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PREVELANCE OF HIGHER GLUTENIN VARIATION IN SYNTHETIC WHEAT GERMPLASM

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ABSTRACT

Genetic improvement in bread wheat (*Triticum aestivum* L., AABBDD, 2n=6x=42) for various economically important characters has remained a challenge for breeders. The present study investigated diversity of the high molecular weight (HMW) as well as low molecular weight (LMW) glutenin subunits in 84 wheat genotypes including Pakistani land races, cultivars and *Aegilops tauschii* derived primary synthetic hexaploid wheats (SHWs). For HMW-GS, 15 x-type and 9 y-type subunits were observed in all three groups. Nei's diversity index was highest for *Glu-D^{1}1* locus in synthetics hexaploid wheats and revealed that the D genome from *Ae. tauschii* carried maximum diversity for HMW-GS. Of the 33 different combinations, the highest combinations observed in SHWs were 'null, 6+8, 2.1+10.5' followed by 'null, 6+8, 1.5+10' and '2*, 6+8, 2+12', while, the Pakistani cultivars and land races harbored 'null, 20, 12+2' and '1, 7+8, 2+12' alleles. For LMW-GS, six alleles were found at *Glu-A3* locus and nine were found at *Glu-B3* locus. Predominant alleles were *Glu-A3c, Glu-A3d* and *Glu-A3b,* which were observed in 41 (50.61%), 19 (23.45%) and 12 (14.81%) genotypes, respectively. At *Glu-B3* locus, at the most prevalent alleles were *Glu-B3h* (18.51%), and *Glu-B3i* (17. 28%). High number of HMW-GS (*Glu-D^{1}1* locus) and LMW-GS combinations reported here highlights the potential use of SHWs for transferring allelic variation from this synthetic stock to bread wheat for broadening genetic base of quality traits. Furthermore, *Ae. tauschii* derived primary SHWs also encoded both x and y-type alleles and offers possibility of these SHWs for the introduction of novel glutenin variability into elite bread wheat cultivars for different end use products.

Key words: Glutenin, HMW, LMW, Synthetic hexaploid wheat, *Ae. tauschii,* quality.

INTRODUCTION

Decades of continuous breeding and selection of the elite germplasm for few desirable traits has reduced the genetic base of existing bread wheat cultivars. This fraction of genetic diversity available is not enough to ensure food security in future, therefore, it is necessary to discover novel genetic sources to develop promising cultivars adaptive to the varying environments. (Mujeeb-Kazi *et al.,* 2008; Mc Couch *et al.,* 2013; Marta *et al*., 2015; Sajjad *et al.,* 2017). Fortunately, the genetic base of wheat may be broadened with quality traits by exploiting the wild and cultivated Triticeae species and derived synthetics (Mujeeb-Kazi and Hettel, 1995; Ali *et al.,* 2016).

Besides their nutritional significance, the storage proteins present in cereal kernels also affect their end use in food manufacturing (Peter *et al.,* 2002). The uniqueness of wheat from cereals is that its milled product *i.e.* the flour is able of making dough due to its gluten content, which retains gas released in the process of fermentation (Bushuk and Wrigley, 1974; Hoseny 1998) and on baking yield well aerated and light bread loaf. This exclusive feature of wheat is due to its two proteins named as glutenins and gliadins, which by adding water result in gluten, the main substance that imparts gas retention property to dough (Gaines, 1991; Souza *et al.*, 1994). Glutenin have two main classes that are HMW-glutenins, having size 80 -130 kDa and the LMW-glutenins, having size 10-70 kDa (Bietz and Walls, 1973). Though, HMW-GS are present in little quantity but plays a major role in determining gluten's elasticity (Payne *et al.,* 1980). The HMW glutenin loci, *Glu-A1, Glu-B1* and *Glu-D1* are multi-allelic genes present on the group 1 long arm chromosomes, and are chief determinant of wheat baking quality. Each HMW glutenin locus on the group 1L arms comprises of x-type and y-type genes; both have originated from an ancient duplication event with subsequent divergence (Anderson *et al.,* 1998). Further, these loci are extremely polymorphic with no evident effects of the external environment (Payne *et al.,* 1981a). Nonetheless, this allelic diversity is the result of various combinations of HMW-GS in different wheat cultivars.

