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Evaluation of allelic variation for HMW glutenin subunits through SDS-PAGE in diverse bread wheats

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Abstract

High-molecular weight glutenin subunits (HMW-GS) play a key role in determining end-use quality of common wheat by estimating the viscoelastic properties of dough and flour. We analyzed the HMW-GS subunit composition and variation at the Glu-1 locus in a core collection of 7 diverse groups of wheat genotypes. Fourteen different wheat standards were utilized for allocating allelic designations to HMW glutenin subunit mobility by SDS-PAGE. Scoring of high HMW-GS subunits in the evaluated genotypes was based on standard electrophoresis patterns using the Payne and Lawrence scoring system. A total of eighteen alleles among the germplasm at Glu-1 loci were detected, 3 at the Glu-A1locus, 11 at the Glu-B1and 4 at the Glu-D1 locus. Thirty four different combinations of HMW-GS alleles were found and higher variations occurred at the Glu-B1 locus compared to Glu-A1 and Glu-D1 loci with relatively high diversity (H=0.83). The distribution of allelic patterns varied among these seven groups and high genetic polymorphism in HMW-GS composition was observed. Together, 45.65% of the alleles detected were rare alleles. Glu-A1a (1Ax1), Glu-A1c (null), Glu-B1a (1Bx7), Glu-B1h (1Bx17 + 1By18), and Glu-D1a (1Dx2 + 1Dy12) alleles were found most frequently at Glu-D1 locus, the frequency of the superior alleles 1Dx5+1Dy10 (50.00%) was observed in maximum genotypes than the inferior allele 1Dx2+1Dy12 (40.83%). The high quality score for HMW glutenin subunits found in the studied genotypes are potential sources of desirable quality traits to be used in main wheat breeding programs for improving bread-baking quality.

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Introduction

Wheat *(Triticum aestivum* L.*)* endosperm contains a major class of storage proteins, where glutenin is considered to be play a major role in bread making quality. Glutenin proteins are the foremost cause for the unique viscoelastic properties of wheat flour and dough. Glutenin possess the rheological characteristics that are vital for a wide range of food products (Shewry *et al.*, 1995; He *et al.,* 2005). End use quality of wheat is influenced by the composition of storage proteins (Dessalegn *et al.,* 2011), its quality and quantity confers elasticity and extensibility necessary for bread making. It contributes 80-85% of the total flour protein (Shewry *et al.,* 1995). This endosperm protein consist of manly two prolamine groups, namely monomericgliadins and polymericglutenin (An *et al.*, 2006). The polymeric glutenin proteins are separated into two group of subunits, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS), according to their mobility's in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne *et al.,* 1979). Glutenins (LMW and HMW) are long polypeptide chains which are linked together by disulfide bonds to form gluten macropolymers (Gras *et al.*, 2000) that are thought to be mostly accountable for the viscoelasticity and extensibility of dough. Glutenin complex subunits contribute about 30-40% to flour protein. Among these the HMW glutenin subunits have the largest effect on bread making quality even though, they comprise only 10% of the total storage proteins as compared to LMW glutenin which contribute about 40% of the total flour protein.

The HMW-GS are encoded by co-dominant genes at Glu-1 loci on the long arms of the homoeologous group 1 chromosomes, designated as Glu-A1, Glu-B1 and Glu-D1, respectively (Payne *et al.,* 1984). Each locus harbors two tightly linked genes (Glu-1-1 and Glu-1-2) encoding two subunits of different size, known as x- type and y-type subunits (e.g., 1Ax and 1Ay), with relatively higher (80-88 KDa) and lower (67-73 KDa) molecular weights (Mackie *et al.,* 1996), respectively.

