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Research Note

Evaluation and characterization of high-molecular weight 1D glutenin subunits from *Aegilops tauschii* in synthetic hexaploid wheats

S.S. Xu [a,*], K. Khan [b], D.L. Klindworth [a], G. Nygard [b]

[a] USDA-ARS, Northern Crop Science Laboratory, 1605 Albrecht Boulevard North, Fargo, ND 58102-2765, USA
[b] Department of Cereal and Food Sciences, Dept 7640, PO Box 6050, North Dakota State University, Fargo, ND 58108-6050, USA

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ABSTRACT

The high-molecular weight (HMW) glutenin subunits of bread wheat are major determinants of end-use quality. The objective of this study was to determine the 1Dx and 1Dy subunits present in 43 synthetic hexaploid wheat (SHW) lines derived by crossing durum 'Langdon' to 43 *Aegilops tauschii* accessions. Protein samples were initially electrophoresed multiple times on SDS-PAGE gels to arrange subunits into similar groups and then were electrophoresed on urea/SDS-PAGE gels. Initial results with SDS-PAGE gels indicated that there were six 1Dx and six 1Dy subunits in these SHW lines. However, results of the urea/SDS-PAGE indicated that some of the subunit groups could be further differentiated into additional subunits. A total of eleven 1Dx and eight 1Dy subunits including the newly designated subunits 1Dx2^t-1, 1Dx2^t-2, 1Dx2^t-3, 1Dx1.5^t-1, 1Dx2.1^t-1, 1Dy10^t-1, and 1Dy12^t-1 were identified, and they composed 17 1Dx and 1Dy combinations in the SHW lines. Eight of the combinations included at least one novel subunit and hence they were novel *Glu-D1* alleles. Our results indicated that urea/SDS-PAGE can be very useful in identifying new HMW glutenin subunits. Quality testing of the SHW lines will determine if any of the alleles are useful in improving wheat-baking quality.

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1. Introduction

The high-molecular weight (HMW) glutenin subunits (GSs) are among the most important determinants of baking quality in bread wheat (*Triticum aestivum* L.). These proteins are controlled by orthologous gene sets, named *Glu-A1*, *Glu-B1*, and *Glu-D1*, located on the long arms of the Group 1 chromosomes. The HMW GSs from the *Glu-D1* locus are particularly important in bread-making quality and about 30 *Glu-D1* alleles have been identified in bread wheat and other hexaploid wheat species (McIntosh et al., 2008). In addition to hexaploid wheat, *Aegilops tauschii* Cosson, the diploid ancestor of the D-genome, conserves many unique *Glu-D1* alleles. So far, 14 x-subunits in *Ae. tauschii* have been identified (An et al., 2009; Gianibelli et al., 2001; Rehman et al., 2008; Yan et al., 2003; Zhang et al., 2008) and 10 of them migrate in order of ascending mobility as 1^t, 2.1^t, 1.5^t, 1.5^{*t}, 2^t, 3^t, 4^t, 5.1^t, 5^t, and 5^{*t}. The migration orders of the four additional x-subunits, 2.2^t, 1.6^t, 5^{*t} and 5.1^{*t}, have only been described relative to some subunits. Gianibelli et al. (2001) listed 10 y-subunits which migrated in the order of 10.3^t, 10.2^t, 10.1^t, 10^t, 11^t, 12^t, 12.1^t, 12.2^t, 12.3^t and 12.4^t. Yan et al. (2003) indicated additional new y-subunits not described by Gianibelli et al. (2001), but the migration order of those new subunits relative to the older subunits is not clear. Rehman et al. (2008) reported that the x- and y-type subunits are combined into 85 different *Glu-D1* alleles.

The unique *Glu-D1* alleles in *Ae. tauschii* can be a valuable source for improving bread-making quality in bread wheat. However, there have been limited tests of the quality effects of the HMW GSs from *Ae. tauschii*. Synthetic hexaploid wheat (SHW) (×*Aegilotriticum* spp.), which is produced from the hybrid between tetraploid wheat (*Triticum turgidum* L.) and *Ae. tauschii*, can serve as useful germplasm for large scale testing of the effects of the HMW GSs from *Ae. tauschii* on quality traits. A set of SHW lines that were previously developed by Dr. L.R. Joppa is currently being evaluated for several traits including pest and disease resistance. The objective of this study was to identify and characterize the HMW glutenin subunits controlled by the *Glu-D1* locus in 43 SHW lines.

2. Experimental

2.1. Plant materials

Common wheats 'Chinese Spring', 'Hobbit', and 'Norquay', durum wheats 'Langdon', Rugby68, and Rugby6, SHW line TA4152-

Abbreviations: GSs, glutenin subunits; HMW, high-molecular-weight; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SHW, synthetic hexaploid wheat.

* Corresponding author. Tel.: +1 701 239 1327; fax: +1 701 239 1369.
E-mail address: steven.xu@ars.usda.gov (S.S. Xu).