Despite the presence of complete orthologous set of x and y-type genes located at the *Glu-1* and *Glu-2* loci in A, B, and D genomes, no report of hexaploid wheat cultivar with all six possible transcriptionally active genes is available; and only a group of three to five

HMW-GS are present at a time due to various gene silencing mechanisms (Wanous *et al.,* 2003). The specific combinations of these subunits are used to envisage the bread making quality using *Glu-1* scoring system (Payne *et al.,* 1987) that is in use since decades. HMW-GS makes the dough elastic and allows entrapping of the gas bubbles that are formed during fermentation, resulting in a soft raised loaf (Cornish *et al.*, 2006). Although, HMW-GS are mainly associated with bread making characteristics of dough, LMW-GS also plays a significant role in dough resistance and dough extension (Cornish *et al.,* 2001). Twice the amount of LMW-GS had been found necessary to attain similar dough properties as obtained with HMW-GS (Wieser and Kieffer, 2001).

In bread wheat, a number of studies have focused on HMW-GS because they play a key role in baking quality with additional advantages of easy identification by electrophoresis. Payne *et al.* (1981a) found relationship among specific HMW-GS and gluten strength by SDS-sedimentation volume test. The allelic diversity of *Glu-D1* locus greatly affects the bread making quality of wheat as compared to diversity present at others *Glu-1* loci. Moreover, several studies showed that 5+10 subunits combination at *Glu-D1* locus provide superior dough quality than 2+12 subunits combination. This property of 5+10 subunits combination is due to an extra cysteine residue in Dx5 subunit that endorses the polymers with large size configuration. Further, the variation in cysteine residues number in 17+18 subunits combination is responsible for large sized polymers with larger configuration in contrast to 20x+20y subunits combination at *Glu-B1* locus. This is also associated with the two extra cysteine residues in 17+18 subunits pair.

In case of LMW-GS, PCR based makers are rapidly replacing SDS-PAGE due to its ease of use and amenable nature. Moreover, characterization and subsequent incorporation of *Glu-3* allelic variation in breeding program will improve bread making quality of targeted wheat genotypes. Liu *et al.* (2010) indicated that PCR based allele specific markers for *Glu-A3* and *Glu-B3* loci offers a simple, precise and cost effective alternative for targeted marker assisted selection (MAS) breeding programs. For LMW-GS, tightly linked PCR based molecular markers have been developed for both *Glu-A3* and *Glu-B3* loci by exploiting the nucleotide sequence variation found in diverse wheat genotypes and applied in efficient MAS breeding programs focusing on backing quality improvement (Zhang *et al.,* 2004; Wang *et al.,* 2009, 2010). Because of the low genetic diversity at *Glu-D3,* no allele specific markers were developed (Liu *et al.,* 2010), and the effect of *Glu-D3* on bread making quality is also negligible as compared to other *Glu-3* loci (Gupta *et al.,* 1989; Zhang *et al*., 2012).

Although, substantial information on wheat protein composition in relation to bread making quality is available, still our understanding of the HMW-GS and LMW-GS composition is limited and needs enhancements. Thus, the present study was designed to explore HMW-GS (by SDS-PAGE) and LMW-GS (by allele specific markers) diversity in three groups of wheat germplasm and the resulting information can be used as guidelines for future selection and development of elite wheat cultivars with improved baking quality.

MATERIALS AND METHODS

A set of 84 wheat genotypes including 10 Pakistani land races, 20 cultivars, 52 *Ae. tauschii* derived primary synthetic hexaploid wheats and their two durum parents (Table S1) were evaluated for their glutenin composition and variability.

HMW-GS Analysis

Protein extraction: Standard procedure of Laemmli (1970) was followed for protein extraction. To take out protein from flour, 10mg of sample was taken into a sterile eppendorf tube and 1ml of protein extraction buffer (5M Urea + 0.5M Tris + 0.2%SDS, pH 8.0) was added to it. The sample was vortexed to homogenize and placed in fridge till the start of electrophoresis.

Electrophoresis: The HMW-GS were analysed using slab type SDS-PAGE with 7.5% polyacrylamide gel. ATTO AE-6400 electrophoresis apparatus was used to carry out the experiment. At the base electro-buffer solution (5M Urea + 0.5M Tris + 0.2%SDS, pH 8.0) was dispensed and gel plates placed in such a way that air bubbles formation was avoided. On the top pool of the apparatus the electro-buffer solution was added using a micro syringe. A volume of 8µl protein extracted sample was load into each well. The apparatus was set at 200V and samples were run until the loaded protein samples reached near the base of gel plates. Electrophoresis followed staining of the polyacrylamide gel for 20-30 minutes and destaining on a shaker until the disappearance of background color. Gels were dried using a gel drying processor for about one hour at 60-70 ^{o}C.