The primary structures of both x-type and y-type subunit are composed of three different domains i.e. longer repetitive domain, highly conserved N-terminal and shorter C-terminal domains. In x-type subunits a unique tri-peptide repeat (GQQ) is present whereas, in y-type subunits the second proline is replaced by a leucine in the GYYPTSPQQ repeat motif (GYYPTSLQQ). Generally most of the x-type subunits have four conserved cysteine residues, whereas y-type subunits commonly possess seven cysteine residues. Difference in the number of these cysteine residues are correlated with the variation in bread-making quality. Bread wheat generally exhibits three common subunits such as 1Bx, 1Dx, and 1Dy whereas some genotypes express two other subunits as well, 1Ax and 1By. Gene encoding the 1Ay subunit ordinarily remains silent in both bread and durum wheat's due to the wheat domestication syndrome. HMW-GS are highly polymorphic in nature and different allelic variants occur at each locus in bread wheat's. Generally there are three common allelic variants (Ax1, Ax2* and Null allele) at Glu-A1 locus in common wheat, although more than 21 alleles have been reported in different diploid and hexaploid wheat. At Glu-B1 locus more than 69 alleles and at Glu-D1 locus 29 allele have been reported in bread wheat (McIntosh *et al.*, 2013).

Allelic variation at each locus was found to be strongly associated with the various high molecular weight glutenin subunits (HMW-GS). The identification of specific HMW-GS alleles is therefore an important target for improving wheat quality (Gale, 2005). Alleles encoding glutenin subunits possess unique functional properties added to various food items and play a key role in dough elasticity and extensibility. The occurrence of particular HMW-GS subunits at Glu-1 loci is positively correlated with bread-making quality. Therefore the identification of specific HMW-GS alleles is an important target for improving wheat quality (Gupta *et al.*, 1999; Gale, 2005). These glutenin proteins are also used as a biochemical marker for identification, differentiation and validation of different wheat genotypes and as a valuable technique to check seed purity.

Today, the most challenging task for wheat breeders is not only yield maximization (Duveiller *et al.,* 2007) but also to boost the grain quality for end use product to meet the requirements of an ever increasing population. Keeping in mind the aim of this work was to characterize storage protein profiles in diverse genotypes of some common wheat's (*Triticum aestivum* L) by SDS-PAGE. A standard reference cultivar is mandatory for the identification and comparison of generated bands during electrophoretic mobility to ensure the right allelic designation for HMW-GSs. In the present study we have utilized and optimized 14 different wheat quality standards for allocation of HMW glutenin subunits at the Glu-1 loci.

Materials and methods

Plant materials

A total of 120 diverse wheat germplasm were used including 38 historical sets, 30 land races, 20 Richard selection, 10 semi-arid wheat screening nursery lines (SAWSN) of CIMMYT, 10 advanced lines, 7 long coleoptile and 5 synthetics hexaploid wheats. Seed of all these materials were maintained after acquisition in the Wheat Wide Crosses and Cytogenetics program in National agricultural research center, Islamabad.

Standard cultivars for identification of HMW-GS

Seeds of the 14 quality standards were obtained from CIMMYT, Mexico. These seeds were stored and planted in the green house of the Wheat Wide Crosses and Cytogenetics Program at NARC during the growing cycle 2014-2015, for increase and possible further distribution upon request at the national and international level. Seed endosperms were used for subunit assay with the corresponding embryos being germinated and respective seedlings used to produce pure seed for future studies.

Extraction of glutenin and SDS-PAGE

Wheats single kernels were milled into white flour by mortar and pestle, 10 mg weighted flour were put into 1.5 ml Eppendorf tubes after removing the seed coat by a spatula. To extract protein from the flour 400µl of protein extraction buffer (0.05 M Tris+0.2% SDS+5M Urea, adjusted to pH 8.0 with HCl) was added into the Eppendorf tubes.

After few minutes 10µl mercaptoethanol was added into micro tube and vortexed (Multi Vertex Biosan) for 5 minutes. Then the samples were centrifuged at 1000 rpm for 5 min and the supernatant containing proteins were loaded on the vertical slab gel system (VS20DSYS Cleaver Scientific UK). The HMW glutenin subunits were fractionated by using 7.5% polyacrylamide gels. Gel apparatus was run at 200 V until the tracking dye reached the bottom of the gel plate. The gels were removed from plates, stained with 0.2% (w/v) Coomassie Brilliant Blue (R-250) solution for 40-60 minutes over a shaker and de-stained with de-ionized water until the background of the gel became clear. HP scan jet 4370 was used to scan the gel for scoring.

