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GLU-D^{T}1 ALLELIC VARIATION IN SYNTHETIC HEXAPLOID WHEATS DERIVED FROM DURUM CULTIVAR 'DECOY' × *AEGILOPS TAUSCHII* ACCESSIONAL CROSSES

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Abstract

Characterization of high molecular weight glutenin subunits is the fundamental approach for categorizing genotypes with good bread making quality. Allelic variation at *Glu-D^{t}1* locus is major determinant of bread wheat end use quality. In synthetic hexaploid wheats (SHWs), the D-genome encodes numerous allelic variants of high molecular weight glutenin subunits that require appropriate identification prior to their exploitation for wheat improvement. This study was conducted to identify allelic variation at *Glu-D^{t}1* locus of 47 accessions of D-genome synthetic wheats derived from the crossing of durum cultivar "Decoy" with different accessions of *Aegilops tauschii*. Biochemical (SDS-PAGE) and molecular marker techniques were used to stringently characterize allelic differentiation. Nine different alleles at *Glu-D^{t}1* locus were observed which formed 13 different subunit combinations. The frequency of inferior quality encoding allele, 1Dx2+1Dy12, was equivalent (21.27%) to the frequency of superior quality encoding allele, 1Dx5+1Dy10 (21.27%). Additional validation was carried out with co-dominant molecular markers for *Glu-A1c* (Null), *Glu-D1d* (1Dx5+1Dy10), *Glu-D1a* (1Dx2+1Dy12) and *Glu-D1-1g* (1Dx2.1) alleles. The high number of glutenin subunits observed in SHWs are suggestive that somewhat narrow genetic base for D-genome encoded glutenin subunits in bread wheat may be broadened by exploiting this diploid genomic grass resource through targeting allelic transfers from synthetic hexaploid genetic stocks. The identification of these new allelic combinations in SHWs provide an option to replace other inferior quality encoding alleles in bread wheat cultivarsby better allelic variants that have become available at the *Glu-D^{t}1* locus of SHWs being inherited from diverse *Ae. tauschii* accessions.

Introduction

The endosperm storage protein of wheat, gluten, forms a continuous protein matrix in dough and confers elasticity and extensibility essential for bread making. High molecular weight glutenin subunit (HMW-GS) is important fractions of glutenins and main determining factor of gluten elasticity. They constitute about 5-12% of the entire grain protein (Payne *et al*., 1987) which is analogous to 1-1.7% of the dry weight of flour. These HMW-GS (80-130 KDa; Bietz & Wall, 1972) are encoded by *Glu-1* loci located on the long arm of homeologous group 1 chromosomes (1A, 1B and 1D) (Payne *et al*., 1980) named as *Glu-A1*, *Glu-B1* and *Glu-D1* respectively. Each locus include 2 genes linked together encoding two different types of HMW-GS, x- and y-type subunits (Payne *et al.*, 1987). In SDS-PAGE, the electrophoretic mobility of x- type subunits is slower due to its high molecular weight than the y- type subunit. Even though 6 genes subsist for HMW-GS, most bread wheat cultivars acquire only three (*1Bx, 1Dx and 1Dy*) to 5 (*1Ax, 1Bx, 1By, 1Dx and 1Dy*) glutenin subunits. Expression of *1Ay* subunits have been reported in *T. monococcum* and *T. urartu* (Waines & Payne, 1987). Recently, some bread wheat with six HMW-GS has been reported (Anjum & Walker, 2000). Allelic variation at *Glu-1* loci is important determinant for bread-making quality in wheat.

Common wheat (*Triticum aestivum* L. 2n=6x=42; AABBDD) originated from a limited number of natural events where *Aegilops tauschii* (D-genome donor) accessions of restricted geographic origin were involved, thus resulting in a narrow genetic diversity within the D-genome (Lagudah *et al.,* 1987). *Ae.tauschii* conserves a rich diversity source for enhancing the genetic variability of glutenin subunits in bread wheat that can significantly improve bread making properties (Pfluger *et al*., 2001). So far, 14 x-type and 10 y-type subunits in *Ae.tauschii* have been identified which combine into 85 different *Glu-D1* alleles. Synthetic hexaploid wheats (*T. turgidum* × *Ae. tauschii;* 2n=6x=42; AABBDD) have been developed by utilizing different *Ae. tauschii* accessions from diverse geographic origins with durum wheat genotypes through standard wide cross hybridization procedures with the objectivity to enrich/widen the wheat gene pool by unique genetic resources and addressing all areas of wheat improvement including grain quality (Mujeeb-Kazi *et al.,* 2008). Therefore these synthetic hexaploids wheats (SHs) are a valuable source for improving bread-making quality which harnesses genetic diversity from *Ae. tauschii*.