Allele identification: Allele identification followed the method of Payne and Lawrence (1983). *Glu-D^{1}1* alleles detection was carried out as per William *et al.* (1993) and Pena *et al.* (1995) and allele naming followed Mac Genes (McIntosh *et al.,* 2008).

Statistical analysis: Nei's index (Nei, 1973) was used to calculate the genetic variation at each locus *i.e.* $H = 1 - \Sigma P_i^2$ where, H is genetic diversity index and P_i is frequency of the number of alleles at the locus. Allelic frequencies were calculated by adding allelic frequencies in each accession, regardless of HMW-GS

composition (heterogeneous or homogeneous) and then dividing the total by number of accessions.

LMW-GS Analysis

Allele specific molecular markers: LMW-GS alleles present on *Glu-A3* and *Glu-B3* loci were amplified with functional markers while, alleles present on *Glu-D3* locus were not recognized due to their non-significant role in bread making quality. A set of 17 closely linked markers were used to study LMW-GS in wheat genotypes. These markers included 7 allele specific markers for *Glu-A3* alleles previously known to verify allelic variation in synthetic hexaploid wheats (Wang *et al.,* 2009) and 10 markers for *Glu-B3* alleles (Wang *et al.,* 2010). The primer sequences, expected product size, melting temperatures and source are enlisted in Table S2.

DNA isolation and allele specific PCR: Kernels of each genotype were germinated for two weeks in growth chamber at 32ºC. Fresh leaves were used for total genomic DNA extraction using phenol chloroform method given by Pallotta *et al.* (2000). DNA quality was checked by running it on 1% agarose gel, while the concentration was estimated with spectrophotometer. PCR reaction was carried out in a total volume of 10µl containing 1x PCR buffer, 50-100ng of genomic DNA, 1.0-1.5mM of MgCl2, 200mM of each deoxyribo nucleotide (dNTP), 5pmol of each primer and 0.3U of Taq DNA polymerase. Experimentally optimized melting temperature and expected product size are given (Table S2). Reproducibility of the PCR amplicon was confirmed by repeating the PCR twice for each primer sets. Electrophoresis of the amplified fragments was carried out on 1.5% agarose gels and sizes were estimated with a DNA marker of 2Kbp size run along PCR products. Ethidium bromide (0.5µg/ml final concentration) was used to stain the gel for 30 minutes whereas Gel documentation system (Bio-Rad) was used to visualize it. The resulted products were scored for absence or presence of the specific allele in all genotypes.

RESULTS AND DISCUSSION

High molecular weight glutenin subunits (HMW-GS) variation: In 84 accessions, 15 x-type and 9 y-type subunits were studied at the three *Glu-A1*, *Glu-B1* and Glu-D1 loci. *Glu-A1* had shown 3 x-type and *Glu-B1* had given 6 x-type and 5 y-type subunits while *Glu-D1* had 6 x-type and 4 y-type subunits (Table 1). A total of 24 alleles were identified in wheat genotypes at *Glu-1* locus, out of which 3 alleles were at *Glu-A1,* 11 were at Glu-B1 and 10 resided at *Glu-D1* locus (Table 3). On the basis of HMW-GS all genotypes clustered into 33 groups. The genetic variation assessed by Nei's index was highest at *Glu-D1* locus (0.83) followed by *Glu-B1* (0.77) and lowest diversity was observed at *Glu-A1* locus (0.64). At *Glu-A1* locus, x-type null subunit encoded by *Glu-A1c* allele was predominantly found in 45.6% accessions and was the most prevalent amongst all alleles at other loci. The y-type subunit at *Glu-A1* locus remained absent.

