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COMPARATIVE ASSESSMENT OF GLUTENIN COMPOSITION AND ITS RELATIONSHIP WITH GRAIN QUALITY TRAITS IN BREAD WHEAT AND SYNTHETIC DERIVATIVES

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Abstract

Baking industry exigencies and wider consumer preferences are highly demanding variable end product use of bread wheat. Diversity in bread wheat to fulfill these demands is primarily dependent on the D-genome encoded glutenins complemented by desirable glutenins from the A- and B- genome. The present study was designed to evaluate and compare glutenin compositions and their effect on key quality parameters in D-genome synthetic hexaploid derivatives (SDW) and conventional bread wheat (CBW) germplasm. The germplasm set selected encompasses the earlier investigated drought tolerant characteristics. Grain quality analyses have provided stringent selection sieve to select the drought tolerant genotypes with desirable end quality characteristics. Several unique D-genome encoded HMW-GS were found along with favorable alleles at A- and B-genomes. D-genome encoded subunit Dx5+Dy10 which is known to encode superior grain quality attributes was observed in 63.64% genotypes followed by 1Dx2+1Dy12 (30.91%). Apart from HMW-GS, PCR based allele specific markers were used to identify allelic variation at *Glu-3* loci (LMW-GS), which had a significant effect on visco-elastic properties of wheat dough. Several combinations of favorable LMW-GS alleles were observed at *Glu-A3* and *Glu-B3* loci. Key quality parameters like protein, sedimentation volume and carotenoids differed significantly within genotypes. Higher values for desirable quality traits were found in synthetic derived genotypes as well as in conventional bread wheat varieties. Our results established significant variability in quality characteristics and glutenin composition among D-genome synthetic-hexaploid wheat derivatives as compared to conventional bread wheat germplasm suggestive of their ability to improve quality traits in bread wheat.

Introduction

Common wheat (*Triticum aestivum* L.) being one of the most important food crops worldwide, needs extensive research with major emphasis on yield improvement as well as its adaptation to various biotic and abiotic stresses. It is believed that only a few accessions of the donor species were involved in the evolution of common wheat. Consequently, genetic diversity was introduced into common wheat by the 'bridge' of synthetic hexaploid (SH) wheat derived from artificial synthesis of hexaploid wheat (*T. Turgidum* × *Ae. tauschii*) in a manner analogous to the evolution of hexaploid wheat (Mujeeb-Kazi *et al.*, 1996).

Various synthetic hexaploid wheats (*T. turgidum* ssp. durum/*Ae.tauschii*) have resulted in significantly superior combinations for biotic/abiotic resistance/tolerances (Mujeeb-Kazi, 2003). But at the same time, baking industry exigencies and wide consumer preferences have driven wheat breeders to incorporate the grain quality related traits as an important preference in current research (Bushuk, 1998). Hence the development of wheat cultivars with good bread making quality is a challenging task in many wheat breeding programmes. Accordingly, grain yield together with grain protein contents by its end use quality contribution are the primary and foremost important characters in the determination of the economic value of a bread wheat crop (Oury & Godin, 2007; Ali *et al*., 2011), particularly if adaptive to a stress environment. Wheat end use quality is influenced by several important traits and the production of specific end-use products can be attributed to high variability among these traits. The quality and quantity of wheat gluten proteins give elasticity and extensibility necessary for bread making and are the key endosperm components that are mainly emphasized regarding end use quality traits (Payne *et al.*, 1987; Kerfal *et al.*, 2010). The gluten contributes about 80-85% of the total flour protein (Shewry *et al*., 1995) and it is comprised of two prolamine groups, gliadins and glutenin. The glutenins, in turn, are long chains of polypeptides linked by disulfide bonds, and comprising further of low molecular weight glutenin subunits (LMW-GS) and high molecular weight subunits (HMW-GS) (Payne & Lawrence, 1983). The HMW-GSs designated as *Glu-A1, Glu-B1 and Glu-D1* are encoded by multi-allelic genes located on the long arms of chromosomes 1A, 1B, and 1D respectively (Payne *et al*., 1984). It has been reported previously that the HMW-GSs of glutenin constitute about 10% of the wheat endosperm storage proteins in comparison to 40 % LMW-GSs but still have a major influence on the bread making properties of flour (Payne *et al*., 1987). Allelic variation in HMW-GS composition was found strongly correlated with differences in bread making quality (Shewry *et al*., 1995). Additionally they also have proven as important genetic markers for exploring genetic diversity in wheat. Therefore, analysis of LMW-GS and HMW-GS is a pre-requisite and an important criterion in breeding for bread making quality improvement.

