with bread making quality, and also to validate the molecular markers required to discriminate the desired alleles for marker assisted selection.

Materials and Methods

The bread wheat germplasm resources in the core collection of the gene pool obtained from the CCRI-Pirsabak, Nowshera (Table 1), were taken as genetic diversity resources for determining the variation of total, Glutenin and Gliadins proteins content in their endosperms through a set of STS markers specific to one HMW-GS (GluB1bcf) and two LMW-GS (GluA3e and GluB3i). The basic research skills and protocols were optimized at Department of Genetics, Hazara University Mansehra, whereas the research was conducted at the Institute of Biotechnology and Genetic Engineering, the University of Agriculture Peshawar during the years 2012-2015. Forty wheat cultivars obtained from the

Cereal Crop Research Institute Pirsabak, Nowshera (Table 1), were grown in accordance with the approved agronomic practices. Similarly, the grains were collected from plants grown under similar conditions in a field-trial at the same location during the same growing season to minimize the influence of environmental factors and experimental errors hereby.

Table 1. Core wheat germplasm collection from CCRI pirsabak used for wheat flour quality and molecular analysis of glutenin sub-units.

S. #	Wheat cultivars	S. #	Wheat cultivars
1.	Pirsabak-04	21.	Lasani
2.	Pak-81	22.	Pirsabak -08
3.	Hasham	23.	Kohat- 2010
4.	Ass- 81	24.	Siran-2010
5.	Bathoor-08	25.	Faisalabad- 08
6.	Upaq	26.	NARC-11
7.	Barsat	27.	Janbaz
8.	Amin-10	28.	Pirsabak -13
9.	Durabi-11	29.	Ghaznavi -98
10.	Suleman-96	30.	Pirsabak -85
11.	Shahkar- 13	31.	Lalma- (NIFA)
12.	Chakwal-50	32.	Inqalib- 91
13.	Atta Habib	33.	Bakhtawar-92
14.	Nowshera	34.	Zam-04
15.	Blue Silver	35.	Saleem -2000
16.	Kyber-87	36.	Kohat -2000
17.	Fakhre-Sarhad	37.	Punjab-11
18.	ARRI	38.	Tatara- 96
19.	Uqab- 2000	39.	Farid -06
20.	Seher	40.	Khatakwal

Seed quality analysis: The quality analyses included total grain protein (TGP), Gliadins, Glutenin content and the ratio of Gliadins to Glutenins in TGP. A standered weight of 10 mg of seed from each cultivar was taken and crushed into fine powder using mortar and pestle. The fine powder was then transferred to new 1.5 ml eppendorf tube and added 1 ml extraction buffer (100 mM Tris HCl, pH 6.6 and 1% SDS). The mixture was homogenized using vortex for 10 minutes and the supernatant was collected as protein fraction after centrifugation at 10,000 rpm for 10 minutes. The total grain protein content of the 40 local wheat cultivars was determined using coomassie brilliant blue (CBB) dye binding method through spectrophotometer (Bradford, 1976). An equal amount of CBB was added to 2 ml of the supernatant and the absorbance was measured at 595 nm by UV spectrophotometer. Total grain protein content was quantified using known concentration of Bovine Serum Albumin (BSA). Approximately 3-5 seeds of each wheat cultivar were crushed finely and added 600 µl distilled water and kept at room temperature (~24-25^{o}C) for 60 min. The homogenate was centrifuged at 12000 rpm for 10 min the ~ 300 µl of the supernatant was collected. The procedure was repeated again and the supernatant was discarded. The pellet was dissolved in 300 µl 0.5N NaCl, briefly vortexed and incubated at 37^{o}C for 30 min. The homogenate was centrifuged at 12000 rpm for 10 min and the supernatant was discarded. The pellet was washed with distilled water and the pellet, after centrifugation at 12000 rpm for 10 min was dissolved in 50% 1-propanol (v/v). The homogenate was incubated for 30 min at 45^{o}C and centrifuged at 12000 rpm for 10 min. The supernatant was collected as gliadins and quantified using the CBB dye binding method as mentioned previously. The pellet was dissolved in 50 % 1-propanol, 25mM DTT and 25mM Tris-HCl pH 8.00 and stored at 4^{o}C for 48 hrs for the extraction of glutenin. After centrifugation at 13000 rpm for 10 min, the supernatant was transferred to new tubes and pellet was re-extracted with the same procedure. The supernatant was pooled and the quantity of glutenin was measured by recording the absorbance at 595 nm using spectrophotometer.