At *Glu-B1* locus, seven different co-dominant alleles were found. The most frequent allele at *Glu-B1* locus was *Glu-B1d* controlling subunit 6+8 (39.5%). The second most common allele at *Glu-B1* was *Glu-B1i* allele controlling 17+18 subunits (17.2%). The other alleles found at *Glu-B1* were *Glu-B1b, Glu-B1f, Glu-B1e* and *Glu-B1c* controlling subunits 7+8, 13+16, 20 and 7+9 with frequency of 14.8%, 13.58%, 11.11% and 2.40%, respectively. Although, the inclusion of these subunits are positively associated with bread making quality, the decrease in diversity may be attributed to their under-utilization or hidden potential of the *Glu-B1* locus. The *Glu-D*^{t}*1d* allele encoding 5+10 subunits being most significant offers superior bread making quality and was identified in 17 genotypes (20. 98%). *Glu-D*^{t}*1a* which encodes 2+12 subunits was identified in maximum genotypes i.e. 22 (27.16%). The second frequent subunits at this locus were 2.1+10.5 controlled by allele *Glu-D1ai* and were found in 12 (14.81%) genotypes. Moreover, other significant subunits at the same locus were 1.5+10, 1.5+12.2, 1.5+12, 1.5+10.5, 2.1+12, 2.1+12.2, 5+12.2 and 3+10 found in 7 (8.64%), 1 (1.23%), 2 (2.46%), 3(3.70%), 6 (7.40%), 1 (1.23%), 1 (1.23%) and 5 (6.17%) accessions, respectively (Table 2). Documentation of quality traits in SHW is not a rare phenomenon and similar results have been reported earlier (Khalid *et al.,* 2013, Masood *et al.,* 2016).

Of the 33 HMW-GS combinations, the most frequent combination was 'null, 6+8, 2.1+10.5' followed by 'null, 6+8, 1.5+10'and '2*, 6+8, 2+12' observed in 6 (11.76%), 5 (9.80%) and 5 (9.80%) synthetic wheats, respectively (Table 1). While, the frequent combination in case of Pakistani cultivars and land races was 'null, 20, 2+12' followed by '1, 7+8, 2+12' found in 5 (16.6%) and 3 (10%) genotypes, respectively (Table 1). The 12 different combinations were rare and each appeared in only one genotype (Table 2). The combination '2*, 17+18/7+8/7+9, 5+10' (*Glu-A1b*, *Glu-B1i*/*Glu-B1b*/*Glu-B1c*, *Glu-D1d*) is considered as positive indicators for better quality bread with *Glu-1* quality score of 9-10 (Sajjad *et al.,* 2012). Similar to our finding in Pakistani germplasm, the combination '2*, 7+9, 5+10', was also reported as the most common in Bulgarian wheat varieties (Atanasova *et al.,* 2009), American hard winter wheat (Graybosch, 1992), Estonian, Nordic and Middle European wheats (Tohver, 2007), CIMMYT bread wheats (Trethowan *et al.,* 2001) and Pakistani varieties (Sajjad *et al.,* 2012; Rehman *et al.,* 2014). While, the combination N, 7+9, 5+10 was found as most common in Slovak varieties (Gregova *et al.,* 2007), Australian varieties (Groger *et al.,* 1997) and in Serbian wheats (Dencic and Kobiljski, 2008). The alleles 1 and 2* at

Glu-A1 are also associated with greater gluten strength and good baking quality (Vazquez *et al*. 2012), while it was found that 17+18 and 7+8 alleles at *Glu-B1* are also associated with high bread volume, especially the 17 allele, which has a positive effect on the rheological properties of the flour (Pena *et al.,* 2005).

The x-type subunit *Dx2.1*^{t} appeared in 19 (51%) followed by *Dx1.5*^{t} found in 13 (25.4%) synthetic hexaploids with all y-type *Glu-D*^{t}*1* encoded subunits. These are very important allele due to their high prevalence. Since synthetic hexaploids are most widely exploited genetic stocks, therefore, it is expected that these subunits may be transferred to bread wheat lines developed from SHWs. *Ae. tauschii* encoded x and y-type alleles are also very important and have the potential to introduce novel gluten variability in wheat varieties for various end use products.

Low molecular weight glutenin subunits (LMW-GS) variation: Distribution of allelic combination of LMW-GS at *Glu-A3* and *Glu-B3* is given in Table 4, while Table 5 is showing allelic frequency for *Glu-A3* and *Glu-B3* loci. In case of LMW-GS, six alleles were found at *Glu-A3* locus and nine at the *Glu-B3* locus. Allelic diversity at *Glu-D3* locus was not assessed due to their trivial role in bread making quality and shared haplotype of several alleles. Allele specific markers by Wang *et al.* (2010) were used to identify alleles at *Glu-A3* locus (Table S2). *Glu-A3a* was not found in any genotype. The most frequent allele observed was *Glu-A3c* found in 41 (50.61%) genotypes followed by *Glu-A3d* and *Glu-A3b* found in 19 (23.45%) and 12 (14.81%) genotypes, respectively. *Glu-A3e* was found only in 2 (2.46%) genotypes. Alleles at *Glu-B3* locus were amplified using markers developed by Wang *et al.* (2009) and 1B.1R translocation specific marker was used to recognize *Glu-B3j* allele. At *Glu-B3* locus, all the wheat genotypes did not contain *Glu-B3a* allele. Maximum frequency was observed at *Glu-B3h* locus that was in 15 (18.51%) genotypes which was followed by *Glu-B3i* found in 14 (17.28%) accessions. While the least frequent allele, *Glu-B3d* was only observed in three genotypes (3.70%).