It has generally been recognized that there is a need to further improve the ability to capture and manipulate diversity (Able *et al.*, 2007). Plant breeders involved in crop improvement efforts in order to meet the ever increasing demand for food are finding appropriate germplasm with desired traits among cultivated crops and in wild, uncultivated plants. High grain yield in wheat

relies upon high yielding varieties possessing resistance and tolerance to various biotic and abiotic stress problems. Recently, Tang *et al.*, (2008, 2010) suggested that grain quality improvement is also possible through the utilization of SHs in breeding programmes.

Utilization of wheat is highly dependent on its end-use quality which in turn relies on traits including protein content, carotenoid contents and SDS-sedimentation volume. The main objective of the present study was to investigate a collection of drought tolerant wheat genotypes comprising of D-genome SH derivatives and conventional bread wheat germplasm, for key grain quality parameters and their genetic composition based on the HMW-GSs and LMW-GSs profiles.

Materials and Methods

Experimental material: The experimental germplasm consisted of a core collection of 50 (designated AA1 toAA50) drought tolerant wheat germplasm comprising 3 groups: i) D-genome synthetic hexaploid derivatives, ii) conventional bread wheat lines and, iii) five elite check cultivars of wheat (Table 1). The germplasm for the study was obtained from wheat wide crosses (WWC) and cytogenetics laboratory, NARC, Islamabad. Its pedigree is given in Ali *et al*., (in preparation), in which the performance of the same germplasm evaluated under drought stress has been reported.

Table 1. Groups and the entry no. of the germplasm used in the study.

S. No.	Group	Entry No.
1.	Synthetic derived bread wheat (SBW)	AA5, AA12, AA13, AA14, AA16, AA17, AA18, AA19, AA20, AA24, AA26, AA27, AA28, AA29, AA31, AA32, AA33 , AA34, AA36, AA39, AA41, AA44, AA45, AA46, AA47, AA48,
2.	Conventional bread wheat (CBW)	AA1, AA2, AA3, AA4, AA6, AA7, AA8, AA9, AA10, AA11, AA15, AA21, AA22, AA23, AA25, AA30, AA35, AA37, AA38, AA40, AA42, AA43, AA49, AA50,
3.	Check cultivars	AA51 (Inqilab 91), AA52 (Seher 2006), AA53 (Chakwal 50), AA54 (NR 372), AA55(Wafaq 2001)

Analysis of High molecular weight glutenin subunits (HMW-GS): Single kernel was crushed by mortar & pestle and 10 mg flour sample was taken in eppendorff tubes. Protein extraction and SDS-PAGE analysis was followed as described in Rasheed *et al*., (2012). Alleles at *Glu-A1* and Glu-*B1* loci were designated according to Payne & Lawrence, (1983) whereas alleles at *Glu-D1* locus were identified according to William *et al*., (1993).

Analysis of Low molecular weight glutenin subunits (LMW-GS)

DNA extraction and PCR amplification: Genomic DNA extraction was done according to phenol-chloroform method as described in Pallotta *et al*., (2000) with some modifications. First 5 to 7 cm long pieces of fresh leaf material were harvested from 2 week-old seedlings, subsequently freeze it in the liquid Nitrogen and then crushed to a fine powder with a knitting needle while still inside the tube. Five hundred µl DNA extraction buffer (1% SDS, 100mM NaCl, 100mM Tris base, 100mM Na_2EDTA, PH: 8.5 by HCl) were added to each eppendorf tube containing the crushed leaf material and mixed well with the help of a knitting needle after which 500 µl phenol: chloroform: isoamylalcohol (in the ratio of 25:24:1) was added to it. Samples were then centrifuged at 5000 rpm for 5 minutes. The aqueous phase (supernatant) was transferred to a fresh tube. To precipitate the DNA 50 µl 3M sodium acetate (pH= 4.8) and 500µl chilled isopropanol was added to the tube and mixed gently. To make the DNA pellet, samples were centrifuged at 5000 rpm for 5 minutes. After pouring supernatant, the pellet was washed with 70% ethyl alcohol and dried at room temperature for an hour, and was resuspended in 40 µl TE buffer (10mM Tris, 1mM EDTA and PH: 8.0). To remove RNA, DNA was treated with 40µg RNAase-A (20 µl of commercially supplied RNAase-A purchased from Gene Link, USA) at 37^{o}C for 1 hour. After RNAase treatment, DNA samples run on 1.0% agarose gel to check the quality of DNA and then were stored at 4^{o}C. To use in Polymerase Chain Reaction (PCR), all DNA samples were quantified spectrophotometrically, and then diluted to a concentration (20ng/µl) with doubled distilled, deionized and autoclaved water. PCR reactions were performed in a total volume of 10 µl containing 50-100 ng of genomic DNA, 1x PCR buffer, 1.0-1.5 mM of $MgCl_2$, 200 mM of each deoxyribonucleotide (dNTP), 5pmol 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 of the *Glu-A3* and *Glu-B3* loci was carried out using the primer sets reported by Wang *et al*., (2010) & Wang *et al.*, (2009), respectively. These analyses are more robust and now considered superior over the earlier conventional technique of SDS-PAGE for identifying LMW-GS. Amplification conditions were similar to those reported by the authors mentioned above. 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 the identification of amplified products.

