Chromosome Variation and HMW Glutenins in Synthetic Hexaploid Wheats (*Triticum turgidum* ssp. *dicoccum/Aegilops tauschii*)

N. Daskalova[1], S. Doneva[2] and P. Spetsov[3]*

[1]Plant Production Department, Technical University, Varna 9010, Bulgaria
[2]Dobroudja Agricultural Institute, 9520 General Toshevo, Bulgaria
[3]College of Konstantin Preslavsky University of Shumen, Dobrich 9302, Bulgaria

Seven synthetic hexaploid wheats (*Triticum dicoccum/Aegilops tauschii*) were subjected for investigation. Numerical variation of chromosome number in F_1 hybrids between three synthetics and common wheat varieties, was recorded. Hexaploid amphiploids (SHW) formed gametes with aneuploid chromosome number at a frequency of 13.2 and 14.8% as male and female parents, respectively. We speculated that the frequency of aneuploids in the generation might depend on variability of BA^u- and D-genomes of synthetic parents, and could be used for increasing the genetic diversity in common wheat. The HMW-glutenins analysis divided two lines in SHW530 and 532 due to different genes present in the B-genome, and increased them to 9 synthetic lines. The subunits 1Dx1.5+1Dy10 was predominantly observed in the synthetics. Two other allelic variants 1Dx2+1Dy11 and 1Dx4+1Dy10.1 were found in four lines and appeared as new genes in SHW originated from *Aegilops tauschii*. The synthetic hexaploid lines could play a significant role as novel germplasm resources for improving the grain quality of bread wheat.

Keywords: *Aegilops tauschii*, tetraploid wheat, mitotic chromosomes, glutenins, synthetic amphiploids

Abbreviations: SHW – synthetic hexaploid wheat; HMW-GS – high-molecular-weight glutenin subunits; IPGR – Institute for Plant and Genetic Resources – Sadovo, Bulgaria

Introduction

Common wheat (*Triticum aestivum* L., 2n = 42, AABBDD) is a globally important food crop and will become even more significant as the world's population increases. Domesticated hexaploid wheat has evolved from two spontaneous hybridization events. Tetraploid *T. turgidum* wheat (2n = 28, AABB) hybridized with diploid *Aegilops tauschii* Coss. (2n = 14, DD) followed by spontaneous chromosome doubling is the commonly quoted concept for the origin of common wheat (Feldman 2001). However, it is widely recognized that only a limited number of individuals of parents were involved in the origin and

*Corresponding author; E-mail: pspetsov@abv.bg

evolution of hexaploid wheat. Thus, the genetic diversity of *T. aestivum* is fairly narrow compared with that of its two donor species, and most of the genetic variation found in tetraploid wheat and *Aegilops tauschii* is not available in the present wheat germplasm.

The genetic resources of tetraploid and diploid wheats, including *Aegilops* species, can be used as primary resources of elite genes for breeding of modern wheat cultivars. The production of synthetic amphiploids is an effective and rapid way of introgressing desirable traits from related species into domesticated wheats (Goncharov et al. 2007). Using the genetic resources of wild relatives is the best strategy to improve the quality and productivity of durum and common wheat. Numerous studies have been carried out to produce and investigate wheat-alien hybrids in order to clarify the inheritance and gene control of important traits in segregating populations. Synthetic hexaploid wheats (SHW) are good example as resulting products of wide hybridization in developing novel genetic lines and SHW-derived wheat varieties (Plamenov and Spetsov 2011; Li et al. 2014). Genes of interest can be introgressed into common wheat by the 'bridge' of re-synthesized hexaploid or amphidiploids, obtained from crossing the tetraploid wheats with *Ae. tauschii*, in a manner analogous to the evolution of hexaploid wheat.

The study aimed to investigate the somatic chromosome number in F_1 hybrids, obtained by crossing three synthetic wheats (*Triticum turgidum* ssp. *dicoccum*/*Aegilops tauschii*) with *T. aestivum* varieties, and identify HMW-glutenin subunits in seven synthetic amphiploids as potential genetic sources for wheat quality improvement.

Materials and Methods

The investigated seven SHW (Nos 32, 106, 107, 530, 531, 532 and 83/27) are presented in Table 1 [genome formulae followed the classification of Goncharov et al. (2009)]. Wheat variety Bezostaya-1 was used as a check in the protein analysis.