Table 1. Combination of *Glu-1* alleles in wheat genotypes.

GluA1	GluB1	GluD1	Accessions	Number
N	6+8	2+12	SH-533, SH-535, SH-551, SH-464	4
N	6+8	2.1+10.5	SH-421, SH-423, SH-519 ,SH-318, SH-400, SH-566	6
N	6+8	1.5+10	SH-52, SH-187, SH-356, SH-419, SH-979	5
N	6+8	1.5+10.5	SH-319, SH-366, SH-372	3
N	7+8	2+12	Chakwal-50, Chakwal-86	2
N	7+8	2.1+10.5	SH-626, SH-641, SH-646	3
N	13+16	2+12	LLR-32	1
N	13+16	5+10	SA-42, LLR-41	2
N	14+15	1.5+12.2	SH-540	1
N	13+16	1.5+10	SH-539, SH-546	2
N	17+18	2.1+10.5	SH-572, SH-542	2
N	20	2+12	LLR-28, LLR-29, LLR-30, LLR-39, LLR-36, Bhittai	6
1	6+8	2.1+10.5	SH-378	1
1	6+8	5+10	SH-905	1
1	7+8	2+12	Abadgar-93, Bhakkar-000, Zindad-000, SH-956	4
1	7+9	5+10	Pak-81	1
1	13+16	5+10	SH-828, SH-829, SH-830	3
1	13+16	1.5+12	SH-182, SH-357,	2
1	17+18	5+10	Faislabad-08, Zamindar-80, SH-833, SH-834	4
1	17+18	2+12	Chenab-70, LLR-33	2
1	20	5+10	LLR-37, LLR-38	2
2*	6+8	3+10	SH-161, SH-389,	2
2*	6+8	2.1+12	SH-12, SH-17, SH-20, SH-23, SH-33	5
2*	6+8	2.1+12.2	SH-375	1
2*	6+8	3+10	SH-856, SH-411, SH-414	3
2*	7+8	5+10	Seher-06, Kirman	2
2*	7+8	2+12	Fareed-06	1
2*	7+9	5+10	Lasani-08	1
2*	13+16	2.1+12	SH-1002	1
2*	17+18	2+12	Parvaz-94, Inqilab-91, Miraj, SH-676, SH-827	5

2*	17+18	5+10	Shafaq-06	1
2*	6+8	5+12.2	SH-412	1
2*	20	2+12	Jauhar	1

Table 2. Allelic variation for HMW-GS.

Locus	Allele	Sub-unit	Accessions	Frequency	Diversity (*H*)
Glu-A1	c	N	37	45.6	
	a	1	20	24.6	
	b	2*	24	29.6	0.64
Glu-B1	d	6+8	32	39.5	
	b	7+8	12	14.8	
	i	17+18	14	17.2	
	-	14+15	1	1.23	
	f	13+16	11	13.58	
	c	7+9	2	2.40	
	e	20	9	11.11	0.77
*Glu-D*1	a	1.5+12.2	1	1.23	
	ah	1.5+10	7	8.64	
	aj	1.5+12	2	2.46	
	-	1.5+10.5	3	3.70	
	ga	2.1+12	6	7.40	
	ai	2.1+10.5	12	14.81	
	-	2.1+12.2	1	1.23	
	a	2+12	22	27.16	
	z	3+10	5	6.17	
	d	5+10	17	20.98	
	-	5+12.2	1	1.23	0.83

Table 3: Frequency of HMW-GS combinations

Locus	x-type	y-type	Combinations
Glu-A1	3	-	3
Glu-B1	6	5	7
Glu-D1	6	4	10
Total	15	9	33

Table 4. Combination of *Glu-3* alleles.