Quality tests: The analysis were carried out in quality lab of CRA-Cereal Research Centre, Foggia, Italy. Wheat grains were milled to flour by Perten Laboratory Mill 3100 installed with 0.8 mm sieve in order to carry out further quality tests. The protein content, carotenoid content and sodium dodecyl sulfate (SDS) sedimentation test were determined by a near-infrared spectrophotometer (FOSS Rapid ContentTM Analyzer) already calibrated according to AACC Method 46-19.01, 56-70.01 and 14-50.01, respectively. Protein content was expressed on a 14% moisture basis. The flour NIR spectra for each sample were taken in five scans and were than averaged.

Results and Discussions

Glutenin composition: The frequency of HMW-GS and LMW-GS alleles identified in germplasm are presented in Table 2. HMW-GSs are key determinants of bread-making qualities of wheat and studied genotypes showed a variable number of alleles. HMW-GS controlled by *Glu-1* loci encoded 11 different alleles across three genomes in these genotypes. At *Glu-A1* locus, three alleles were observed, of which *Axnull* allele was predominantly found in 46 (83.64%) genotypes. At *Glu-B1* locus, the subunit 17+18 encoded by *Glu-B1b* was found in maximum (54.55%) genotypes. Similarly, G*lu-D1d* which encodes Dx5+Dy10 subunit was observed in (63.64%) genotypes which is an important good quality subunit encoding high molecular weight glutenin allele. Maximum allelic diversity was found at *Glu-B1* (0.61) locus followed by *Glu-D1* (0.50) and *Glu-A1* (0.29). This is primarily due to the allelic richness (5 alleles) observed at *Glu-B1* locus, however the other two loci (*Glu-A1* and *Glu-D1*) had same allelic richness (3 alleles on both loci) but the distribution of allele frequencies at *Glu-D1* locus contributed towards more diversity than that of alleles at *Glu-A1* locus. The HMW-GS observed are presented as Fig. 1.

Table 2. Allelic variation at *Glu-1* (HMW-GS) and *Glu-3* (LMW-GS) loci in wheat germplasm under study.

Locus	Allele	Subunit	Number of accessions	Frequency (%)	H (Nei's Index)
Glu-A1	a	1	3	5.45	
	b	2*	6	10.91	
	c	Null	46	83.64	0.29
Glu-B1	a	7	2	3.64	
	b	7 + 8	30	54.55	
	d	6 + 8	3	5.45	
	i	17 + 18	16	29.09	
	f	13 + 16	4	7.27	0.61
Glu-D1	a	2 + 12	17	30.91	
	d	5 + 10	35	63.64	
	z	3 + 10	3	5.45	0.50
Glu-A3	a		2	3.64	
	b		15	27.27	
	c		25	45.45	
	d		3	5.45	
	f		7	12.73	
	g		3	5.45	0.70
Glu-B3	b		2	3.64	
	d		6	10.91	
	e		5	9.09	
	f		7	12.73	
	g		4	7.27	
	h		12	21.82	
	i		8	14.55	
	j		8	14.55	0.87