Five *Aegilops tauschii* accessions were successfully involved in breeding of synthetic wheats. Mitotic chromosome counting was performed in hybrid seedlings, obtained between single plants of Nos 530, 531 and 532 and some Bulgarian and Czech bread wheat varieties. A sample of 50 grains per line was crushed and ground to powder. Extraction of

Table 1. Pedigree of the synthetic hexaploid wheats employed in the study

Breeding No.	Pedigree
32	F_1 (44961/Zagorka[a]/45432)/*Ae. tauschii* No 19089
106	F_2 (44961/Zagorka/45432)/*Ae. tauschii* No 22744
107	45398/*Ae. tauschii* No 22744
530	510F_1 (45390/45398)/*Ae. tauschii* No 19088
531	510F_1 (45390/45398)/*Ae. tauschii* No 30422
532	510F_1 (45390/45398)/*Ae. tauschii* No 22744
83/27	*T. dicoccum* Khapli-III/*Ae. tauschii* No 001

[a]Bulgarian *T. durum* variety; *T. dicoccum* accessions 44961, 45390, 45398 and 45432 were obtained from the ICARDA, Syria; *Aegilops tauschii* accessions 001, 19088, 19089, 22744 and 30422 originated from the IPGR – Sadovo, Bulgaria.

Table 2. Somatic chromosome number of F_1 hybrids, obtained by crossing three SHW (Nos 530, 531 and 532) to bread wheat cultivars

Cross	NPC	Chromosome number					
		41	41^t	42	42^t	43	O
Aglika/530-3	12	2	1	7	1		1
Milena/530-1	1			1			
Pobeda/530-4	10			10			
Antitsa/530-3	6	1		5			
Alana/530-1	5			4	1		
Sulamit/530-2	1			1			
Vlasta/530-4	7			4		3	
Slaveya/530-3	7			5	1	1	
Vlasta/530-2	9			8		1	
Trakiya/530-4	12			12			
Svilena/530-4	7			7			
Enola/530-3	3			1		2	
Polena/530-4-0	11			10		1	
Slaveya/530-4	15			15			
Milena/532-2	1			1			
Slaveya/532-2	3			3			
Vlasta/532-6	11			11			
Total	121	3 (2.5)	1	105 (86.8)	3	8 (6.6)	1
530-3/Korona	4			1		3	
530-5-6/Enola	6			6			
530-1-2/Karat	7			7			
530-4-0-1/Aglika	9	2		7			
530-4-2-2/Aglika	12			11		1	
531-2-2/Korona	8	1		7			
532-1-1/Polena	16			16			
532-1-4/Aglika	13			13			
532-6-1-1/Meritto	13	5		7			1
Total	88	8 (9.1)		75 (85.2)		4 (4.6)	1

NPC – number of plants checked; t – telosome; O – others. Means (in %) of hybrids with 2n = 42 are not statistically different.

HMW-glutenins was performed on vertical apparatus followed the classical one-dimensional 12% polyacrylamide gel, SDS–PAGE (Singh et al. 1991; Lafiandra et al. 1993). Universal system for arrangement and numbering of HMW-GS in wheat was employed and a method for *Glu-1* score assessment as a criterion for wheat quality, too (Payne 1987).

The data were statistically evaluated by analysis of variance with *t*-test using Assistat version 7.7 beta (www.assistat.com). The genetic diversity at each locus was calculated using Nei's index (Nei 1973) and allelic frequencies were determined by summing the frequencies of alleles in the individual lines, dividing this total by the number of amphiploids.

Results

Chromosome number of hybrids obtained by crossing single plants of SHW530, 531 and 532 with some common wheat varieties, was counted (Table 2). A set of 121 hybrids, received from crosses with SHW as pollinators, were checked and 105 (86.8%) proved to have 42 chromosomes. The rest hybrids exhibited different number of chromosomes: 8 (6.6%) plants contained 43 chromosomes, 3 (2.5%) – 41 chromosomes, and the remaining 5 individuals possessed different number of chromosomes with a telosome. From the reciprocal combination, 75 (85.2%) plants of 88 investigated hybrids had 42 chromosomes. Four (4.6%) plants possessed 43 chromosomes and eight (9.1%) – 41 chromosomes. Some other karyotypes contained 40, 41 and 41^t chromosomes. The difference between hybrids with 2n = 42 in the reciprocal crosses was not statistically proven.

