

## Short Communication

# High-molecular-weight (HMW) glutenin subunit composition of the Elite-II synthetic hexaploid wheat subset (*Triticum turgidum* × *Aegilops tauschii*; $2n = 6x = 42$ ; AABBDD)

Amna Bibi<sup>1</sup>, Awais Rasheed<sup>2\*</sup>, Alvina Gul Kazi<sup>3</sup>, Tariq Mahmood<sup>2</sup>, Saifullah Ajmal<sup>1</sup>, Iftikhar Ahmed<sup>4</sup> and Abdul Mujeeb-Kazi<sup>5</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi, Pakistan, <sup>2</sup>Plant Biochemistry and Molecular Biology Lab, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan, <sup>3</sup>National University of Science and Technology (NUST), Islamabad, Pakistan, <sup>4</sup>National Agriculture Research Center (NARC), Islamabad, Pakistan and <sup>5</sup>National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Received 5 August 2011; Accepted 19 October 2011 – First published online 14 November 2011

### Abstract

Characterization of high-molecular-weight (HMW) glutenins is an important criterion for identifying genotypes with good bread-making quality. In synthetic hexaploids (SHs), the D-genome encodes several allelic variants of HMW glutenins that require proper identification prior to their utilization for bread wheat (BW) improvement. In this study, SHs with promising agronomic features were characterized for HMW glutenin composition. Seven different allelic variants were observed at the *Glu-D<sup>1</sup>* locus, three of which (1Dx1.5 + 1Dy10, 1Dx1.5 + 1Dy12.2 and 1Dx2.1 + 1Dy10) have not been previously reported in existing BW germplasm. The results also showed a variety of D-genome-encoded subunits along with superior glutenin alleles in the B-genome (1Bx7 + 1By8, 1Bx6 + 1By8 and 1Bx13 + 1By16). About 63% of these SHs encoded favourable allelic variants of HMW glutenins, which make them a good choice for improvement in wheat bread making. *Glu-D<sup>1</sup>* encoded favourable allelic variants (1Dx5 + 1Dy10 and 1Dx1.5 + 1Dy10) that are frequently observed in SHs can be easily incorporated into BW through recombination breeding.

**Keywords:** *Aegilops tauschii*; *Glu-1* loci; HMW glutenin subunits; synthetic hexaploids

### Experimental

#### *Plant material*

The Elite-II subset studied is comprised of 33 primary synthetic hexaploid wheats. These synthetic hexaploids (SHs) were derived from the combinations of 14 durum wheats

---

\*Corresponding author. E-mail: awaispbg@gmail.com

and 32 *Aegilops tauschii* accessions. Their production and the protocol have been reported earlier (Mujeeb-Kazi *et al.*, 1996). Six wheat varieties (Chinese Spring, Pavon-76, C-591, SH-231, SH-61 and SH-139) known to carry identified high-molecular-weight (HMW) glutenin subunits were used as controls. The 14 durum parents within these SHs were also characterized separately for the *Glu-A1* and *Glu-B1* subunits to confirm the respective subunits in the synthetic hexaploid wheats.

### Electrophoresis and nomenclature

Protein extraction and electrophoresis procedures were carried out according to the method of Xu *et al.* (2010). The nomenclature assigned to *Glu-A1* and *Glu-B1* was adopted from Payne and Lawrence (1983). The alleles at the *Glu-D<sup>1</sup>* locus were identified according to William

*et al.* (1993). All the allele names were obtained from MacGenes. The *Glu-D<sup>1</sup>*-encoded  $\gamma$ -type subunit, initially named T2, was replaced by 12.2 according to Gianibelli *et al.* (2001).