SDS-PAGE is a traditional method that has been used extensively to identify glutenin alleles and their effect on bread quality (Gupta *et al*., 1994).On the other hand specific glutenin based DNA molecular markers have been designed to assist in selection for improved dough rheology (Gale, 2005). D'Ovidio & Anderson, (1994) developed primers to detect 1Dx5-1Dy10 (*Glu-D1d*) whereas Smith *et al*., (1994) intended primers to differentiate Dx2+Dy12 (*Glu-D1a*) alleles, however *Dx5* assay has been used to distinguish these 2 major alleles found at *Glu-D1* locus (Varghese *et al*., 1996). The main objective of present study was to characterize *Glu-*

*D^{t}1*allelic variation by SDS-PAGE and allele-specific markers in D-genome synthetic hexaploids derived from durum cultivar 'Decoy' × *Aegilops tauschii* accessional crosses.

Materials and Methods

Plant material: Synthetic hexaploids evaluated in the present study were developed by crossing durum cultivar Decoy with different accessions of *Ae. tauschii*.and protocol and methodology has been described by Kazi *et al*., (1996). Chinese spring, Chenab-2000, Dirk, Marvi, E-1-16, E-1-57 and some accessions of *Aegilops tauschii* accessions (Rasheed *et al.*, 2013) having known *Glu-D1* alleles were used as standards for HMW identification and comparison of generated bands.

Protein extraction and SDS-PAGE: Total protein was extracted from single grain of each SH by grinding it in to fine powder. Approximately 400 µl of Protein extraction buffer (0.05M Tris + 0.2% SDS + 5M Urea, adjusted to pH 8.0 with HCl) was used for each sample. After vortex the samples, it was centrifuged at 1000 rpm for 5 minutes. The supernatant containing protein was separated which was used to analyze HMW glutenin subunits through slab type SDS-PAGE using 7.5% polyacrylamide gel.

Allelic identification and nomenclature: The allelic identification and the numbering of HMW-GS were based on the classification system of Payne and Lawrence, (1983). The identification and nomenclature of D-genome was done according to system for *Ae. tauschii* proposed by Lagudah *et al*., (1987); William *et al*., (1993) and Gianibelli *et al*., (2001). The allelic names of *Glu-D^{t}1* were designated from MacGenes (McIntosh *et al*., 2010). Some identified *Glu-D^{t}1* subunit pairs are not documented in MacGenes consequently their allelic names were the combination of allelic names of both subunits.

Molecular validation: Genomic DNA extraction was done according to phenol-chloroform method of Pallota *et al*., (2000) with some modification. PCR reactions were performed in a total volume of 10 µl containing 50-100ng of genomic DNA, 1x PCR buffer, 1.0-1.5mM of $MgCl_2$, 200mM of each deoxyribonucleotide (dNTP), 100ng of each primer and 0.3 U of *Taq* DNA polymerase. PCR reactions were repeated twice for all primer sets to confirm the results of the amplified products. Allele-specific PCR marker for *Glu-A1*alleles was carried out using the primer set reported by Liu *et al.*, (2008). Similarly, allele specific PCR for *Glu-D1* subunits Dx2, Dx5, Dy10 and Dy12 was carried out by primers reported by Liu *et al.*, (2008) and for Dx2.1 allele, primer pair reported by Lu *et al.*, (2005) was used. The amplification condition and primer sequences have been given in the aforesaid reports. The amplified fragments were separated on 1.5% agarose gel, stained with ethidium bromide and visualized using the Gel Documentation System (Bio-Rad). The DNA marker of 100bp size (Fermentas Cat # SM0321) was used for identification of amplified product.