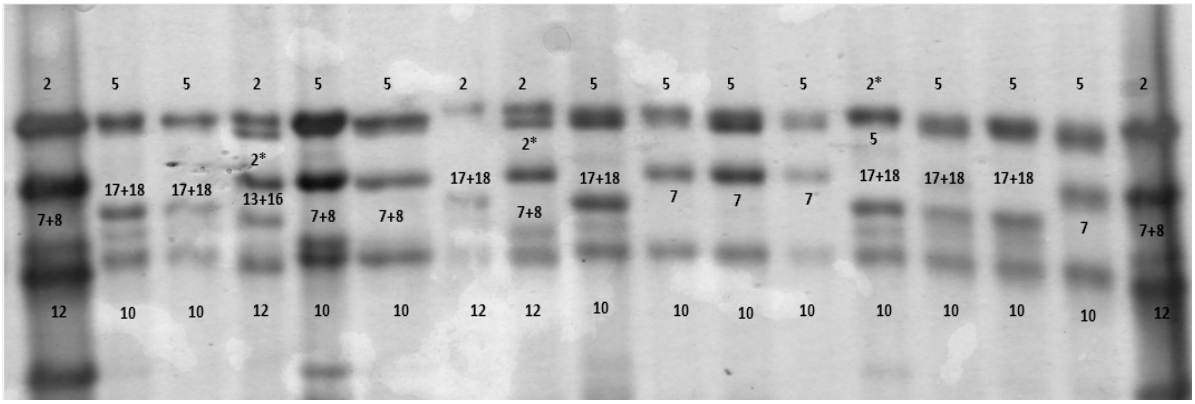

Fig. 1. HMW glutenin subunit profile of germplasm
Lane (From left): 1: CS (Check), 2: AA11, 3: AA15, 4: AA37, 5: AA17, 6: AA18, 7: AA27, 8: AA31, 9: AA38, 10: AA22, 11: AA213, 12: C291 (Check), 13: Pavon (Check), 14: AA41, 15: AA45, 16: C591 (Check), 17: CS (Check).

In Table 2 are reported the allele frequency and the genetic diversity of alleles at each LMW-GS locus. Allelic variations at the *Glu-3* loci encoding LMW-GS have a pronounced effect on dough visco-elastic properties (Gupta & MacRitchie, 1994; Maucher *et al*., 2009). Given the discrepancy reported in some studies about the correct identification of the alleles coded at LMW-GSs loci using SDS-PAGE (Ikeda et al., 2008), in this study, alleles were identified using an analysis-based on PCR. In effect, the recent development of allele-specific markers for *Glu-A3* (Wang *et al*., 2010) and *Glu-B3* (Wang *et al*., 2009) alleles have profoundly increased the efficiency, accuracy and reduced the cost for allelic characterization in bread wheat germplasm (Liu *et al*., 2010). The PCR profiles of LMW-GS alleles are presented as Fig. 2. Unfortunately, no functional markers for the *Glu-D3* locus were developed due to the very small variations among alleles (Liu *et al*., 2010), but its impact on dough quality is relatively small in comparison with the *Glu-A3* and *Glu-B3* loci (Gupta *et al*., 1989). Six different alleles at *Glu-A3* locus and eight alleles at *Glu-B3* locus were identified by allele specific markers (Table 2). At *Glu-A3*, the allele *Glu-A3c* was present in majority of the genotypes (45.45%), while *Glu-A3a* was present only in two genotypes (AA39 and AA41). The predominant frequency of *Glu-A3c* has been observed in Indian cultivars (Ram *et al*., 2011) and other studies also showed its presence in diverse wheat genotypes representing different regions (Liu *et al*., 2010; Wang *et al.*, 2010; Zhang *et al*., 2004). The frequency of other alleles *Glu-A3b, Glu-A3d, Glu-A3f* and *Glu-A3g* was found to be 27.27%, 5.45%, 12.73% and 5.45%, respectively. The major alleles found at *Glu-B3* locus was *Glu-B3h* which appeared in 12 genotypes (21.81%) followed by *Glu-B3i* and *Glu-B3j* (14.54%) each. Maximum allelic diversity was found at *Glu-B3* (0.87) locus followed by *Glu-A3* (0.70) which is primarily due to the allelic richness (8 alleles) observed at *Glu-B3* locus. There are many reports indicating different allelic frequencies representing the *Glu-B3* locus in genotypes from different regions. Among Indian cultivars, frequency of *Glu-B3b* was highest (29.3%) followed by *Glu-B3j* (27.1%) and *Glu-B3h* (13.8%). Similarly, Branlard *et al*., (2003) reported *Glu-B3b* in 10.0% of cultivars in France, *Glu-B3g* in 49.0% and Glu-B3d in 3.5%. Other reports have also indicated the presence of *Glu-B3b* and *Glu-B3g* alleles in large numbers of cultivars (Igrejas *et al*., 2010; Jackson *et al*., 1996; Wang *et al*., 2009). *Glu-B3g* has been shown to have a positive effect on gluten strength (Liang *et al*., 2010; Maucher *et al*., 2009; Oury *et al*., 2010).