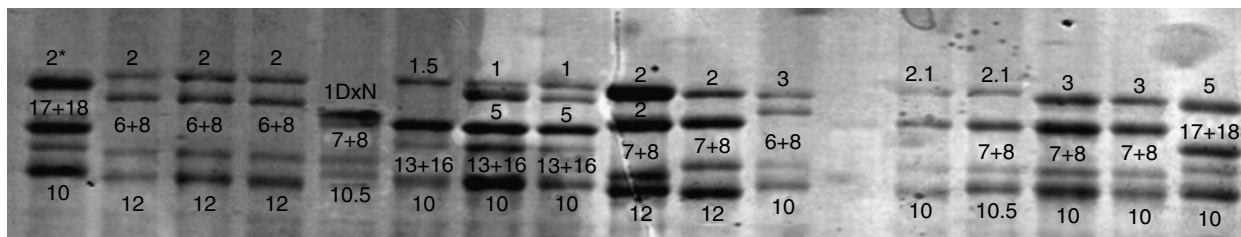
### Discussion

Although an Elite-I set has a wide global distribution and utilization, the Elite-II set has had lesser emphasis but has been structured based on multiple stress resistance that is crucial for crop improvement. Recent investigations have reported the resistance to barley yellow dwarf virus (Saffdar *et al.*, 2009) and stripe rust (Tariq-Khan and Ul-Haq, 2011) in the Elite-II set and suggested its wider use in breeding. This study focuses on the delimitation of the 33 entries based on quality components for

**Table 1.** High-molecular-weight glutenin subunit combinations in Elite-II D-genome synthetic hexaploids (*Triticum turgidum* × *Aegilops tauschii*;  $2n = 6x = 42$ ; AABBDD)

Name	Pedigree	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D<sup>1</sup></i>
E-II-1 <sup>a</sup>	CETA/ <i>Aegilops squarrosa</i> (392) <sup>b</sup>	Null	6 + 8	2 + 12
E-II-2	GAN/ <i>Ae. squarrosa</i> (335)	Null	6 + 8	2 + 12
E-II-3	CETA/ <i>Ae. squarrosa</i> (417)	Null	6 + 8	2 + 12
E-II-4	SKARV_2/ <i>Ae. squarrosa</i> (304)	Null	7 + 8	Null + 10.5
E-II-5	STY-US/CELTA//PALS/3/SRN_5/4/ <i>Ae. squarrosa</i> (431)	Null	13 + 16	1.5 + 10
E-II-6	D67.2/P66.270// <i>Ae. squarrosa</i> (497)	1	13 + 16	5 + 10
E-II-7	D67.2/P66.270// <i>Ae. squarrosa</i> (308)	1	13 + 16	5 + 10
E-II-8	CETA/ <i>Ae. squarrosa</i> (533)	Null	6 + 8	3 + 10
E-II-9	SORA/ <i>Ae. squarrosa</i> (323)	Null	7 + 8	2.1 + 10
E-II-10	DVERD_2/ <i>Ae. squarrosa</i> (214)	Null	7 + 8	2.1 + 10/10.5
E-II-11	CROC_1/ <i>Ae. squarrosa</i> (210)	Null	7 + 8	3 + 10
E-II-12	SORA/ <i>Ae. squarrosa</i> (192)	Null	7 + 8	3 + 10
E-II-13	ARLIN_1/ <i>Ae. squarrosa</i> (218)	Null	7 + 8	5 + 10
E-II-14	TK SN1081/ <i>Ae. squarrosa</i> (222)	Null	7 + 8	1.5 + 12.2
E-II-15	GAN/ <i>Ae. squarrosa</i> (236)	Null	6 + 8	3 + 10
E-II-16	LCK59.61/ <i>Ae. squarrosa</i> (693)	Null	7 + 8	1.5 + 10
E-II-17	CETA/ <i>Ae. squarrosa</i> (1025)	Null	6 + 8	1.5 + 12
E-II-18	DOY1/ <i>Ae. squarrosa</i> (1027)	Null	6 + 8	2.1 + 10
E-II-19	CETA/ <i>Ae. squarrosa</i> (386)	Null	6 + 8	2.1 + 10
E-II-20	CPI/GEDIZ/3/GOO//JO/CRA/4/ <i>Ae. squarrosa</i> (1018)	Null	20	5 + 10
E-II-21	CETA/ <i>Ae. squarrosa</i> (1031)	Null	6 + 8	5 + 10
E-II-22	CETA/ <i>Ae. squarrosa</i> (1038)	Null	6 + 8	5 + 10
E-II-23	CETA/ <i>Ae. squarrosa</i> (1046)	Null	6 + 8	3 + 10
E-II-24	CETA/ <i>Ae. squarrosa</i> (1053)	Null	6 + 8	5 + 10
E-II-25	CROC_1/ <i>Ae. squarrosa</i> (212)	Null	7 + 8	5 + 10
E-II-26	CETA/ <i>Ae. squarrosa</i> (368)	Null	6 + 8	2 + 12
E-II-27	ARLIN_1/ <i>Ae. squarrosa</i> (430)	Null	7 + 8	1.5 + 12.2
E-II-28	D67.2/P66.270// <i>Ae. squarrosa</i> (1015)	1	13 + 16	5 + 10
E-II-29	GAN/ <i>Ae. squarrosa</i> (206)	Null	6 + 8	5 + 10
E-II-30	ARLIN_1/ <i>Ae. squarrosa</i> (335)	Null	7 + 8	2 + 12
E-II-31	68.111/RGB-U//WARD RESEL/3/STIL/4/ <i>Ae. squarrosa</i> (385)	Null	6 + 8	1.5 + 12
E-II-32	68.111/RGB-U//WARD RESEL/3/STIL/4/ <i>Ae. squarrosa</i> (432)	Null	6 + 8	3 + 10
E-II-33 <sup>a</sup>	DOY1/ <i>Ae. squarrosa</i> (534)	Null	6 + 8	2.1 + 10