Results

Composition and allelic variation at *Glu-D1* by SDS-PAGE analysis: SDS-PAGE analysis was conducted to study allelic variation at *Glu-D^{t}1* of HMW-GS in 47 accessions of SHs (Fig. 1). *Glu-D^{t}1* subunit in individual SH and molecular validation of some of the important alleles is depicted in Table 1. Durum cultivar 'Decoy' contributes A- and B-genome in these synthetic hexaploids. Therefore all these synthetics have *Axnull* and *Bx6+By8* alleles at *Glu-A1* and *Glu-B1* loci, respectively. The frequency of HMW-GS at *Glu-D^{t}1* locus observed in this germplasm set is given in Table 2. At *Glu-D^{t}1* locus, five x-type subunits and four y-types subunits resulted in a combination of 13 different alleles. The most frequent subunit was*Glu-D1a* and *Glu-D1d* (1Dx5+1Dy10) found in 10 (21.27%) genotypes. The x-type subunit *Glu-D1-1a* (1Dx2) showed combination with three y-type subunits, the most common of which was *Glu-D1a* (1Dx2+1Dy12). The other combinations were Dx2+Dy10 and Dx2+Dy12.2. Similarly, Dx5 which had the fastest mobility among x-type subunits in SDS-PAGE, was found to be associated with only Dy10. The x-type allele *Dx2.1* was found to be associated with Dy10.5and Dy12 in 5 and 3 SHs, respectively. Another x-type, *Ae. tauschii* specific subunit, Dx1.5, was appeared with four-type subunits viz. Dy10, Dy12 Dy10.5 and Dy12.2.

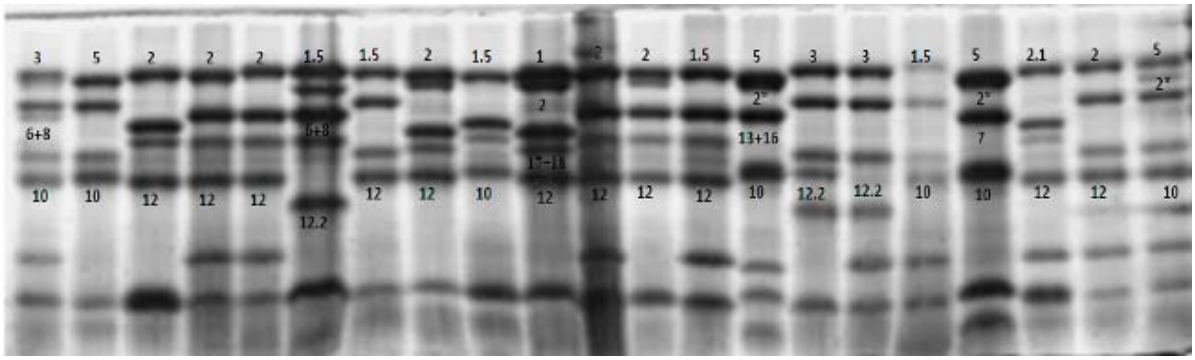

Fig. 1. SDS-PAGE profile of HMW-GS alleles in Decoy— *Ae.tauschii* synthetic hexaploid wheats
Lane (From left): 1: Elite 1-57 (Check), 2: SH-907, 3: SH-852, 4: SH-678, 5: SH-677, 6: Elite 1-16 (check), 7: SH-675, 8: SH-672, 9: SH-649, 10: Chenab 2000 (Check), 11: SH-648, 12:E1-47, 13:E1-86, 14: Dirk (Check), 15: SH-509, 16: SH-505, 17: SH-128, 18: Marvi (Check), 19: SH-447, 20: SH-373, 21: SH-441.

Table 1. High molecular weight *Glu-D*^{t}*1* subunits in Decoy-*Ae.tauschii*synthetic hexaploid wheat.