DNA extraction and PCR amplification: Extraction of the DNA was done from fresh leaves, using the method of Doyle & Doyle (1990). Quantity and quality of the extracted DNA were checked with Nanodrop (Nanodrop^{TM} 1000, Fischer, USA). Amplification of the DNA was done using specific primers for *GluB1bcf, GluA3e* and *GluB3i* through gene specific primers reported by Anderson *et al.,* (1989), D'Ovidio & Anderson (1994), Smith *et al.,* (1994) and Ma *et al.,* (2003). The reaction mixture and thermal conditions used for 20 ul PCR reactions comprised of 100mM KCl, 20mM Tris-HCl (pH 7.8), 200µM of each dNTP, 3mM $MgCl_2$, 250nM Primer, 2.5 units of *Taq* polymerase and 50 ng of DNA. The amplification conditions were an initial denaturation at 94°C for 6 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 58-64°C for 1 minute and elongation at 72°C for 1 minute. The PCR cycles were followed by a final extension step at 72°C for 5 min. The 5*µl* PCR product was separated on 2% agarose gel and visualized with ethidium bromide stain.

Statistical analysis

The data obtained was subjected to two different statistical analysis tools. The seed quality parameters were analyzed for one-way ANOVA (Analysis of Variance), followed by LSD (the least significant difference multiple comparison test), using Gen-Stat Ver 3.0. The presence of different Glutenin sub-units and their allele frequencies were manually calculated from photograph of the agarose gel of the PCR product. The correlation analysis between GPC and Glutenin and Gliadins as well as seed quality parameters and the HMW-GS *GluB1bcf* alleles was carried out using Microsoft Excel 2010. Odd ratios were calculated by counting the number of genotypes with mean seed quality character above or less than the population mean with the presence of different alleles at *GluB1bcf* locus.

Results and Discussion

Wheat research has recently been diversifying (Zimin *et al.,* 2017; Ali *et al*., 2016; Ali *et al.,* 2014) and is more target oriented regarding its utilization beside its use as flour for traditional bread, noodles and a number of other bakery products. Wheat is also used for

industrial products like starch and bio-ethanol production as well as animal feeds. This diverse use of wheat seed/flour necessitates the re-characterization of the core germplasm collection of CCRI Pirsabak. The genotypes of the core collection have never been grouped into different categories according to the use or bread making quality. Further, yield and stress resistance are the major objective and seed quality parameters and molecular markers are seldom used in the wheat breeding programs at CCRI Pirsabak.

Seed quality analysis: The total grain protein content (TGP) of all the forty local wheat cultivars was 16.5 percent of seed dry weight (Table 3). Elevated TGP content and its quality influences the flour extraction, loaf volume and other bread making properties of wheat but not the grain yield (Mesfin *et al*., 2000; Oury *et al*., 2003 . Maximum TGP on dry weight basis was noted in Zam-04 cultivar (20.8%), followed by Barsat (20.54%) whereas least TGP was noted in Farid-2006 (11.3%). Wheat TGP is reported to range between 8-20% (Goesaert *et al*., 2005), which is similar to the TGP calculated for different genotypes in the current experiment. Previously, significant variation has been reported in the TGP content in Pakistani wheat genotypes (Minhas *et al.,* 2014).

Further, the gluten proportion of the TGP was also determined to understand dough quality differences among the germplasm. The glutenin constituted an average of 23.68% of the TGP. Maximum glutenin were noted in Atta-Habib (31.63%), followed by Bathoor-2008 (31.85%) and Suleman-96 contained the least proportion of glutenin (13.30%) in the TGP (Table 3). Similarly, gliadins constituted an average of 17.50% of the TGP. Maximum gliadins as seed protein were noted in Hasham (19.16%), followed by Saleem-2000 (18.88%) and gliadins were in least quantity (14.95%) in the TGP in Zam-2000 (Table 3). The low gliadins to glutenin ratio are essential for better bread making quality. In the wheat germplasm collection from CCRI Pirsabak, least gliadins/glutenin ratio was noted for ARRI and Durabi-11 (0.54), followed by Atta-Habib and Kohat-2010 (0.56) whereas maximum gliadins/glutenin ratio (1.39) was noted in Hasham (Table 3).