<sup>a</sup>E-II-1: to be read as Elite two entry number 1 up to Elite two entry number 33. <sup>b</sup>The accession numbers of *Ae. squarrosa* in the Wheat Wide Crosses working collection at CIMMYT, Mexico and NARC Islamabad, Pakistan are indicated in parentheses.



**Fig. 1.** Allelic composition of Elite-II D-genome synthetic hexaploids (*Triticum turgidum* × *Aegilops tauschii*;  $2n = 6x = 42$ ; AABBDD). Lane 1 (from left): Pavon (Check), 2: E-II-1, 3: E-II-2, 4: E-II-3, 5: E-II-4, 6: E-II-5, 7: E-II-6, 8: E-II-7, 9: C-591 (Check), 10: Chinese spring (Check), 11: E-II-11, 12: blank, 13: E-II-12, 14: E-II-13, 15: SH-61 (Check), 16: E-II-14, 17: Pavon (Check).

targeted use in breeding, emphasizing the significance of quality diversity along with stress resistance.

The results regarding the HMW glutenin composition in SHs are presented in Table 1. The allelic variants observed in these SHs are presented in Fig. 1. Fourteen different allelic variants at *Glu-1* loci were observed in these SHs. At the *Glu-A1* locus, only two alleles, 1A<sub>x</sub>Null and 1A<sub>x</sub>1, were found. The other subunit generally found in bread wheat germplasm, designated as 1A<sub>x</sub>2\*, was absent in these SHs. For the *Glu-B1* allelic variants, both the x- and y-type subunits were observed except for 1B<sub>x</sub>20, which was found only in one SH. Although it was initially considered to occur with a 1B<sub>y</sub> null gene, analysis using reverse phase high-performance liquid chromatography showed the presence of a y-type subunit, which accounts for about one-third of the total protein and co-migrates with the subunit 1B<sub>x</sub>20 on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Margiotta *et al.*, 1993). In the observed allelic variants, the subunit 1B<sub>x</sub>6 + 1B<sub>y</sub>8 encoded by the allele *Glu-B1d* was predominant, appearing in 17 (50%) SHs. This subunit has been reported to have a positive influence on most of the bread-making quality parameters (Tang *et al.*, 2010). The other subunits observed were 1B<sub>x</sub>7 + 1B<sub>y</sub>8 in 11 (32.2%) and 1B<sub>x</sub>13 + 1B<sub>y</sub>16 in 4 (12.1%) SHs.