SH lines	Pedigree	*Glu-D*^{t}*1*	AxNull$_{362bp}$	Dx2.1$_{564bp}$	Dx10$_{397bp}$	Dx12$_{415bp}$	Dx5$_{281bp}$	Dx2$_{299bp}$
SH – 6	DOY1 / AE.SQUARROSA(188)	2.1+10.5	+	+	-	-	-	-
SH – 43	DOY1 / AE.SQUARROSA(216)	2.1+12	+	+	-	+	-	-
SH – 115	DOY1 / AE.SQUARROSA(446)	1.5+10.5	+	-	-	-	-	-
SH – 117	DOY1 / AE.SQUARROSA(447)	2.1+12	+	+	-	+	-	-
SH – 123	DOY1 / AE.SQUARROSA(488)	1.5+10.5	+	-	-	-	-	-
SH – 128	DOY1 / AE.SQUARROSA(510)	1.5+10	+	-	+	-	-	
SH – 129	DOY1 / AE.SQUARROSA(511)	1.5+10	+	-	+	-	-	-
SH – 131	DOY1 / AE.SQUARROSA(515)	5+10	+	-	+	-	+	-
SH – 302	DOY1 / AE.SQUARROSA(293)	3+10	+	-	+	-	-	-
SH – 323	DOY1 / AE.SQUARROSA(333)	3+12.2	+	-	-	-	-	-
SH – 326	DOY1 / AE.SQUARROSA(349)	2+12.2	+	-	-	-	-	-
SH – 330	DOY1 / AE.SQUARROSA(370)	2+12.2	+	-	-	-	-	-
SH – 341	DOY1 / AE.SQUARROSA(428)	1.5+10.5	+	-	-	-	-	-
SH – 349	DOY1 / AE.SQUARROSA(458)	5+10	+	-	+	-	+	-
SH – 361	DOY1 / AE.SQUARROSA(507)	3+10	+	-	+	-	-	-
SH – 363	DOY1 / AE.SQUARROSA(532)	2+10	+	-	+	-	-	-
SH – 373	DOY1 / AE.SQUARROSA(177)	2+12	+	-	-	+	-	+
SH – 377	DOY1 / AE.SQUARROSA(255)	2+12	+	-	-	+	-	+
SH – 380	DOY1 / AE.SQUARROSA(256)	2.1+10.5	+	+	-	-	-	-
SH – 381	DOY1 / AE.SQUARROSA(258)	5+10	+	-	+	-	+	-
SH – 385	DOY1 / AE.SQUARROSA(267)	5+10	+	-	+	-	+	-
SH – 395	DOY1 / AE.SQUARROSA(318)	5+10	+	-	+	-	+	-
SH – 396	DOY1 / AE.SQUARROSA(322)	2.1+12	+	+	-	+	-	-
SH – 398	DOY1 / AE.SQUARROSA(334)	2.1+12	+	+	-	+	-	-
SH – 401	DOY1 / AE.SQUARROSA(372)	2.1+12.2	+	+	-	-	-	-
SH – 403	DOY1 / AE.SQUARROSA(390)	1.5+10.5	+	-	-	-	-	-
SH – 409	DOY1 / AE.SQUARROSA(418)	2.1+10.5	+	+	-	-	-	-
SH – 422	DOY1 / AE.SQUARROSA(516)	5+10	+	-	+	-	+	-
SH – 424	DOY1 / AE.SQUARROSA(517)	2+12	+	-	-	+	-	+
SH – 426	DOY1 / AE.SQUARROSA(526)	5+10	+	-	+	-	+	-
SH – 434	DOY1 / AE.SQUARROSA(1016)	2+12	+	-	-	+	-	+
SH – 441	DOY1 / AE.SQUARROSA(1024)	5+10	+	-	+	-	+	-
SH – 444	DOY1 / AE.SQUARROSA(1027)	2+12	+	-	-	+	-	+
SH – 447	DOY1 / AE.SQUARROSA(1018)	2.1+12	+	+	-	+	-	-
SH – 500	DOY1 / AE.SQUARROSA(1026)	1.5+10	+	-	+	-	-	-
SH – 505	DOY1 / AE.SQUARROSA(1029)	3+12.2	+	-	-	-	-	-
SH – 509	DOY1 / AE.SQUARROSA(172)	3+12.2	+	-	-	-	-	-
SH – 618	DOY1 / AE.SQUARROSA(295)	1.5+12.2	+	-	-	-	-	-
SH – 638	DOY1 / AE.SQUARROSA(359)	2+12	+	-	-	+	-	+
SH – 648	DOY1 / AE.SQUARROSA(360)	2+12	+	-	-	+	-	+
SH – 649	DOY1 / AE.SQUARROSA(518)	1.5+12	+	-	-	+	-	-
SH – 672	DOY1 / AE.SQUARROSA(534)	2+12	+	-	-	+	-	+
SH – 675	DOY1 / AE.SQUARROSA(1030)	1.5+12	+	-	-	+	-	-
SH – 677	DOY1 / AE.SQUARROSA(540)	2+12	+	-	-	+	-	+
SH – 678	DOY1 / AE.SQUARROSA(1010)	2+12	+	+	-	+	-	-
SH – 852	DOY1 / AE.SQUARROSA (632)	5+10	+	-	+	-	+	-
SH – 907	DOY1 / AE.SQUARROSA (1067)	5+10	+	-	+	-	+	-

However, we observed some ambiguous mobility of x-type subunits. In SH-302, x-type subunit had slightly faster mobility than Dx3 and slower than Dx5 and we finally designated it as Dx3. Similarly, in SH-401 and SH-678, the intermediate mobility of x-type subunit between Dx2 and Dx2.1 was ambiguous.