Allele identification and nomenclature

Allelic variation for high molecular weight glutenin subunits (HMW-GS) at Glu-1 loci was identified and numbered according to the Payne and Lawrence (1983), scoring system. The overall quality scores of HMW- GS for individual germplasm were calculated by adding the scores of individual subunits according to Glu-1 quality method (Payne *et al*., 1987).

Statistical analysis

The genetic diversity at Glu-A1, Glu-B1 and Glu-D1 loci was calculated on the basis of Nei's index (Neis, 1973), by using formula: $H = 1 - \Sigma Pi^2$ where H and *Pi* denote the genetic variation index and the frequency of the number of alleles at the locus, respectively. Allelic frequencies were determined by adding the frequencies of alleles of each genotypes, regardless of whether the HMW-GS combination was homogeneous or heterogeneous, and then dividing this total by the number of genotypes.

Results

SDS-PAGE profiles of standard cultivars

Quality standards used for allocating allelic designations for HMW glutenin subunits are described in the table 1. Unit allelic variability and frequencies at Glu-1 loci in these standards at 7.5% gel are given in the Fig. 1, 2 and table 2, respectively.

Table 1. Standards used for allocating allele designations of HMW glutenin subunits.

S. No	Standard Cultivar	Subunit composition	Source
1	ACA 303	2* 7+8 5+10	Liu *et al.*, 2008
2	Chinese Spring	N 7+8 2+12	Das *et al.*, 2001
3	Amadina	1 7+9 5+10	Liu *et al.*, 2008
4	Blue Sky	2* 7+8 5+10.	Cornish, 2005
5	Darius	2* 7+8 2+12	-
6	Opata M-85	2* 13+16 2+12	Rabinovich *et al.*, 2000
7	Gabo	2* 17+18 2+12	Branlard *et al.*, 2003
8	Glenlea	2* 7+8* 5+10	Ng & Pogna,1989
9	Halberd	1 20 5+10	Anon., 1998
10	Pavon	2* 17+18 5+10	Liu *et al.*, 2008
11	Insignia	1 20 5+10	Branlard *et al.*, 2003
12	Marquis	1 7+9 5+10	Graybosh, 1992
13	Norin61	2* 7+8 2.2+12	Nakamura, 2000
14	Seri M-82	1 7+9 5+10	Payne & Pena, 2006

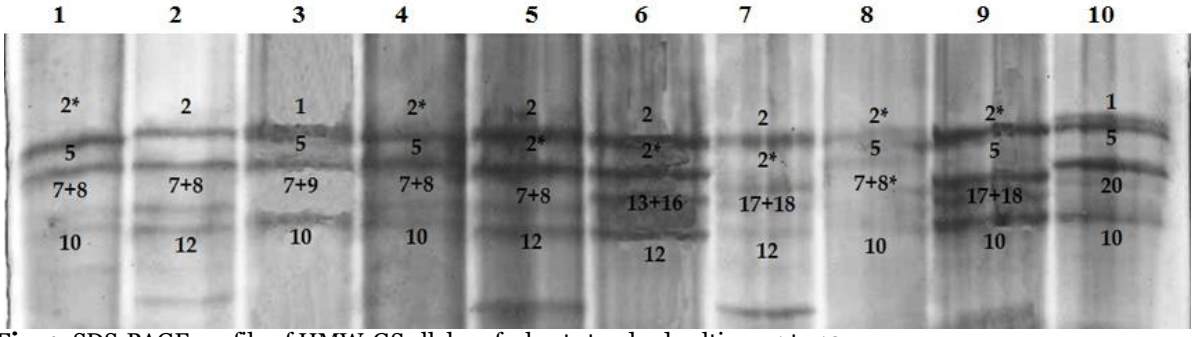

Fig. 1. SDS-PAGE profile of HMW-GS alleles of wheat standard cultivars 1 to 10.