Results from previous studies on the relationship between glutenin subunits and end-use quality have confirmed that HMW-GS are highly correlated with bread baking quality (Payne *et al*., 1987; Gupta *et al*., 1989; Carrillo *et al*., 1990; Sontag-Strohm *et al*., 1996). LMW-GS and gliadins were also found to influence the bread-making quality (Payne *et al*., 1987) but with inconsistent results. This is mostly due to the use of different genetic materials with various genetic backgrounds in different studies. For example, when measuring the effect of the subunits 2* on SDS-sedimentation volume, a value of =1 was observed from using the British-grown wheat varieties (Payne *et al*., 1987), whereas a value of >1 was found by Mao *et al*., (1995) using different wheat varieties, and a value of <1 was obtained by Liu *et al*., (2005) using 251cultivars and advanced lines. Therefore genotype selection based on HMW-GS and LMW-GS for bread-making quality traits is reliable and is considered as a crucial analysis of the germplasm (Xiyong *et al*., 2012).

Quality parameters: The key quality parameters studied in this germplasm include protein contents (%), SDS-sedimentation volume and carotenoids and their values in individual genotypes are depicted in Table 3. Protein contents ranged from 11.2% to 19.9% with an average of 13.6%. The highest protein content was observed in AA53 (Chakwal-50) which is a rainfed cultivar released in Pakistan and in this study was used as control for comparing the other experimental germplasm. The lowest protein content was found in AA55 which is also cultivated in Pakistan. Eighteen genotypes (32.7%) were found to have more than 14% protein content which is significantly a promising result. In the same way for SDS (AACC standard methods 2001), when the value is greater than 4ml wheat flour is considered to be of good quality, while with value between 3ml and 3.5 ml is of medium quality. The SDS sedimentation volume in the studied germplasm ranged from 2.4ml to 5ml with an average 3.4ml. Chakwal-50 which is known to have good glutenin composition and protein contents had SDS-sedimenation value of 5 ml. At the same time, other lines including AA20 and AA48 which are synthetic derivatives exhibited SDS-sedimenation volume of 4.3 ml and 4.1 ml respectively. Carotenoids ranged from 4.4 ppm to 9.9 ppm with an average of 6.5 ppm. Among these quality traits, proteins content was most consistent with 9.37% CV, followed by SDS-sedimentation (CV%, 11.89) and carotenoids (CV%, 18.04). This indicated the maximum variability for carotenoids was found among these genotypes. Results with NIR spectroscopy for grain quality traits of wheat was found promising and economical as well. Wheat flour SDS sedimentation volume together with gluten strength is correlated with dough rheology (Mondal *et al*., 2009). Since, wheat is focused mainly from end-use quality point of view a better understanding of the genetics underlying specific quality parameters is essential to enhance selection during the breeding process (Carter *et al.*, 2012). The results help us improve understanding of the relationships among glutenin compositions and grain quality traits (Tabasum *et al*., 2011).

Comparative assessment of both germplasm sets: Quality traits and diversity for glutenin alleles were compared between conventional bread wheat germplasm and D-genome synthetic hexaploid derivatives (Table 4). In synthetic derivatives, more diversity for *Glu-A1* (0.38) and *Glu-D1* (0.59) was observed while no comparison was found for *Glu-B1* and *Glu-B3* loci. Similarly, protein contents (13.8±0.9) and SDS-sedimentation volume (3.5±0.3) were slightly higher in synthetic derivatives as compared to bread wheat cultivars. Carotenoids were slightly lower in synthetic derivatives, which may be statistically non-significant.

Table 3. HMW-GS, LMW-GS and quality characteristics of wheat genotypes under study.