Furthermore, in order to determine the influence of TGP on the gluten proteins fraction a regression analysis was conducted (Fig. 1). It is evident from the regression analysis that there is an increase in the gluten protein fractions with an increase in TGP. Each mg increase in the TGP resulted in approximately 1.23 % increase in glutenin and 6.96 % increase in the gliadins content. Similarly, the correlation between the TGP and both the glutenin and gliadins was positive. However, this correlation was much stronger (r = 0.882) and statistically significant (t = 11.54; p>0.001*) between the TGP and gliadins compared with the correlation between TGP and glutenin (r = 0.238, t = 1.82, p = 0.076). Significant genetic contribution in variation in the quantities of TGP, Glutenin and gliadins was previously noted (Plessis *et al.,* 2013). A positive correlation between the TGP and gluten content has previously been reported and an increase in TGP results in increase the gluten content. Further, the increase in the amount of glutenin and gliadins is desirable for better bread making quality and is in agreement with previous report (MacRitchie, 1984). Similarly, the proportion of gliadins and glutenin and the gliadins to glutenin ratio were not modified by the environment but genetically determined (Plessis *et al.,* 2013).

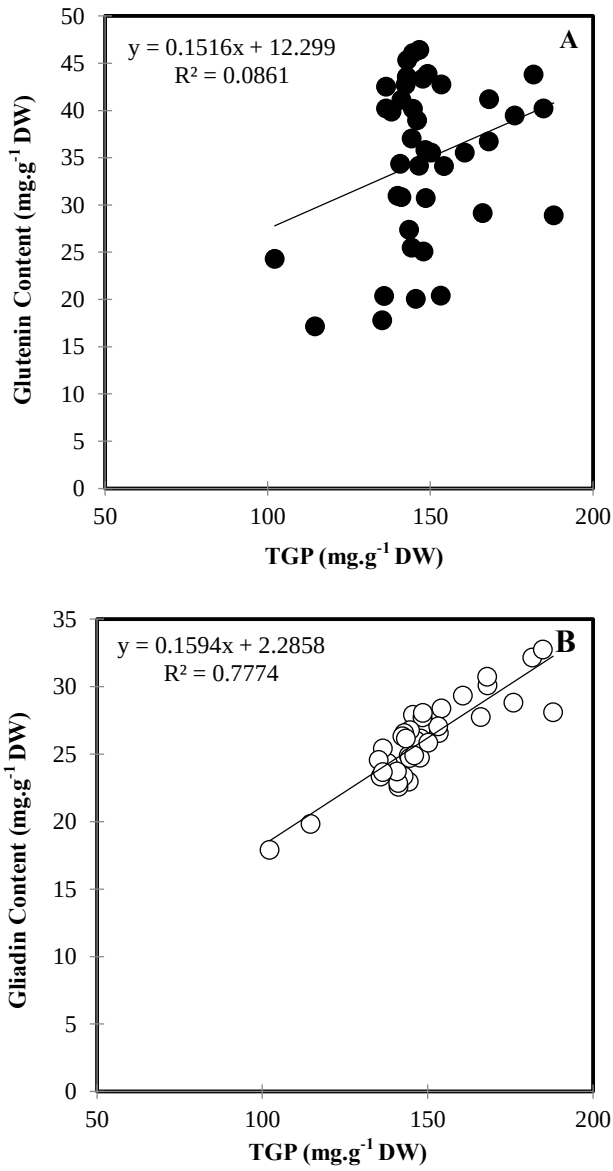

Fig. 1. Correlation between TGP and glutenin or gliadins content in TGP of wheat flour in the wheat germplasm collection from CCRI Pirsabak.

Allele frequencies of HMW-GS *GluB1bcf* and LMW-GS *GluA3e* and *GluB3i*: The allelic composition of different HMW-GS and LMW-GS can be determined through functional markers developed from gene sequences (Liu *et al*., 2012). The HMW-GS *GluB1bcf* and LMW-GS *GluA3e* and *GluB3i* were amplified through allele specific PCR resulting in band size of 709/662, 158 and 621 bp respectively (Fig. 2A and D). Among the HMW-GS, the *GluB1* locus showed higher level of polymorphism by contributing 7 different types of allelic variants (Yan *et al*., 2007). Amplification of the 662 bp band with *GluB1bcf* primers was specific for the *By9* allele at *GluB1* locus