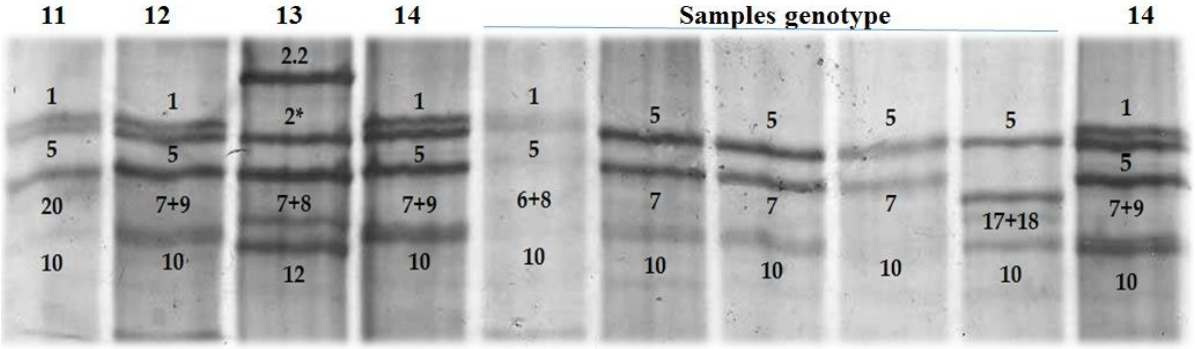

Fig. 2. SDS-PAGE profile of HMW-GS alleles of wheat standard cultivars 11-14 and diverse wheat's sample genotypes.

Table 2. Allelic frequencies (HMW-GS) and diversity at Glu-1 loci in 14 standards materials.

Locus	Allele	Subunit	No of Genotypes	Frequency %	H (Nei·s index)
Glu-A1	A	1	5	35.31	
	B	2*	7	50.00	
	C	Null	2	14.28	0.61
Glu-B1	B	7+8	4	28.57	
	C	7+9	3	21.42	
	E	20	2	14.28	
	F	13+16	1	7.14	
	I	17+18	2	14.28	
	D	7+8*	2	14.28	0.81
Glu-D1	A	2+12	3	0.04	
	D	5+10	10	0.51	
	F	2.2+12	1	0.005	0.44

Analysis of HMW-GS by SDS-PAGE

Allelic variations for HMW-GS were identified in a set of bread wheat germplasms shown in table 3. The HMW-GS encoded by Glu-1 loci showed a variable number of alleles. Eighteen different alleles have been found across the three genomes.

At Glu-A1 locus three x-type subunits 1, 2* and null, controlled by alleles Glu-A1a, Glu-A1b and Glu-A1c, respectively were found. The null allele was predominantly found in 67 (55.83%) genotypes followed by subunit 1Ax1 in 29 (24.16%) and 1Ax2* in 24 (20.00%) germplasm.

The allelic variation for this locus was 0.59 shown in table 4. The y-type subunit at this locus was always absent.

At Glu-B1 locus, 7 x type subunit viz., 7, 7*, 6, 20, 13, 14 and 17, and 6 y-type subunits viz., 8, 8*, 9, 16, 15 and 18 were detected (table 3) in different combinations.

The subunit 1Bx7 was found in 23 (19.16%) germplam and also found in association either with 1By8 or 1By9 subunits (Table 3). At Glu-B1 locus, the subunit 1Bx17+1By18 encoded by Glu-B1i was found in maximum number of genotypes, 31 (25.83%).

The remaining alleles identified at this locus were 1Bx7 (19.16%), 1Bx7+1By8 (16.66%), 1Bx7+1By9 (13.33%), 1Bx20 (13.33) 1Bx6+1By8 (0.83%) encoded by alleles Glu-B1a, Glu-B1b, Glu-B1c, Glu-B1e, and Glu-B1d, respectively.

The rare alleles at this locus, 1Bx7* 1Bx14+1By15, 1Bx6+1By8* and 20x (0.83%) were found in the same proportion across all the genotypes at Glu-B1 locus (table 3).

Table 3. Number of alleles and combinations at Glu-1 loci in diverse germplasm of wheat.