Genotype	Type	HMW			LMW		Protein	Carotenoid	SDS-sedimentation
(AA)		*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	(%)	Ppm	ml
1	CBW	Null	7+8	2+12	b	J	13.3	6.2	3.1
2	CBW	Null	13+16	2+12	b	J	13.3	8.1	3.5
3	CBW	Null	7+8	5+10	b	g	12.5	6.6	3.1
4	CBW	Null	17+18	5+10	d	f	13.2	8.1	3.2
5	SBW	Null	6+8	2+12	d	i	13.4	6.5	3.3
6	CBW	Null	7+8	5+10	c	h	13.0	8.3	3.3
7	CBW	Null	7+8	5+10	f	h	13.9	7.4	3.5
8	CBW	Null	7+8	5+10	c	h	12.3	4.5	3.0
9	CBW	Null	17+18	5+10	c	i	12.9	5.9	2.4
10	CBW	Null	7+8	2+12	f	J	12.0	7.2	2.9
11	CBW	Null	17+18	5+10	g	d	12.7	8.4	2.7
12	SBW	Null	13+16	3+10	d	i	13.5	9.9	3.6
13	SBW	Null	7+8	3+10	b	f	12.0	5.2	3.5
14	SBW	Null	7+8	5+10	g	J	12.5	5.5	3.1
15	CBW	Null	17+18	5+10	b	d	12.4	8.0	3.2
16	SBW	Null	7+8	2+12	c	i	13.7	5.5	3.9
17	SBW	Null	7+8	5+10	f	c	13.3	7.2	3.7
18	SBW	Null	7+8	5+10	c	h	15.0	8.3	3.4
19	SBW	Null	7+8	5+10	c	h	13.4	5.2	3.2
20	SBW	Null	7+8	5+10	c	f	14.5	6.1	4.3
21	CBW	Null	7+8	5+10	b	J	14.2	8.7	3.3
22	CBW	Null	7	5+10	a	d	13.6	8.5	3.4
23	CBW	Null	7	5+10	c	f	13.4	7.7	4.1
24	SBW	Null	7+8	2+12	b	i	13.7	7.0	3.2
25	CBW	Null	7+8	5+10	a	d	13.5	7.0	3.6
26	SBW	Null	6+8	2+12	c	f	14.9	5.7	3.6
27	SBW	Null	17+18	2+12	f	e	14.3	6.9	3.7
28	SBW	2*	17+18	5+10	c	f	15.3	8.2	3.6
29	SBW	Null	7+8	5+10	c	f	15.3	5.3	3.6
30	CBW	Null	17+18	2+12	f	J	15.3	5.9	3.4
31	SBW	2*	7+8	2+12	c	h	13.6	6.9	3.4
32	SBW	1	7+8	2+12	b	e	14.4	5.8	3.5
33	SBW	1	6+8	2+12	b	h	13.9	6.2	3.3
34	SBW	Null	7+8	5+10	b	h	12.7	6.2	2.9
35	CBW	Null	7+8	5+10	c	h	13.0	5.3	2.8
36	SBW	Null	7+8	5+10	b	b	12.0	6.6	3.3
37	CBW	Null	13+16	2+12	f	d	13.3	6.4	3.5
38	CBW	Null	17+18	5+10	c	e	12.9	6.5	3.8
39	SBW	2*	17+18	5+10	b	b	13.0	6.3	3.5
40	CBW	2*	17+18	2+12	b	i	12.7	5.1	3.0
41	SBW	Null	17+18	5+10	c	h	12.8	5.1	3.5
42	CBW	Null	7+8	5+10	b	d	13.5	6.0	3.0
43	CBW	Null	7+8	5+10	f	h	13.8	5.5	3.4
44	SBW	2*	17+18	5+10	c	J	14.8	7.7	3.6
45	SBW	Null	17+18	5+10	c	i	13.4	6.4	3.6
46	SBW	Null	7+8	2+12	c	e	13.8	5.9	3.4
47	SBW	Null	7+8	3+10	c	h	14.1	6.0	3.6
48	SBW	Null	13+16	2+12	b	i	14.6	6.0	4.1
49	CBW	Null	17+18	5+10	c	e	15.3	5.9	3.3
50	CBW	Null	7+8	5+10	c	j	14.1	7.0	4.1
51	CBW*	Null	17+18	2+12	c	g	14.0	6.7	3.5
52	CBW*	Null	7+8	5+10	g	g	14.7	6.0	3.2
53	CBW*	Null	7+8	5+10	c	c	19.9	5.1	5.0
54	CBW*	Null	17+18	5+10	c	c	12.2	4.4	3.0
55	CBW*	2*	7+8	5+10	c	g	11.2	5.3	3.2
Average							13.6	6.5	3.4
St. Dev							1.28	1.18	0.41
CV(%)							9.37	18.04	11.89
Max							19.9	9.9	5.0
Min							11.2	4.4	2.4