At the *Glu-D'1* locus, seven different allelic variants were observed. All these alleles were the combinations of the x- and y-type subunits except in the case of the entry SKARV\_2/*Aegilops squarrosa* (304) where only the y-type subunit was observed. Due to the superior bread-making quality attributes of 1D<sub>x</sub>5 + 1D<sub>y</sub>10 encoded by the allele *Glu-D1d*, it was important to observe its high frequency in ten (29.4%) SHs. The inferior subunit at the *Glu-D1* locus, designated as 1D<sub>x</sub>2 + 1D<sub>y</sub>12, which resulted in poor bread-making quality, was observed in five (14.7%) SHs. This subunit is generally predominant in bread wheat germplasm, and its replacement is only possible when other allelic variants of this locus are deployed frequently. The x-type subunit 1D<sub>x</sub>1.5 was found in seven SHs and its association with the y-type subunit 1D<sub>y</sub>12

perceived in two SHs. The other two y-type subunits associated with 1D<sub>x</sub>1.5 include 1D<sub>y</sub>10 and 1D<sub>y</sub>12.2, which were also perceived in two SHs. The *Glu-D'1ab* (1D<sub>x</sub>1.5 + 1D<sub>y</sub>10) allele encodes positive quality attributes and has the potential to improve end-use quality as validated by Peña *et al.* (1995) and Tang *et al.* (2008). Similarly, the x-type subunit 1D<sub>x</sub>2.1 was found in five SHs associated in all cases with the y-type subunit 1D<sub>y</sub>10. This subunit has also been previously reported in a landrace from Afghanistan (Lagudah *et al.*, 1987). The *Glu-D1z*-encoded subunit 1D<sub>x</sub>3 + 1D<sub>y</sub>10 was found in six (17.7%) SHs. This allele has been reported to contribute significantly to extensible gluten and bread loaf volume (Peña *et al.*, 1995).

The *Glu-D'1*-encoded allelic variants are very important for their contribution to bread-making quality. The narrow genetic base of the *Glu-D1* locus in bread wheat is usually accredited to the presence of 1D<sub>x</sub>2 + 1D<sub>y</sub>12 or 1D<sub>x</sub>5 + 1D<sub>y</sub>10. The occurrence of the array of the described D-genome-encoded subunits with many B- and A-genome subunits allows this germplasm set to be investigated thoroughly for its important functional properties, which in turn allows their possible utilization for improvement in grain quality. On the other hand, the subunit 1D<sub>x</sub>1.5 + 1D<sub>y</sub>12.2, encoding dough stickiness and low bread volume (Hsam *et al.*, 2001), was observed in only two SHs.

There is always a search for new alleles having favourable functional properties for bread-making qualities (Xu *et al.*, 2010). In this context, SHs are very important, as they possess a large number of allelic variants of HMW glutenin subunits (Rehman *et al.*, 2008). Most wheat breeding programmes focus on allelic diversity for crop improvement, and when it comes to *Ae. tauschii* as a novel source, the preference has been towards exploiting its synthetic hexaploid wheats. SH wheats have unique AB-genome compositions complemented by diverse D-genome accessions. Thus, using SHs for wheat improvement allows their incorporation across all three wheat genomes. Hence, SH wheat utilization via 'bridge crosses' has been preferred, as it

augments diversity across all three wheat genomes. However, where precision is required to target specific D-genome transfers, the 'direct crossing' holds significance. In such cases, a limited backcross strategy where the A- and B-genomes remain constant while the D-genome recombines, gives precise information about any D-genome transfers. This strategy is less exploited but has excellent scientific precision that could be explored by using the accessional *Ae. tauschii* information generated through the present findings on glutenin subunits associated with a D-genome chromosome.