Locus	Number of alleles	x-type	y-type
Glu-A1	3	3	-
Glu-B1	11	7	6
Glu-D1	4	2	3
Glu-1 Combinations	34		

Similarly at Glu-D1 locus two x type subunit (Dx2 and Dx5) and three y type subunit were identified (Dy10, Dy12 and Dy12.2).

The most frequent subunit, 1Dx5+1Dy10 encoded by the allele Glu-D1d was identified in 60 (50.00%) genotypes followed by 1Dx2+1Dy12 encoded by allele.

Glu-D1a in 49 (40.83%) genotypes. 1Dx5+1Dy10 was considered a superior subunit and found in maximum genotypes in the selected germplasm which may be regarded as the best genotypes in the studied germplasm.

The other subunits at this locus were 2+10 encoded by allele Glu-D1e and 2+12.2 encoded by allele Glu-D1x were detected in 9 (7.5%) and 2 (1.66%) genotypes respectively.

Combination of HMW-GS Glu-1 quality score

The SDS-PAGE profiles of the HMW-GSs combination and Glu-1 quality scores are shown in Table 5. The HMW-GS allelic composition found most frequently (12.5%) was N, 7+8, 2+12 in 15 germplasm.

The other allelic combination, N 7 5+10 (12), N 17+18 2+12, 1 7+9 5+10, and 2*, 17+18, 2+12 were found in 9 genotypes. At Glu-A1 locus 1Ax1 and 1Ax2* subunit were observed in 7 and 9 genotypes, respectively which are more important than locus having null allele at this locus, because the null allele score is not desirable for quality trait contribution.

The rare allele of HMW glutenin subunit 1Bx14+1By15, 1Bx7*, 1Bx6+1By8* were found at Glu-B1 locus, and their allelic combination are given in the table 4.

Table 4. Allelic frequencies (HMW-GS) and diversity at Glu-1 loci in core collection of diverse bread wheat.

Locus	Allele	Subunit	No of Genotypes	Frequency %	H (Nei·s index)
Glu-A1	a	1	29	24.16	
	b	2*	24	20.00	
	c	Null	67	55.83	0.59
Glu-B1	a	7	23	19.16	
	b	7+8	20	16.66	
	c	7+9	16	13.33	
	d	6+8	1	0.83	
	e	20	16	13.33	
	f	13+16	9	7.5	
	h	14+15	1	0.83	
	i	17+18	31	25.83	
	-	6+8*	1	0.83	
	-	20x20y	1	0.83	
	-	7*	1	0.83	0.83
Glu-D1	a	2+12	49	40.83	
	d	5+10	60	50.00	
	e	2+10	9	7.5	
	x	2+12.2	2	1.66	0.58

Superior subunits 1Dx5+1Dy10 was found in different allelic combinations viz., 1Ax1, 1Ax2*, 1Bx17+1By18, 1By20 and 1Bx7+1By9 in 60 genotypes. These sixty genotypes may be regarded as best lines for quality contributing traits because 1Dx5+1Dy10 contributes to superior bread-making quality. The inferior subunit 1Dx2+1Dy12 was also found in different allelic combinations, the most common of which was Glu-D1a (1Dx2+1Dy12) shown in table 4. Other combinations at Glu-D1a locus identified were 2+10 and 2+12.2 found in 10 and 2 genotypes respectively. Glu-1 quality score of all the genotypes ranged from 4-10 with an average of 7.3 shown in table 5. The quality scores of 17 selected germplasm could not be identified due to the presence of rare alleles in their allelic combination and warrant further investigated.

Table 5. HMW-GS allelic compositions and Glu-1 quality score in diverse germplasm ofwheats.