SBW, synthetic-derived bread wheat**; CBW,** conventional bread wheat and Check cultivars

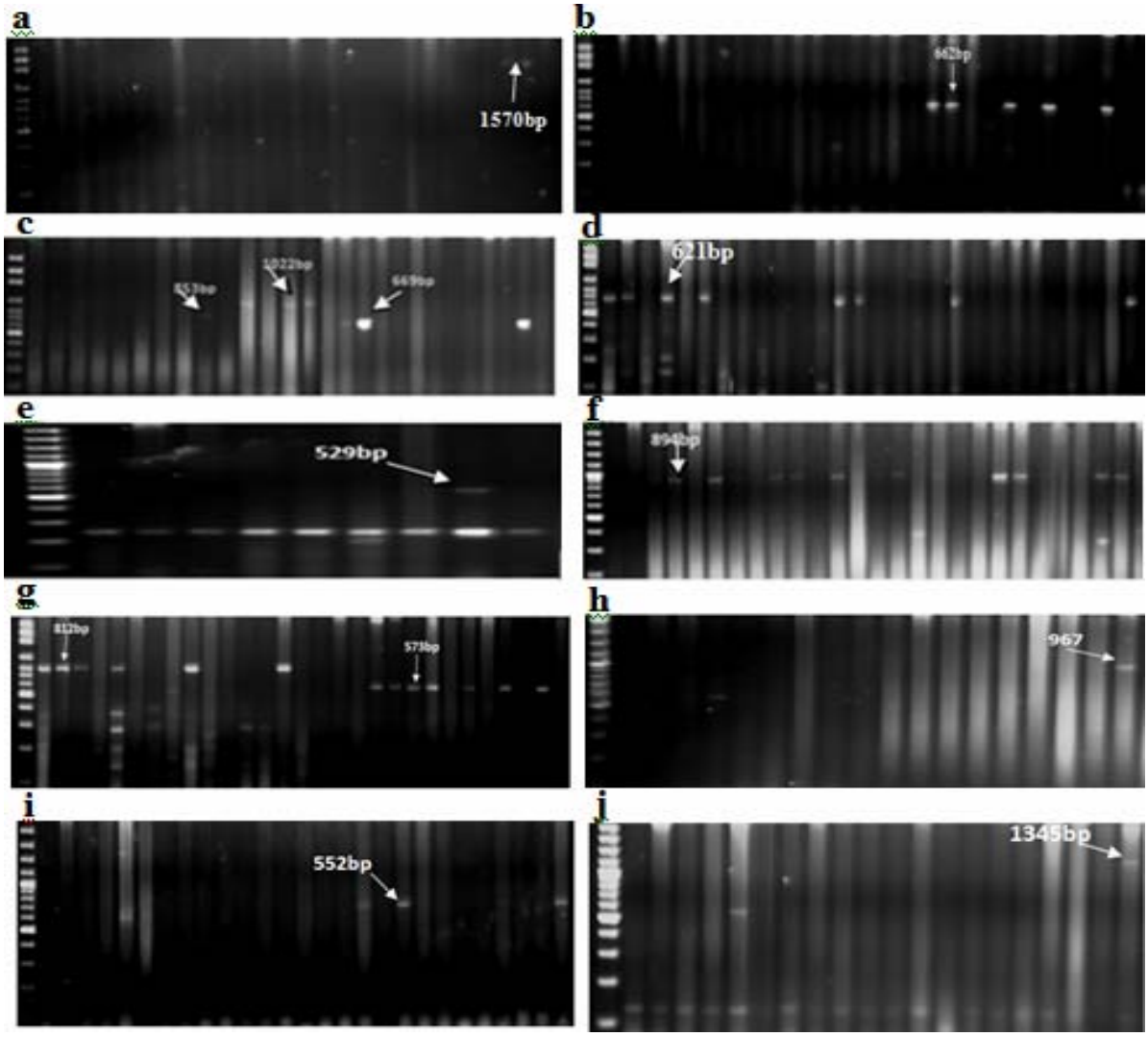

Fig. 2. Electrophoresis of some of the PCR products amplified from the studied germplasm on agarose gel using allele specific markers for: a *gluB3b*, b *gluB3d*, c *gluB3g*, *gluB3h*, *gluB3e* , d *gluB3i*, e *gluA3a*, f *gluA3b*, g *gluB3f*, *gluA3c*, h *gluA3d*, i *gluA3f*, j *gluA3g*. Materials used as PCR templates were as follows: (a) *B3b*- AA36, AA39 (b) *B3d*-Aa11, AA15, AA22, AA25, AA37, (c) *B3g-AA29*, *B3h*-AA18, AA19, AA31, *B3e-AA27, AA32, AA46,* (d) *B3i*- AA5, AA9, AA12, AA16, AA24, AA40, AA45, AA48, (e) *A3a*-AA22 (f) *A3b*-AA1, AA3, AA13, AA15, AA21, AA32, AA33, AA34, AA36, AA39, AA40, AA42, AA48, (g) *B3f*-AA4, AA13, AA20, AA26, AA28, AA29; *A3c*-AA16,.AA18, AA19, AA20, AA26, AA28, AA29, AA31, (h) *A3d*-AA30, (i) *A3f*-AA7, AA10, AA27,(j) *A3g*-AA11.

Table 4. Comparison of quality traits and glutenin diversity between D-genome synthetic derivatives and conventional bread wheat.

Traits	SBWs (n=26)	CBW (n=29)
Protein (%)	13.8 ± 0.9	13.5 ± 1.5
SDS-Sedimentation	3.5 ± 0.3	3.3 ± 0.4
Carotenoids	6.5 ± 1.1	6.6 ± 1.25
Diversity (*H*) at:		
Glu-A1	0.38	0.19
Glu-B1	0.59	0.6
Glu-D1	0.59	0.36
Glu-A3	0.64	0.64
Glu-B3	0.82	0.84

D-genome synthetic hexaploids originated from *Ae. Tauschii.* The potentiality of this diploid grass for improving quality was unclear (Lagudah *et al*., 1987) and its introgression for quality improvement not preferred. Later, Yueming *et al.,* (2003) proposed that introgressed novel genes from the D-genome may improve wheat quality. After this proposal, Gedye *et al*., (2004) reported novel haplotypes for grain hardiness genes (puroindolines) in D-genome synthetic hexaploids with potential impact on kernel texture in wheat. They suggested that the new puroindoline alleles from *Ae. tauschii* all gave soft endosperm, although textural differences were apparent and attributable to interactions between the durum and *Ae. tauschii* genomes. But contrastingly, Lillemo *et al*., (2006) found significant but small differences in grain hardness amongst the hardness genes using a larger collection of synthetics and their derivatives. However there was not much progress observed for the functional effects of the new *Ae. tauschii* derived