whereas the 709 bp product was specific for other alleles at this locus (Table 2). The genetic analysis with specific *GluB1bcf* primers revealed that HMW-GS *By9* was present in a high frequency at *GluB1* locus in the core collection from CCRI Pirsabak. Both the alleles were amplified in 20 genotypes each (*f* = 0.50; Fig. 2A and D). Jin *et al*., (2016) reported an allele frequency ranging between 8.2-100% for the *By9* allele at the *GluB1* locus in genotypes from different countries. The high frequency of *By9* allele of the *GluB1* locus in the current study is in agreement with that reported by Kang *et al*., (2007), Gregova *et al*., (2006). However, Singh *et al*., (2007), Tahir *et al*., (1996) and Tabassum *et al*., (2011) have reported higher frequency for *By18* compared with *By9* allele at *GluB1* locus. Furthermore, the *By9* allele at the *GluB1* locus of the HMW-GS is associated with higher grain yield and protein content (Aktas & Baloch, 2017). Higher grain yield is an important consideration in the wheat breeding program at CCRI Pirsabak and could thus be one reason for the higher frequency of *By9* allele in the core germplasm collection.

The LMW-GS are 4–5 times more abundant than HMW-GS and are encoded by a large multigene family (D'Ovidio & Masci, 2004). The *Glu A3* and *Glu B3* loci are important because of the significant effect on the bread making quality (Khan *et al*., 2016). Among these LMW-GS, *Glu A3e* allele is considered inferior and *GluB3i* allele as superior for bread making quality (Zhang *et al*., 2012). The LMW-GS *GluA3e* was amplified in 13 genotypes (*f* = 0.33; Fig. 2B and D) and *GluB3i* was amplified in 19 genotypes (*f* = 0.48; Fig. 2B and D) of the wheat germplasm core collection from CCRI Pirsabak. The allele frequency of both the *GluA3e* and *GluB3i* ranged between 0.00-0.33 in wheat cultivars from different countries (Jin *et al*., 2011). Furthermore, the frequencies of both these LMW-GS were higher in spring then in winter wheat genotypes.

Correlation of seed quality parameters with HMW-GS *GluB1bcf*: The wheat genotypes with or without the Glutenin sub-unit By9 at the *GluB1bcf* locus in the CCRI core germplasm collection were noted to differ significantly in the TGP, Glutenin and gliadin content as well as the Gli/Glu ratio and the presence of alleles other than By9 at *GluB1bcf* locus resulted in a decrease in the TGP, Glutenin and gliadin content but an increase in the gli/glu ratio (Fig. 3). In the presence of Glutenin sub-unit By9 at the *GluB1bcf* locus, the mean TGP content in the wheat genotypes was 17.30% whereas it was 15.71% in genotypes containing other alleles at this locus (Fig. 3A). Thus the presence of By9 allele at this locus resulted in 9.19% increase in the TGP content. The glutenin and gliadin content of the seed was 36.02 and 26.96 $mg.g^{-1}$ DW in the presence of By9 compared with 33.65 and 24.99 $mg.g^{-1}$ DW in the presence of the other alleles at *GluB1bcf* locus (Table 4). The absence of the Glutenin By9 sub-unit at the *GluB1bcf* locus has thus resulted in 6.62 and 7.28% decrease in the glutenin and gliadin content, respectively in the core germplasm collection from CCRI Pirsabak (Fig. 3B and 3C). The differential decrease in the glutenin and gliadin content resulted in an increase in the gli/glu ratio in the genotypes containing the different alleles at *GluB1bcf* locus. The mean gli/glu ratio was 0.78 in genotypes containing glutenin By9 sub-unit the *GluB1bcf* locus whereas it was 0.80 in genotypes with the other alleles at the *GluB1bcf* locus, thus there was a minor 2.36% decrease in the gli/glu ratio when the By9 allele at the *GluB1bcf* locus was present (Fig. 3D). Furthermore, the relationship between the *GluB1bcf* locus and seed quality parameters was further probed by calculating the odd ratios and $\chi 2$ values. The data indicated that the TGP content of seed was found to be more strongly associated with the By9 allele of the *GluB1* locus (OR=4.33, 95%CI=1.15-16.32) than the gliadins and Glutenin content or gli/glu ratio.