Locus	Subunit Combinations	Alleles	No of Genotypes	Glu-1 Score
Glu-A1,B1,D1	N, 7, 5+10	c, a, d	12	6
	N 7* 2+12	c, u, a	1	-
	N 7 2+10	c, a, e	3	-
	N 7+8, 2+12	c, b, a	15	6
	N 7+8 2+12.5	c, b, -	1	-
	N 7+8* 2+12	c, - a	2	-
	N 7+8 2+10	c, b, e	2	-
	N 7+9 2+12	c, c, a	2	5
	N 7+9 2+10	c, c, e	2	-
	N 6+8* 5+10	c, - d	1	-
	N 20 2+12	c, e, a	1	4
	N, 20, 5+10	c, e, d	3	6
	N, 13+16, 2+12	c, f, a	3	6
	N, 13+16, 5+10	c, f, d	2	8
	N 13+16 2+10	c, f, e	1	-
	N 17+18 2+12	c, i, a	9	7
	N 17+18 5+10	c, i, d	7	8
	1, 7, 5+10	a, a, d	3	6
	1 7+9 5+10	A, c, d	9	7
	1 20 5+10	a, e, d	6	8
	1 17+18 5+10	a, i, d	6	10
	1 13+16 5+10	a, f, d	2	10

	1 14+15 2+12	a, h, a	1	-
	2*, 7, 5+10	b, a, d	3	9
	2,* 7, 2+12	b, a, a	2	6
	2*, 7+8, 2+12	b, b, a	1	8
	2* 7+9 5+10	b, c, d	2	9
	2* 7+9 2+12	b, c, a	3	7
	2*, 13+16, 2+12.2	b, f, x	1	-
	2*, 17+18, 2+12	b, i, a	9	8
	2*, 17+18 5+10	b, i, d	2	10
	2* 20 -	b, e, -	2	-
	1 6+8 5+10	a, d, d	1	8
	1 7+9 2+12	a, c, a	1	7
Average				7.3

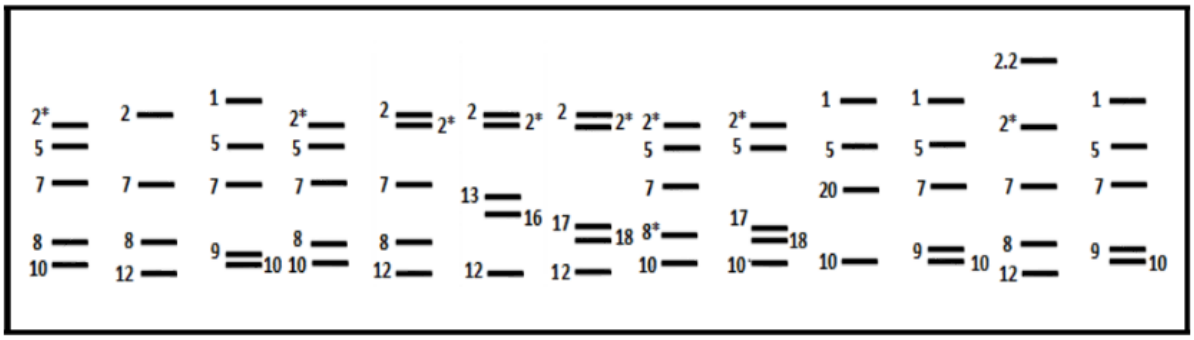

Fig. 3. SDS-PAGE profile of HMW-GS alleles of wheat standard cultivars at three gene loci (left to right 1-14 standards, 10 & 11 are similar).

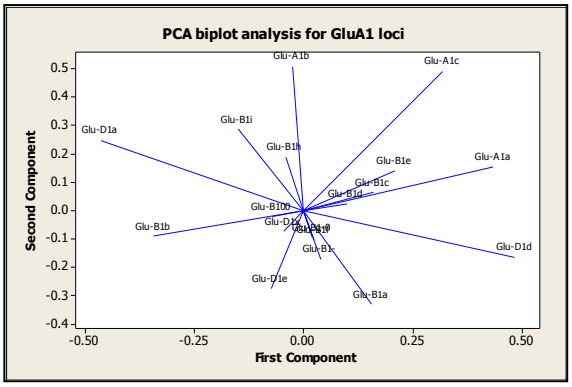

Fig 4. PCA analysis was made by making data matrix for all the scored genotypes to show the allelic diversity at the Glu-A1 loci.