Eleven allelic variants of HMW-GS were detected in the synthetics (Table 3 and Fig. 1). The analysis differentiated two lines in Nos 530 and 532 due to different genes

Table 3. Allelic frequencies of HMW-GS at *Glu-1* loci of nine SHW (including two lines in Nos530 and 532)

Locus	Allele	Subunit	Glu-1 quality score	Number of lines	Frequency %
Glu-A1 H* = 0.49	a	1	3	2	22.2
	x	1.1	–	6	66.6
	c	Null	1	1	11.1
Glu-B1 H* = 0.72	b	7+8	3	4	44.4
	f	13+16	3	1	11.1
	i	17+18	3[b]	1	11.1
	h	14+15	3[a], 2[b]	2	22.2
	k	22	1	1	11.1
Glu-D^{t}1 H* = 0.59	ah	1.5+10	–	5	55.5
	–	2+11	–	2	22.2
	–	4+10.1	–	2	22.2

[a]Branland and Dardevet 1985.
[b]Bahraei et al. 2004.
*Index for genetic diversity (Nei 1973).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	2*	2	1.1	1.1	1	1		1.1	1.1	1.1	1.1		1.1	1.1	1.1			1.1
	5	2			2			4	4	1.5	1.5	1.5	1.5	1.5	1.5	2*		
	7+9	17+18	22	7+8	7+8	7+8	4	7+8	7+8	7+8	13+16		7+8	14+15	14+15	14+15	14+15	14.1
	10	11	11	11	11		10.1	10.1	10.1	10	10	10	10	10	10			22*
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
	Bez	83/27	30422	45390	531	45398	19088	530-1	530-2	532-1	532-2	22744	107	106	32	Zag	44961	45432
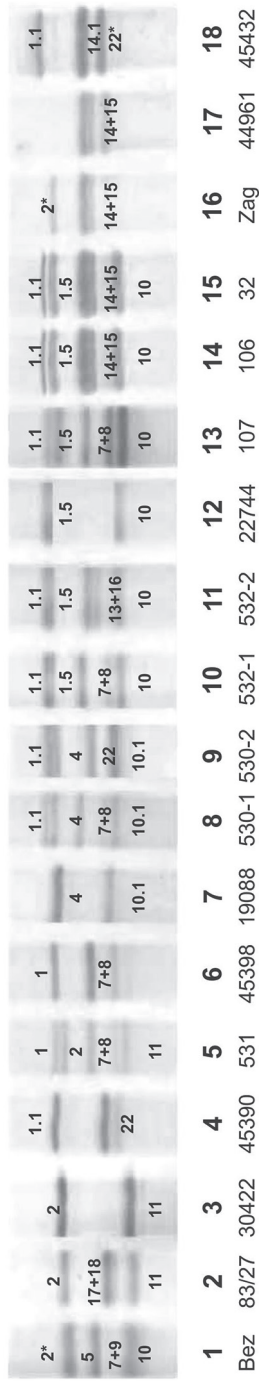
Figure 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis pattern of high-molecular-weight glutenin subunits (HMW-GS) in nine SHW from the crosses of *Triticum turgidum* ssp. *dicoccum* (including hybrid tetraploid parents) with *Aegilops tauschii*. Lane 1, hexaploid wheat variety Bezostaya-1 (Bez, HMW-GS combinations 2*, 7+9, 5+10); Lane 2, 83/27 (*T. turgidum* ssp. *dicoccum* Khapli-III/No001); Lane 3, No 30422 (*Ae. tauschii*); Lane 4, No 45390 (*T. turgidum* ssp. *dicoccum*); Lane 5, 531 (45390/45398/30422); Lane 6, No 45398 (*T. turgidum* ssp. *dicoccum*); Lane 7, No 19088 (*Ae. tauschii*); Lane 8, 530-1 (45390/45398/19088); Lane 9, 530-2 (45390/45398/19088); Lane 10, 532-1 (45390/45398/22744); Lane 11, 532-2 (45390/45398/22744); Lane 12, No 22744 (*Ae. tauschii*); Lane 13, 107 (45398/22744); Lane 14, 106 (44961/Zagorka/45432/22744); Lane 15, 32 (44961/Zagorka/45432/19089); Lane 16, Durum variety Zagorka (Zag, HMW-GS combinations 2*, 14+15); Lane 17, No 44961 (*T. turgidum* ssp. *dicoccum*); Lane 18, No 45432 (*T. turgidum* ssp. *dicoccum*)