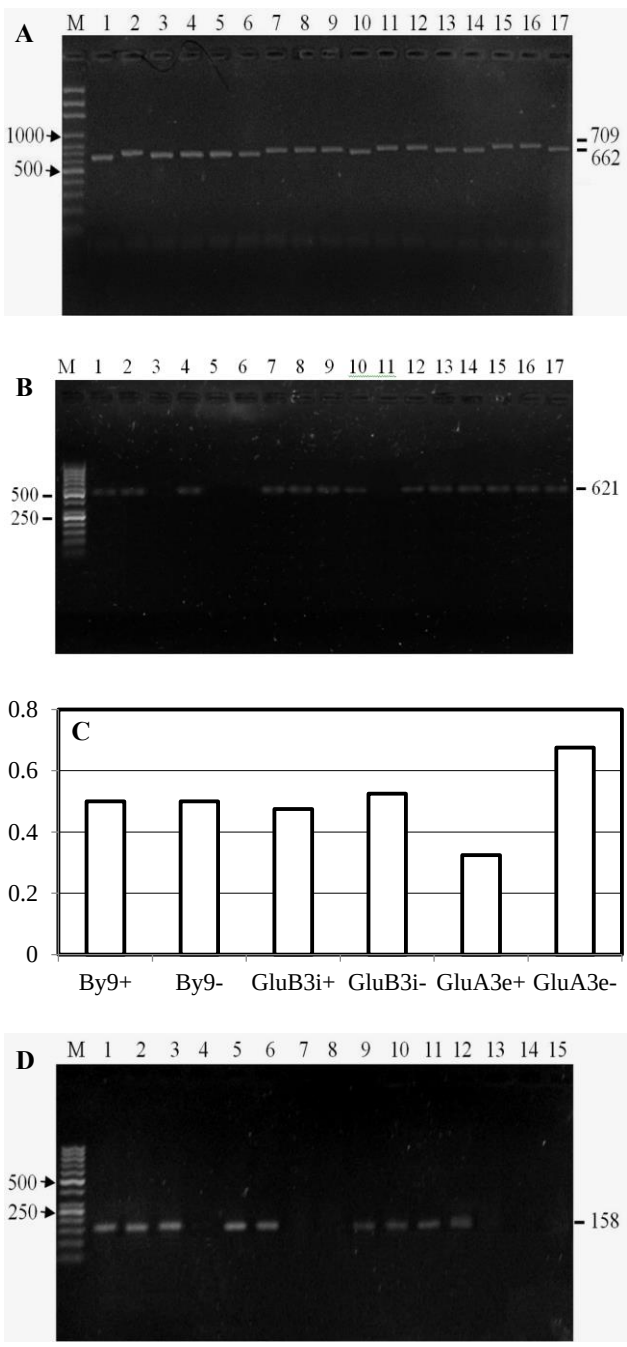

Fig. 2. PCR amplification pattern of HMW By9 *GluB1* (A), *GluB3i* (B) and GluA3e (C) loci and the Allele Frequencies (D) of the different glutenin subunits. M = Molecular weight marker (Number are similar to the serial numbers of different genotypes given in Table 1).

Table 2. Primer sequences, expected product size and annealing temperature for amplification of different HMW and LMW-GS.

Marker	Sequence	Product size	Annealing temp.
GluB1bcf	5'-GCATCAACAACAAATAGTACTAGAA-3' 5'-GGCGGGTCACACATGACA-3'	707, 662	60
GluA3e	5'-CAATGAAAACCTTCCTCGTCTG-3' 5'-GATGCCAACGCCTAATGGCACAC-3'	158	64
GluB3i	5'-TATAGCTAGTGCAACCTACCAT-3' 5'-TGGTTGTTGCGGTATAATTT-3'	621	58

Table 3. TGP (Percent), glutenin, gliadins in TGP and the ratio of gliadins to glutenin (Gli/Glu) in the core wheat germplasm collection at CCRI Pirsabak.