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16 (Altar 84/*Ae. tauschii* CPI 219) (Mujeeb-Kazi et al., 2000), and *Ae. tauschii* accessions (Gianibelli et al., 2001) known to carry the previously identified HMW subunits were used as the controls (Table 1). The seed samples of TA4152-16 and the *Ae. tauschii* accessions were obtained from Wheat Genetic and Genomic Resource Center, Manhattan, KS. The TA and CPI accession numbers for *Ae. tauschii* accessions were cross-referenced from USDA-ARS National Plant Germplasm System (http://www.ars-grin.gov/npgs/) and AusPGRIS – Australian Plant Genetic Resource Information Service (http://www2.dpi.qld.gov.au/extra/asp/auspgris/). Forty-three SHW lines were derived from partially fertile F_1 hybrids between durum 'Langdon' and 43 *Ae. tauschii* accessions (Table 2). The *Ae. tauschii* parents of the SHW lines were included as checks to verify the pedigree of the SHW lines.

2.2. Electrophoresis

Protein samples were initially analyzed using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE), which were carried out using the SE 600 (GE Healthcare, Piscataway, NJ) vertical apparatus with either 8% or 12% separating gels as described by Xu et al. (2004). After review of the initial results, additional tests were conducted in which SHW lines that appeared to have similar bands were re-run in groups with appropriate checks. Following identification of the subunits on 8% and 12% gels, the samples were prepared for electrophoresis on 12% urea/SDS-PAGE (Goldsbrough et al., 1989) gels to detect additional differences in the subunits. The gel images were captured using the Kodak Logic 100 system and were analyzed using Kodak 1D Image Analysis Software Version 3.6.1 (Eastman Kodak Company, Rochester, NY).

Table 1
High-molecular weight (HMW) subunits reported to be present in checks used in this study.

Species	Line and Accession No.[a]	HMW subunits[b]	Alleles[c]
T. aestivum			
	Chinese Spring	2 + 12	*Glu-D1a*
	Hobbit (PI 428521)	3 + 12	*Glu-D1b*
	Norquay	5 + 10	*Glu-D1d*
Ae. tauschii			
	TA1588 (CPI 110615)	$1^t + 12^t$	*Glu-D1ax*
	TA1591 (CPI 110618)	$1^t + 10^t$	*Glu-D1aw*
	TA1622 (CPI 110630)	$2^t + 12.1^t$	*Glu-D1bf*
	TA1645 (CPI 110646)	$2.1^t + 12.3^t$	
	TA1659 (CPI 110657)	$3^t + 10.2^t$	*Glu-D1bg*
	TA1662 (CPI 110660)	$5^t + 12^t$	
	TA1672 (CPI 110669)	$3^t + 12.2^t$	*Glu-D1y*
	TA1694 (CPI 110689)	$1.5^t + 10.3^t$	*Glu-D1bc*
	TA2377 (CPI 110715)	$2^t + 12.2^t$	
	TA2414 (CPI 110750)	$2.1^t + 12.4^t$	*Glu-D1be*
	TA2461 (CPI 110796)	$2.1^t + 10.2^t$	*Glu-D1au*
	TA2463 (CPI 110798)	$5^t + 10^t$	
	TA2479 (CPI 110814)	5^{*t} + null	*Glu-D1bm*
	TA2529 (CPI 110860)	$5^t + 10^t$	
×*Aegilotriticum* spp.			
	TA4152-16	$1.5^t + 12.2^t$	

[a] Line and accession number: TA, PI, and CPI are the accession numbers in Wheat Genetic and Genomic Resource Center, Manhattan, KS, USDA National Small Grains Collection, Aberdeen, ID, and Australian Plant Genetic Resource Information Service (http://www2.dpi.qld.gov.au/extra/asp/auspgris/, accessed on September 4, 2009), respectively.

[b] The HMW subunits of common wheat cultivars and synthetic hexaploid wheat line TA4152-16 were based on MacGenes (McIntosh et al., 2008) and Mujeeb-Kazi et al. (2000), respectively, and the subunits of *Ae. tauschii* accessions were mainly based on Gianibelli et al. (2001). The HMW subunits in all the checks were verified in this study.

[c] The allele names were obtained from MacGenes (McIntosh et al., 2008).

Table 2
High-molecular weight Glu-D1 subunits in durum Langdon–*Ae. tauschii* synthetic hexaploid wheat lines.