presented at the *Glu-B1* locus, and increased them to 9 synthetic lines. Three of the variants were at the *Glu-A1* locus, five-in *Glu-B1* and three-in *Glu-D^{t}1*. At the *Glu-A1* locus, the composition of alleles were only contributed by x-type subunits, viz. 1Ax1, 1Ax1.1 and Null, which are controlled by *Glu-A1a, Glu-A1x* and *Glu-A1c.* The *x* allele was the most frequent (encoded for subunit 1.1) in six genotypes, followed by *a* allele and *c* allele. Five allelic variants were detected at the *Glu-B1* locus. The most frequent was *b* allele, controlled the pair 7 + 8 in four genotypes, followed by *h* allele, responsible for subunits 14 + 15 in two synthetic lines.

From all HMW-GS variants, the D-genome alleles are essential for wheat dough and flour properties. The *Glu-D^{t}1* locus was contributed by three alleles with the combination of three x-type and y-type subunits (Table 3). The x-type subunits included 1.5, 2 and 4 variants, whereas the y-type subunits exerted the composition of 10, 10.1 and 11. The most frequent subunit pair 1Dx1.5 + 1Dy10 encoded by allele *Glu-D1ah*, was observed in five synthetic amphiploids including SHW32 and 106, which have the same female hybrid parent (Table 1, Fig. 1).

Discussion

SHW and their synthetic derivative lines have been used as a means of introducing novel genetic variation into bread wheat (McIntyre et al. 2014; Rasheed et al. 2014). Beneficial SHW traits, such as large grains and high tiller number, were transferred into Sichuan varieties (Li et al. 2014; Ma et al. 2014). Mestiri et al. (2010) analyzed first generations of synthetic allohexaploids and found progenitor-dependent meiotic irregularities, such as incomplete homologous pairing, resulting in univalent formation and leading to aneuploidy in the subsequent generation. High variation of chromosome number in newly synthesized hexaploid wheats were also recorded by Niwa et al. (2010). We investigated somatic chromosome number in 209 hybrids between Nos 530, 531 and 532, and 15 bread wheat cultivars. It is known that bread wheat varieties usually form gametes with 21 chromosomes. Thus, some of the synthetic plants as male or female in crosses, produced gametes with different chromosome number, at a frequency of 13.2–14.8%. We speculated that the frequency of aneuploids in the generation might depend on variability of BA^u- and D-genomes of synthetic parents, and could be used for increasing the genetic diversity in hybrid populations.

No y-type subunits at the *Glu-A1* locus were found, although some authors reported their associations with good bread-making quality in wheat (Rasheed et al. 2012). Subunits 1.5 + 10 was discovered in SHW by William et al. (1993) and proved to have significant influence on bread-making traits (Tang et al. 2008).

Giraldo et al. (2010) analyzed 165 Spanish wheat landraces and found four, nine and three subunits at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. Subunits 1.1, 14 + 15, 22, 1.5 + 10, 2 + 11 and 4 + 10.1, found in this study, were not among them. Yasmeen et al. (2015) analyzed 242 lines of wheat, including landraces from the provinces of Punjab and Baluchistan, as well as the commercial varieties of Pakistan, and observed rare and uncommon variants in the *Glu-B1* locus. Five subunits, reported in this study, including

the three pairs in the *Glu-D*^{t}*1*, were not detected by the authors. Ribeiro et al. (2013) studied the Portuguese wheat Barbela and reported a new x-type HMW-GS encoded at the *Glu-A1* locus that was named *1Ax1.1.*, while Ravel et al. (2014) discovered *Glu-B1-1* protein *22* in Fruh-weizen (13310) wheat accession. We investigated nine SHW and reported 11 subunits (3 at the *Glu-D*^{t}*1* locus) as compared to Rasheed et al. (2012) analyzing ninety-five Elite-1 synthetic hexaploid wheats (*Triticum turgidum*/*Aegilops tauschii*) with 22 glutenins (13 at the *Glu-D*^{t}*1* locus). Five subunits (1.1, 14 + 15, 22, 2 + 11 and 4 + 10.1) reported here, were not present in the Elite-1 subset. Two of them, the pairs 2 + 11 and 4 + 10.1, encoded at the *Glu-D*^{t}*1* locus, are the HMW-GS published for the first time.

Trait variation in the SHW lines may contribute beneficial grain quality to modern wheat cultivars through introgression of novel allelic diversity.

Acknowledgement

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