Genotypes	TGP	Glutenin	Gliadins	Gli/Glu
Pirsabak-04	16.03 HI	17.65 CD	15.91 B	0.90 CD
Hasham	16.18 H	13.77 D	19.16 A	1.39 F
Upaq	20.19 B	24.10 B	17.69 AB	0.73 B
Barsat	20.54 AB	21.74 C	17.71 AB	0.81 BC
Amin-10	15.69 J	21.82 C	15.99 BC	0.73 BC
Chakwal-50	16.58 GH	29.40 AB	17.83 AB	0.61 AB
Atta Habib	16.30 H	31.63 A	17.82 AB	0.56 A
Blue Silver	15.34 JK	28.88 AB	17.62 AB	0.61 AB
Kyber-87	16.42 H	29.35 AB	16.74 B	0.57 A
Fakhre-Sarhad	17.05 F	27.85 AB	17.30 B	0.62 A
Uqab-200	19.56 C	22.42 B	16.36 BC	0.73 BC
Seher	16.04 HI	25.64 B	17.24 B	0.67 B
Lasani	17.14 F	22.10 C	18.39 AB	0.83 C
Serin-10	15.56 J	22.11 C	16.85 BC	0.76 BC
Pirsabak-13	18.46 DE	17.53 CD	16.70 BC	0.95 CD
Lalma- (NIFA)	17.85 E	22.13 C	18.26 AB	0.83 C
Inqalib-91	16.44 H	16.94 D	17.67 AB	1.04 DE
Zam-04	20.88 A	15.38 D	14.95 C	0.97 D
Punjab-11	18.68 D	24.50 BC	17.89 AB	0.73 BC
Tatara-96	15.16 K	31.15 AB	18.63 AB	0.60 AB
Pak-81	15.03 K	19.80 CD	18.16 AB	0.92 CD
Aas-81	15.09 K	15.00 D	17.17 B	1.14 E
Bathoor-08	16.07 HI	31.85 A	17.07 B	0.54 A
Durabi-11	16.29 H	23.29 B	17.72 AB	0.76 BC
Suleman-96	17.03 F	13.30 D	17.65 AB	1.33 F
Shahkar-13	16.22 H	26.70 B	17.06 B	0.64 AB
Nowshera	18.67 D	21.85 C	18.28 AB	0.84 C
ARRI	15.87 IJ	30.50 AB	16.35 BC	0.54 A
Pirsabak -08	16.50 GH	24.10 BC	18.65 AB	0.77 BC
Kohat- 2010	15.68 J	29.13 AB	16.19 BC	0.56 A
Faisalabad-08	15.64 J	24.39 BC	16.84 B	0.69 B
NARC-11	16.70 G	23.65 BC	17.20 B	0.73 BC
Janbaz	15.16 K	29.45 AB	17.33 B	0.59 A
Ghaznavi-98	16.08 HI	27.75 AB	18.49 AB	0.67 B
Pirsabak-85	15.88 IJ	31.70 A	18.57 AB	0.59 A
Bakhtawar-92	15.82 IJ	30.00 AB	18.48 AB	0.62 AB
Saleem -2000	16.51 GH	20.67 CD	18.88 AB	0.91 C
Kohat-2000	15.94 I	19.06 CD	18.22 AB	0.96 C
Farid-06	11.36 M	23.78 BC	17.51 AB	0.74 BC
Khattakwal	12.73 L	14.95 D	17.29 B	1.16 E
Mean	16.51	23.68	17.50	0.78
LSD value	0.212	2.432	0.910	0.068
p-value	0.0001 **	0.0001 **	0.0001 **	0.0001 **

Table 4. Association between HMW-GS *GluB1bcf* locus and seed quality characters.

Seed Quality character	Locus	No of Genotypes		OR	95 % CI	χ^2 value
		Above mean	Below mean			
TGP	*By9*+	14	6	4.33*	1.15 ~ 16.32	4.91*
	By9-	7	13			
Gli	*By9*+	13	7	2.27	0.64 ~ 8.11	1.62 ^{ns}
	By9-	9	11			
Glu	*By9*+	12	8	1.23	0.35 ~ 4.31	0.10 ^{ns}
	By9-	11	9			
Gli/Glu	*By9*+	8	12	0.81	0.22 ~ 2.91	0.11 ^{ns}
	By9-	7	13			