Molecular validation of subunits: Several allele-specific markers for HMW-GS genes have been developed from the nucleotide sequence of cloned genes (Liu *et al.*, 2012). In this study, we used four allele-specific markers for important D-genome and A-genome encoded subunits, as an additional validation. Allele-specific PCR marker, UMN19, which can differentiate genotypes having *AxNull* allele from others by producing the amplicon of 362bp (Liu *et al*., 2008) was used. In durum cv. Decoy and its all derived synthetic hexaploids, this marker generated the amplicon of 362bp (Figs. 2a, 2b), while the other amplicon of 344bp was found in cv. Bobwhite which was used as standard and had Ax2* subunit. Similarly, the amplicon of 564bp was observed in 10 SHs which indicated the presence of *Dx2.1*subunit in these genotypes. All these genotypes were also identified to have *Dx2.1*subunit by SDS-PAGE. The other most important validation was the confirmation *Dx5+Dy10* and *Dx2+Dy12* subunits by two primer pairs UMN25 and

UMN26. The results achieved fromUMN26 confirmed the presence of *Dy10* and *Dy12* in 35 genotypes. Out of these genotypes 16 gave positive results for *Glu-Dx10*by producing fragment size of 397bp and remaining 18 genotypes gave positive results for *Glu-Dx12*by producing 415bp amplicon. The PCR amplification products of this primer are presented in Fig. 2c. The markers for *Glu-Dx2.1* gene may have sequence homology with other allelic variants at this *Glu-D^{t}1* locus therefore it produced an amplicon in most of genotypes found negative for *Dx2.1* in SDS-PAGE. However, it produced the amplicon of 546bp in all SHs known to have *Dx2.1* detected by SDS-PAGE.

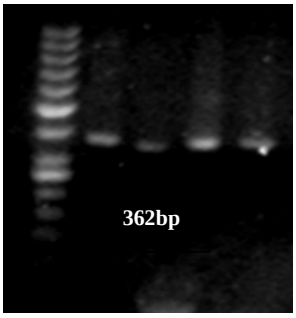

Fig. 2a. Allele-specific PCR product of *Glu-A1c (null)* allele with UMN-19 marker having fragment size of 362bp.Lane (From left): 1:50 bp ladder,2:Decoy, 3: BobWhite (1x2*) 4: SH852, 5: SH618

Discussion

Baking industry exigencies and wide consumer's preferences have driven wheat breeders to improve the bread making quality. After exploring the genetic variability and ascertaining the importance of *Glu-D1* encoded protein in bread wheat through breeding (Lagudah *et al*., 1987; William *et al*., 1993) it becomes important to characterize germplasm for HMW prior to their utilization. In the present study, allelic variation was observed at *Glu-D^{t}1* locus in SHs having uniform durum background and superior alleles were selected from the recorded combinations. As the A- and B- genome contributing parents was same, therefore same alleles i.e., *Glu-A1c* (Null) at *Glu-A1* and *Glu-B1d* (6+8) at *Glu-B1* were detected in all selected genotypes. The major occurrence of null allele was reported by various scientists (Peña *et al*., 1995; William *et al*., 1993). However, its frequency was minimal in European spelt wheat genotypes as recorded by An *et al*., (2005). Recently, Li *et al*., (2009) reported higher frequency of this allele in Chinese genotypes. The subunit *Bx6+By8* along with superior subunits from *Glu-A1* and *Glu-D1* impart overall positive effects and is very common in SHs and durum wheats yet their frequency is very low in bread wheat. The quality effects of 1Bx6+1By8 on 21 quality and noodles test parameters were reported by Tang *et al*., (2010).