Discussion

The HMW-GS proteins are important parts of the gluten complex in wheat. They are encoded by a highly variable gene family expressing different HMW GS subunits, separated on the basis of protein polymorphism by using SDS-PAGE analysis. Presence or absence of specific allelic variants of HMW-GS are correlated with bread-making quality (Wieser & Zimmermann, 2000).The results of the present study have confirmed different allelic combination in the selected germplsm.

The combined occurrence of different alleles from the three loci is important in the accumulation of scores and also in determining quality (Payne *et al.,* 1987). Exploration of genetic variability in breeding programs regarding loci encoded glutenin proteins is an important tool to characterize germplasm for their utilization. Different allelic combinations were found in the selected germplasm controlling quality scores in wheat endosperm proteins.

The combined occurrence of different alleles from Glu1 loci is an important in the accumulation of scores and also in determining quality (Payne *et al.*, 1987). Allelic variation in HMW-GS composition at each locus was found to be strongly associated with various high molecular weight glutenin subunits (HMW-GS). Different subunits have been identified and characterized, and the significant association between some subunits and quality traits has been found (Gianibelli, 2001). These subunits have been used for screening since longer time because these glutenin proteins are highly polymorphic in nature without environmental influence (Payne *et al.*, 1981).

For the allocation of right allelic designation to HMW-GS, 14 different quality standards were utilized and optimized for the characterization and identification of studied genotypes. All these standards matched their subunit designation (except Darius) and were used as reference for generated bands during electrophoresis. These base line standard arrays will be helpful for screening and characterizing large genomic stocks in future.

In the present study, Glu-1A locus contributed three alleles Null, Ax1 and Ax2* with the dominancy of the null subunit (55.83%) that is also analogous to the earlier reports of Nakamura (2000) and Popa *et al*., (2003). Pena *et al.,* (1995), William *et al*., (1993) and other have also determined the occurrence of the major null allele. However, its frequency was minimal in European spelt wheat genotypes as reported by An *et al*., (2005). Li *et al*., (2009) recently proposed higher frequency of this allele in Chinese genotypes. The presence of subunit 1 was relatively higher as compared to 2* but lower than the null alleles. Valizadeh *et al.,* 2001 and Chaparzadeh *et al*., (2008) revealed the lack of subunit Ax1 in their experimental wheat genotypes. These differences may be due to difference in the germplasm used or due to lesser number of varieties included in the previous experiments. Our experimental material were diverse hence we observed the subunit Ax1 in maximum number of genotypes as compared to Ax2* subunit. Therefore the genotypes possessing these alleles at Glu-1A locus may be used in breeding programs for quality improvement and broadening the genetic background of local varieties.

Maximum number of alleles have been observed at Glu-B1 locus (table 3) in different combinations. Sajjad *et al.,* (2012) identified 9 different alleles in CIMMYT materials at Glu-B1 locus in different combination (7, 8, 7+8, 7+9, 13+19, 13+16, 17+18, 14+15, & 20). The most frequent alleles found among these CIMMYT materials were 7+8 and 7+9, a frequency of 16. Subunits 14+15, 13+16 and 20 occurred in the least genotypes. Sultana *et al.,* (2007) and Masood *et al.,* (2004) reported a higher proportion of 1Bx17+1By18 and 1Bx7+1By9 alleles in land races and cultivated varieties of Pakistan.