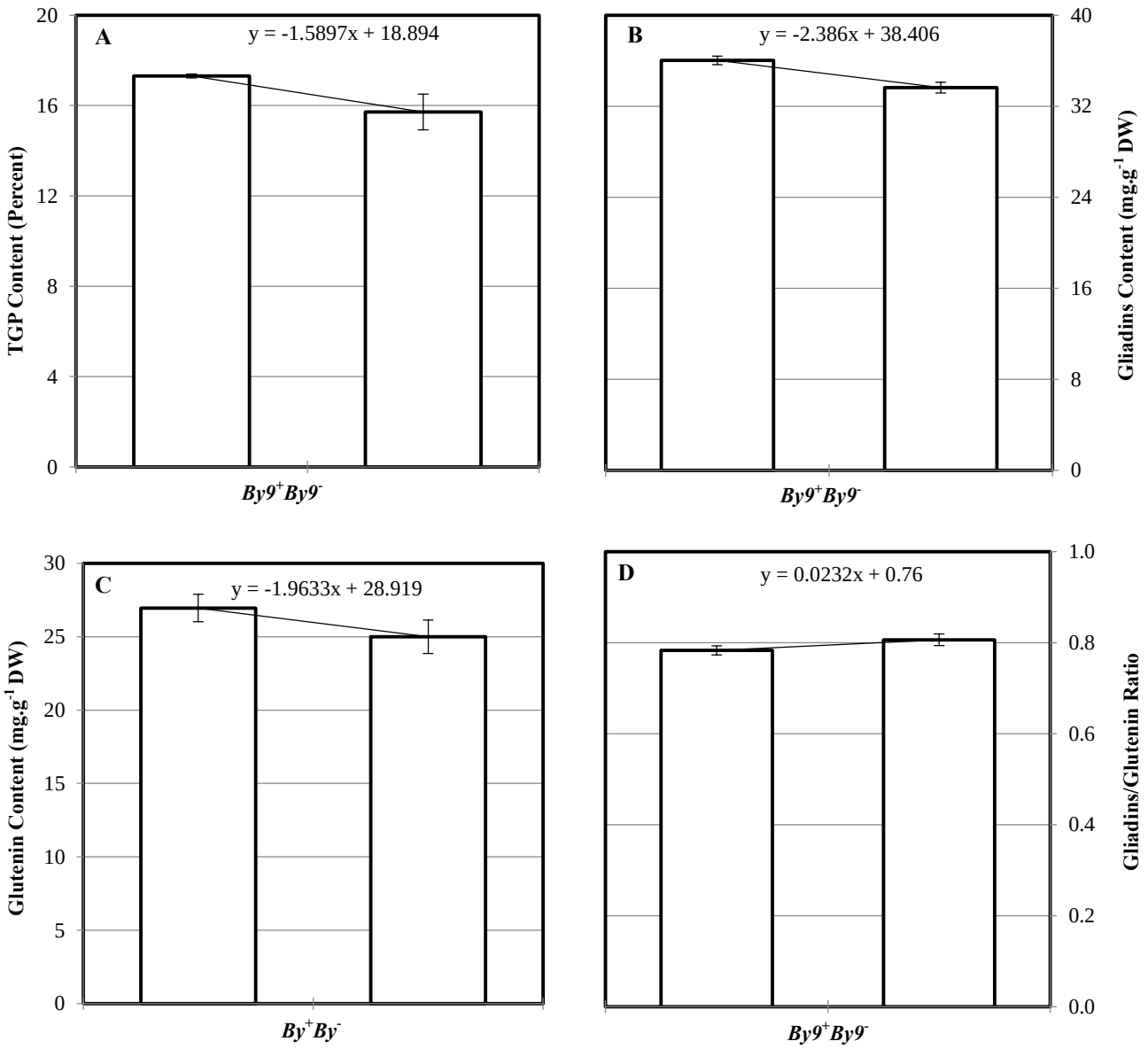

Fig. 3. TGP Content (A), Glutenin (B), Gliadins content (C) and Gliadins to Glutenin ratio (D) in the wheat lines in the presence or absence of sub-unit *By9* at the HMW *GluB1* locus.

The genetic make-up of the wheat cultivars is a major determinant of the composition of wheat seed storage proteins and its bread making quality mainly through variation in the types of HMW-GS. Significant variation in the TGP, Glutenin and gliadins content as well as gli/glu ratio were noted in the core wheat germplasm collection from CCRI Pirsabak that could be manipulated in the future for a desired level of the protein components. Utilizing the more reliable allele specific molecular markers, significant variation was noted in the presence of HMW-GS *GluB1* and LMW-GS *GluA3* and *GluB3* alleles in the core germplasm collection. Similarly, association between the presence of different Glutenin sub-units and seed quality

parameters have not been reported in the past. A strong association was also noted in the TGP and HMW glutenin GluB1 sub-unit which can be used to gene pyramiding and quality improvement of wheat using molecular markers assisted breeding. However, further studies should be carried out by analyzing the other gluten proteins and a wider selection of genotypes to verify these findings.

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