## References

- Gianibelli MC, Gupta RB, Lafiandra D, Margiotta B and MacRitchie F (2001) Polymorphism of high  $M_r$  glutenins in *Triticum tauschii*: characterisation by chromatography and electrophoretic methods. *Journal of Cereal Science* 33: 39–52.
- Hsam SLK, Kieffer R and Zeller FJ (2001) Significance of *Aegilops tauschii* glutenin genes on bread-making properties of wheat. *Cereal Chemistry* 78: 521–525.
- Lagudah ES, Flood RG and Halloran GM (1987) Variation in high molecular weight glutenin subunits in landraces of hexaploids wheat from Afghanistan. *Euphytica* 36: 3–9.
- Margiotta B, Colaprico G, D'Ovidio R and Lafiandra D (1993) Characterization of high  $M_r$  subunits of glutenin by combined chromatographic (RP-HPLC) and electrophoretic separations and restriction fragment length polymorphism (RFLP) analyses of their encoding genes. *Journal of Cereal Science* 17: 221–236.
- Mujeeb-Kazi A, Rosas V and Roldan S (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. Non L.) in synthetic hexaploid wheats (*T. turgidum* L. s. lat.  $\times$  *T. tauschii*;  $2n=6x=42$ , AABBDD) and its potential utilization for wheat improvement. *Genetic Resources and Crop Evolution* 43: 129–134.
- Payne PI and Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for the high-molecular weight subunit of glutenin whose in hexaploid wheat. *Cereal Research Communications* 11: 29–35.
- Peña RJ, Zarco-Hernandez J and Mujeeb-Kazi A (1995) Glutenin subunit compositions and bread making quality characteristics of synthetic hexaploid wheats derived from *Triticum turgidum*  $\times$  *Triticum tauschii* (Coss.) Schmal Crosses. *Journal of Cereal Science* 21: 15–23.
- Rehman A, Evans N, Gianibelli MC and Rose RJ (2008) Allelic variation in high and low molecular weight glutenins at the *Glu-D1* locus of *Aegilops tauschii* as a potential source for improving bread wheat quality. *Australian Journal of Agricultural Research* 59: 399–405.
- Saffdar H, Ashfaq M, Hameed S, Ul-Haq I and Mujeeb-Kazi A (2009) Molecular analysis of genetic diversity in Elite-II synthetic hexaploid wheat screened against barley yellow dwarf virus. *African Journal of Biotechnology* 14: 3244–3250.
- Tang Y, Yang W, Tian J, Li J and Chen F (2008) Effect of HMW-GS 6 + 8 and 1.5 + 10 from synthetic hexaploid wheat on wheat quality trait. *Agriculture Sciences in China* 7: 1161–1171.
- Tang Y, Yang W, Wu Y, Li C, Li J, Zou Y, Chen F and Mares D (2010) Effect of high molecular weight glutenin allele, *Glu-B1d*, from synthetic hexaploid wheat on wheat quality parameters and dry, white Chinese noodle-making quality. *Australian Journal of Agricultural Research* 61: 310–320.
- Tariq-Khan M and Ul-Haq I (2011) Elite-II synthetic hexaploids wheats as a potential source of resistance against yellow rust. *Archives of Phytopathology and Plant Protection* 44: 1165–1170.
- William MDHM, Peña RJ and Mujeeb-Kazi A (1993) Seed protein and isozyme variations in *Triticum tauschii* (*Aegilops squarrosa*). *Theoretical and Applied Genetics* 87: 257–263.
- Xu SS, Khan K, Klindworth DL and Nygard G (2010) Evaluation and characterization of high-molecular weight 1D glutenin subunits from *Aegilops tauschii* in synthetic hexaploid wheats. *Journal of Cereal Science* 52: 333–336.