puroindoline alleles in wheat quality and has still to be determined. From evaluation of about 200 synthetics it appears that the *Ae. tauschii* parent has a much larger influence on the quality of the synthetic hexaploid than the durum parent (Van Ginkel & Ogbonnaya, 2007). About 20% of synthetic hexaploids could be classified as attaining the Australian hard and Australian prime hard quality classification based on examination of the polymeric glutenins proteins (Van Ginkel & Ogbonnaya, 2007). This study reports the glutenin diversity and good end-use quality profile of synthetic derivatives which is the practical example for improving quality traits of bread wheat using synthetic hexaploids in breeding programs. This is consistent with the results of Lage *et al*., (2006), who reported significant genetic variation among synthetic hexaploids for protein content and quality, grain weight and plumpness. However the quality sensitive breeding program can also use synthetic hexaploids with poor bread-making quality but otherwise outstanding traits by crossing and backcrossing to high quality conventional wheats, while other synthetics with novel proteins should be studied to determine their potential value in terms of improved quality characteristics. In recent studies at CIMMYT, it has become abundantly clear that synthetic derivatives carrying excellent bread-making quality can indeed be bred if the common bread wheat parent(s) in the cross have good bread quality traits. High molecular weight and low molecular weight glutenin profiles of the primary synthetic hexaploids (Bibi *et al.,* 2012; Rasheed *et al.,* 2012) can be used to determine promising crosses, and to identify the best quality lines in their progeny. Nelson *et al*., (2006) reported that some lines from the ITMI (International Triticeae Mapping Inititative) population showed quality values consistently superior to those of the parental lines. Despite these observations, some breeders remain skeptical about using synthetics to resolve non-quality related constraints in their target production environment, because they are afraid of compromising wheat quality, and mores studies of this type are needed.

Conclusively, this germplasm set which is known to have drought tolerant characteristics possessed desirable glutenin alleles and grain quality characteristics. The diverse origin of the genotypes in this germplasm set will enhance the genetic base of breeding programs and will contribute to new alleles from D-genome synthetic hexaplioids, for both drought and grain quality.

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