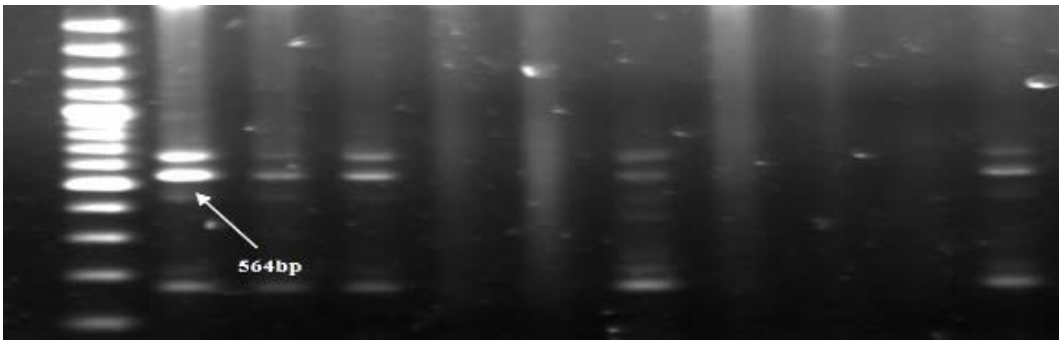

Fig. 2b. Allele-specific PCR product of *Glu-Dx2.1* allele with SNP based marker having fragment size of 564bp
Lane (From left): 1:100bp ladder, 2:SH-6, 3: SH-43, 4:SH-117, 5: SH-302-, 6: SH-323, 7: SH-447, 8: SH-672, 9: SH-675, 10: SH-677, 11: SH-678.

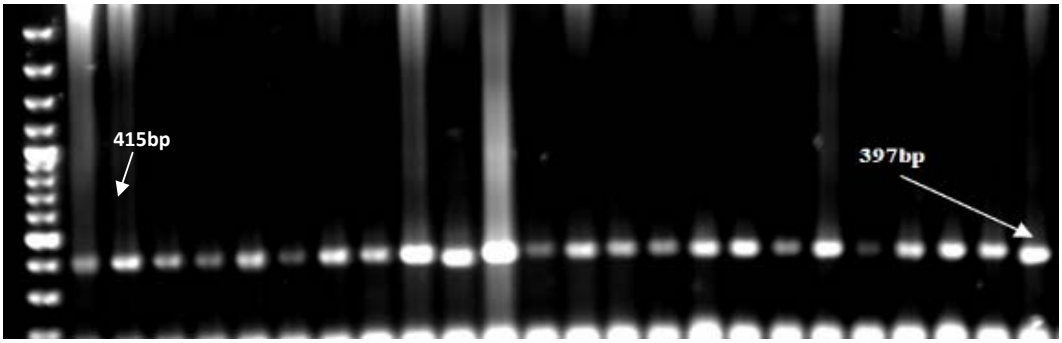

Fig. 2c. Electrophoresis of the PCR products amplified from Decoy-*Ae. tauschii* synthetic hexaploid wheats on agarose gel using UMN-26 marker for *Glu-Dx10* (397bp) and *Glu-Dx12* (415bp).
Lane (from left): 1: Marker 100bp, 2: SH-43, 3: SH-117, 4:SH-373,5: SH-377,6: CS, 7: SH-396. 8: SH-398, 9:SH-424, 10: Elite 1-57, 11: SH-131, 12: Dirk, 13:SH-434,14:SH-444,15: SH-447, 16: SH-638, 17:SH-648, 18: SH-649, 19:SH-672, 20:SH-675, 21: Elite 1-16,22:SH-677, 23 SH-678, 24:C591,25:SH-349,

Table 2. Frequency of the individual HMW-GSallele at *Glu-D^{t}1* locus found in Decoy-*Ae. tauschii* synthetic hexaploid wheat.

Subunits	Alleles	No. of genotypes	Accessions	Frequency (%)
2+12	*Glu-D1a*	10	SH-373, SH-377, SH-424, SH- 434, SH-444, SH- 638, SH-648, SH-672, SH-677, SH-678	21.27
5+10	*Glu-D1d*	10	SH-131, SH-349, SH-381, SH-385, SH-395, SH- 422, SH-426, SH-441, SH-852, SH-907	21.27
2.1+10.5	*Glu-D1ai*	3	SH-6, SH-380, SH-409,	6.38
2.1+12	*Glu-D1-1g+Glu-D1-2a*	5	SH-43, SH-117, SH-396, SH-398, SH-447,	10.63
1.5+10.5	*Glu-D1-1l+Glu-D1-2p*	4	SH-115, SH-123, SH-341, SH-403,	8.51
1.5+10	*Glu-D1ah*	3	SH-128, SH-129, SH-500	6.38
3+10	*Glu-D1z+Glu-D1-ac*	2	SH-302, SH-361	4.25
2+12.2	*Glu-D1x*	2	SH-326, SH- 330	4.25
2+10	*Glu-D1e*	1	SH-363	2.12
2.1+12.2	*Glu-D1x+Glu-D1-ae*	1	SH-401	2.12
3+12.2	*Glu-D1y*	3	SH-323, SH-505, SH-509	6.38
1.5+12.2	*Glu-D1-ag*	1	SH-618	2.12
1.5+12	*Glu-D1-aj*	2	SH-649, SH-675	4.25