As our studied genotypes comprised of diverse germplasm a higher proportion (55.82%) of these subunits (1Bx7+1Bx8, 1Bx7+1By9 and 1Bx17+1By18) were observed. The association of these subunits with end-use quality characteristics has been formerly determined by Tang *et al*., (2010). Payne and Lawrencen (1983), reported the frequencies of the Glu-B1a, Glu-B1b, Glu-B1c and Glu-B1d alleles in the worldwide collection were 19%, 25%, 30% and 18% respectively. Morgunov *et al*., (1993) reported that frequencies of Glu-B1a, Glu-B1b, Glu-B1c and Glu-B1d were 13%, 25%, 31%, 10% respectively. Rare alleles, 7*, 6+8*, 14+15 and 20 encoding by Glu-B1 locus were also found across the selected genotypes. Allelic frequencies and combination are shown in table 4 and 5 respectively. The subunit Bx7* was first described by Pogna *et al.,* (1989) in Cheyenne variety and considered as a standard cultivar like Chinese spring but the electrophoretic mobility of Cheyenne Bx7* is slighter higher than the Chinese Spring (Bx7) standard subunit. Dong *et al.,* (2009) characterized and identified the same rare subunit (Bx7* and By8*) through SDS-PAGE and RP-HPLC technique in Jing 411 bread wheat cultivar. Tang *et al*., (2010) evaluated quality assets of 6+8 on 21 quality and noodle test parameters. The ordinary effect of 6+8 subunit was positive influencing most of the quality parameters essentially if combined with superior subunits from Glu-A1 and Glu-D1. Rasheed *et al.,* (2012) proposed the subunit 6+8 very common in synthetic hexaploids and durum wheat but its frequency was very low in bread wheat. The other unique band (20) encoded by Glu B1 locus was observed in only one genotype (table 5). Dong *et al.,* (2009) was reported a similar unique band of 20x20y at Glu-B1 locus in the durum (Bidi 17) wheat genotype.

Our results show that sixty five genotypes had subunits 5+10 and the maximum number was found in the Richard selection (95%) followed by entries of the historical set (57%), land races (36.6%) and SAWSN (50%). 41 genotypes had 2+12 subunit at the Glu-D1locus across the seven groups, maximum numbers of this subunit being found in the three groups.

Generally, the presence of subunit 5+10 is considered as a superior and desirable trait for good quality (Payne *et al*., 1987; Kasarda, 1999) and a maximum score is allocated for its presence. It has been reported that genes coding for D genome subunits play an important role in determining the bread making quality (Rodriguez-Quijano, 2001), and good bread quality is mostly associated with the presence of subunit 5+10 at locus Glu-D1 (Bushuk,1998; Rabinovich,1998).

Our study revealed that the subunit 5+10 mostly occurred in combination with 7 (52.9%), 7+9 (26.5%), 13+16 (11.7%) and 17+18 (38.2%) alleles of Glu-B1, which resulted in the accumulation of high scores for most of the genotypes. Luo *et al*., (2001) reported the association of 5+10 subunit with sedimentation volume and longer pelshenke time and also determine that genotypes having 5+10 subunit revealed greater whole meal flour protein. Rare alleles at the Glu-D1 locus were found in 11 genotypes i.e. 2+10 and 2+12.2 in 9 and 2 Genotype across the selected germplasm respectively. Hua *et al.,* (2005) has previously identified 2+10 subunit in Chinese land races collected from Xinjiang District. Allelic frequencies of these rare subunits encoded by Glu-D1e were 2.1% in the two group of Chinese land races. Rasheed *et al.,* (2012) also reported the presence of a similar allele, 1Dy12.2 at Glu-Dt 1 locus in twelve synthetics either in combination with 1.5 or 2 subunits. The effect of the 12.2 subunit on end use quality of wheat is not yet determined as their quality effects are yet to be recognized.

The inferior subunits (2+12) encoded by Glu-D1d associated with deleterious effects on quality appeared in different combinations, with frequent subunits encoded by Glu-B1 viz., 7, 7+8, 7+9, 13+16 and 17+18 and found in the selected germplasm (table 5). Nakamura (2000), examined the electrophoretic profile of HMW glutenin subunits of 274 hexaploid Chinese wheat cultivars through sodium dodecyl sulfate polyacrylamide gel electrophoresis and reported 27 different major HMW glutenin subunits. The frequency of subunit 2+12 encoding by Glu-D1a allele was high and majority of the Chinese varieties were dominated by this subunits.

Conclusion

Our result indicated that the genotypes under study have valuable alleles across the three loci (Glu-A1, Glu-B1 and Glu-D1), subunits 1, 2*, 7+8, 7+9, 13+16, 17+18 and 5+10 were found among the genotypes which can be exploited in breeding programs for improvement and high grain quality.

Seed of the 14 optimized quality standards utilized in the study matched their allelic designation of HMW-GS and will beneficial to use as a standard set for characterization of other germplasm.

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