Glu A3	Glu B3	Accessions	Number
B	b	SH-533	1
B	d	SH-551	1
B	g	SH-23, SH-33, SH-318	3
B	i	Lasani-08, SH-182, SH-535, SH-572	4
B	h	Chakwal-86	1
B	h	SH-833,SH-834,	2
B	j	SH-1002	1
C	d	LLR-39, LLR-41	2
C	c	SH-161, SH-400, SH-905, SH-423,	4
C	e	SH-464, SH-540	2
C	f	SH-956, SH-539	2
C	g	Inqilab-91, Fareed-06, Miraj-08, Shafaq-06	4
C	h	SH-52, SH-187, SH-319, SH-542, SH-546	5
C	i	LLR-28, LLR-29, LLR-30, LR-32, LLR-33, LLR-36, LLR-37, LLR-38, Chakwal-50	9
C	j	Pak-81, SH-12, SH-17, SH-20	4

C	b	Seher-06, SH-626, SH-641, SH-646	4
C	f	SA-42, Abadgar-93,SH-828, SH-829, SH-830	5
D	b	Chenab-70, Bhittai	2
D	g	Bhakkar-000, SH-357, SH-366, SH-372, SH-375, SH-378, SH-389	7
D	i	Zamindar-80	1
D	j	SH-676, SH-827, SH-356	3
D	h	Kirman, Parvaz-94, SH-856, SH-411, SH-412, SH-414	6
E	b	SH-421	1
E	h	Faislabad-83	1
F	e	SH-356, SH-519	2
F	g	SH-979	1
G	b	Jauhar	1
G	f	Zindad-000, SH-566	2

Table 5: Allelic variation for LMW-GS

Locus	Allele	Marker	Accessions	Frequency (%)
Glu-A3	A	*gluA3a*	-	-
	B	*gluA3b*	12	14.81
	C	*gluA3ac*	41	50.61
	D	*gluA3d*	19	23.45
	E	*gluA3e*	2	2.46
	F	*gluA3f*	3	3.70
	G	*gluA3g*	3	3.70
Glu-B3	A	*gluB3a*	-	-
	B	*gluB3b*	9	11.11
	C	*gluB3c*	4	4.93
	D	*gluB3d*	3	3.70
	E	*gluB3bef*	4	4.93
	F	*gluB3fg*	9	11.11
	G	*gluB3g*	12	14.81
	H	*gluB3h*	15	18.51
	I	*gluB3i*	14	17.28
	J	*gluB3j*	8	9.87

The combinations of *Glu-A3* and *Glu-B3* significantly affect bread making quality and appearance of favorable alleles at both loci is expected to encode better quality characteristics. In case of synthetic hexaploid wheats 12 different allelic combinations were observed, of which the most frequent combination was *Glu-A3c+Glu-B3h* appeared in 6 (11.76%) genotypes followed by *Glu-A3c+Glu-B3c* found in 4 (7.8%) synthetic hexaploid wheats. While the combinations, *Glu-A3b+Glu-B3b* and *Glu-A3b+Glu-B3d, Glu-A3b+Glu-B3j, Glu-A3e+Glu-B3b* and *Glu-A3f+Glu-B3g* were observed only in one synthetic hexaploid, hence less frequent and rare. In case of land races and Pakistani cultivars most frequent combination were *Glu-A3c+Glu-B3i* (80%) and *Glu-A3c+Glu-B3g* (20%) among their groups, respectively. While, *Glu-A3b+Glu-B3h, Glu-A3d+Glu-B3i, Glu-A3e+Glu-B3h* and *Glu-A3g+Glu-B3b* were found in only one Pakistani cultivar. The least frequent combination in case of land races was *Glu-A3c+Glu-B3d,* found in only two accessions. A related sub-units composition of the glutenin in Pakistani cultivars and land races was observed by Sajjad *et al.* (2012) and Rehman *et al*. (2014). Twelve new combinations were found in synthetic wheats that could serve as a conduit and highlights the immense potential of SHWs genes to be incorporated in existing wheat cultivars for widening their genetic base as well as for improvements in bread making quality.

Conclusion: Wheat glutenins analysis is known to be a prevailing tool for the evaluation of genetic resources. The study revealed that the selected synthetic hexaploid wheats have impending value in wheat breeding programs for quality. The comparison of SHWs with the local cultivars and land races showed that novel HMW-GS and LMW-GS are present in SHWs which can be used to broaden narrow genetic base for quality characters in bread wheat.

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