The *Glu-D1d* allele encoding the subunit *1Dx5+1Dy10* was most frequent (21.27%) among all the studied subunits at this locus. This subunit was reported as superior than all other alleles at *Glu-1* loci (Li *et al*., 2009). Luo *et al*., (2001) revealed that this subunit is associated with large sedimentation volume, longer pelshenke time and greater whole meal flour protein. The varieties characterized by subunit 1Dx5+1Dy10 possess superior quality thus imparting greater visco-elastic and dough characteristics and are considered good for bread making (Redaelli *et al*., 1997). Various researchers concluded that this subunit associated with good quality of bread, making in commercial wheat cultivars grown in Canada (Bushuk, 1998), Germany (Wieser & Zimmermann, 2000), UK (Payne *et al*., 1987), Norway (Uhlen, 1990), Syria (Mir Ali *et al*., 1999), United States (Dong *et al*., 1991), New Zealand (Luo *et al*., 2001) and in SH wheats (Peña *et al*., 1995).

The inferior subunit 1Dx2+1Dy12 encoded by *Glu-D1a* allele was also found in 10 (21.27%) genotypes. This allele has mainly negative relationship with quality of protein due to weak gluten and low value of sedimentation (Ulhen, 1990). This allele can be replaced by any other superior allele at this locus using D- genome SH from *Ae. tauschii*. The *Glu-D1ah* (1Dx1.5+1Dy10) allele has positive quality attribute and has the potential to improve end use quality as reported by Peña *et al*., (1995) & Tang *et al*., (2008). The *Glu-D1z* (Dx3+Dy10) contributes significantly to extensible gluten and larger bread loaf volume than Dx2+Dy10 (Peña *et al*., 1995).The allele *Glu-D1ag* encoding subunit 1Dx1.5+1Dy12.2 impart stickiness to dough and low bread volume (Hsam *et al*., 2001). High level of genetic variability at *Glu-D^{t}1* locus is invaluable genetic reserve to improve bread making quality because the allelic variation of HMW-GS strongly influence the variability in bread making quality (William *et al*., 1993; Pfluger *et al*., 2001; Bibi *et al.,* 2012; Rasheed *et al.,* 2012). Recent findings of Xu *et al.,* (2010) include a wide array of D-genome encoded subunit in synthesized wheat germplasm with same durum background.

The PCR based markers validation of HMW-GS was found consistent with SDS-PAGE results and identified as a reliable technique for high throughput screening of important *Glu-D1* subunits. However, there is need to develop more accurate markers for Glu-D^{t}1 subunits which can distinguish the allelic variation in the breeding material assembled from the wild ancestors, like SHs. The SHs genotypes reported here will be useful materials for testing the quality effects of the HMW-GSs from *Ae. tauschii*. Because all the SHs genotypes are in the same durum background, their quality differences are likely contributed by the *Ae. tauschii* accessions. Most of these lines can be tested directly; however, some lines have poor agronomic characteristics, such as hybrid chlorosis, grassy dwarfness, or very poor yield. Studies of the quality differences attributed by the new subunits (Dx1.5, Dx2.1, Dy10.5 and Dy12.2) relative to those found in bread wheat (Dx2, Dx5, Dy10 and Dy12) will provide new insights into the contribution of protein primary structure on wheat quality.

Conclusion

In conclusion, SHs are very important for enhancing the quality of bread due to presence of a large number of allelic variants at *Glu-D^{t}1* locus.SHscan contribute to new variability towards bread-making quality attributes and their exploitation in breeding programmes can become a priority choice for the breeder emphasizing on wheat breeding for high grain quality without compromising genetic variability.

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