Line No.	Pedigree[a]	Glu-D1 subunits
SW1	Langdon/*Ae. tauschii* Clae 1	$2^t + 10^t$-1
SW2	Langdon/*Ae. tauschii* Clae 5	$2^t + 10^t$-1
SW3	Langdon/*Ae. tauschii* Clae 9	$2.1^t + 10^t$
SW4	Langdon/*Ae. tauschii* Clae 11	$2.1^t + 10^t$
SW5	Langdon/*Ae. tauschii* Clae 14	2^t-2 + 10^t-1
SW7	Langdon/*Ae. tauschii* Clae 22	2.1^t-1 + 12^t
SW8	Langdon/*Ae. tauschii* Clae 25	$1.5^t + 12.2^t$
SW9	Langdon/*Ae. tauschii* Clae 26	2^t-3 + 12.2^t
SW10	Langdon/*Ae. tauschii* H80-101-4	2^t-1 + 10^t-1
SW11	Langdon/*Ae. tauschii* H80-114-1	2^t-2 + 10^t-1
SW12	Langdon/*Ae. tauschii* H80-115-3	2^t-1 + 12^t-1
SW14	Langdon/*Ae. tauschii* PI 220641	$2^t + 10^t$-1
SW15	Langdon/*Ae. tauschii* PI 317392	2^t-1 + 10^t-1
SW16	Langdon/*Ae. tauschii* RL5003	$2^t + 10^t$-1
SW17	Langdon/*Ae. tauschii* RL5214	$2^t + 10^t$-1
SW19	Langdon/*Ae. tauschii* RL5259	$2^t + 10^t$-1
SW20	Langdon/*Ae. tauschii* RL5261	2^t-1 + 10^t-1
SW21	Langdon/*Ae. tauschii* RL5263	$2^t + 10^t$-1
SW22	Langdon/*Ae. tauschii* RL5266-1	$2^t + 10^t$-1
SW23	Langdon/*Ae. tauschii* RL5271	$4^t + 10^t$
SW24	Langdon/*Ae. tauschii* RL5272	$5^t + 12^t$
SW25	Langdon/*Ae. tauschii* RL5286	$1.5^t + 12.2^t$
SW26	Langdon/*Ae. tauschii* RL5392	$2^t + 10^t$-1
SW27	Langdon/*Ae. tauschii* RL5393	$5^t + 12^t$
SW28	Langdon/*Ae. tauschii* RL5492	2^t-2 + 10^t-1
SW29	Langdon/*Ae.tauschii* RL5498	2^t-2 + 10^t-1
SW30	Langdon/*Ae.tauschii* RL5527	$2^t + 10^t$-1
SW32	Langdon/*Ae. tauschii* RL5532	2^t-1 + 10.3^t
SW34	Langdon/*Ae. tauschii* RL5544	$5^t + 12^t$
SW35	Langdon/*Ae. tauschii* RL5552	$2^t + 10^t$-1
SW36	Langdon/*Ae. tauschii* RL5555	$2^t + 10^t$-1
SW38	Langdon/*Ae. tauschii* RL5560	$5^t + 12^t$
SW39	Langdon/*Ae. tauschii* RL5561	2^t-1 + 10^t-1
SW40	Langdon/*Ae. tauschii* RL5562	1.5^t-1 + 10.1^t
SW41	Langdon/*Ae. tauschii* RL5570	$2^t + 12^t$
SW44	Langdon/*Ae. tauschii* PI476874	2^t-1 + 10^t-1
SW52	Langdon/*Ae. tauschii* Clae 17	$5^t + 10^t$
SW53	Langdon/*Ae. tauschii* PI 268210	$2.1^t + 10.1^t$
SW55	Langdon/*Ae. tauschii* RL5257	$2^t + 10^t$-1
SW56	Langdon/*Ae. tauschii* RL5258	$2^t + 10^t$-1
SW57	Langdon/*Ae. tauschii* RL5270	2^t-1 + 10^t-1
SW57-1	Langdon/*Ae. tauschii* RL5270	5 + 10.2^t
SW58	Langdon/*Ae. tauschii* AL8/78	$2.1^t + 10.2^t$
SW59	Langdon/*Ae. tauschii* Clae 19	2.1^t-1 + 12^t

[a] *Ae. tauschii* accessions: PI/Clae and RL numbers are the accessions from USDA National Small Grains Collection, Aberdeen, ID and Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada, respectively.

2.3. Nomenclature of HMW subunits

We identified subunits first based on their mobilities in 8% and 12% SDS-PAGE gels. To do this, the classification system of Lagudah and Halloran (1988) was followed, in which the subunits of *Ae. tauschii* are identified by the numerical designation of Payne and Lawrence (1983), and followed by the superscript designation – *t*- to indicate their origin from *Ae. tauschii*. Following classification on normal SDS-PAGE gels, subunits were further differentiated by urea/SDS-PAGE. This was done by adding to each designation a dash and a numeral. For example, if on the SDS-PAGE gels, the 1Dx subunits of two SHW lines migrated similar to 1^t of the *Ae. tauschii* accession used as the check, both were initially assigned as 1^t. After observing the results of urea/SDS-PAGE, if the SHW line had a 1Dx subunit that migrated similar to the 1Dx of the check *Ae. tauschii* accession, its designation remained as 1^t. If the SHW line had a 1Dx subunit that migrated differently than the 1Dx of *Ae. tauschii*, it was assigned the subunit designation of 1^t-1.