Turkish Journal of Agricultural and Natural Sciences
Special Issue: 2, 2014

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

Allelic Variation and Composition for Seed Proteins in Synthetic Hexaploid Wheats (2n=42, BBA^{u}A^{u}DD)

[a]Sonya DONEVA*, [a]Diana YORDANOVA, [b]Nadia DASKALOVA, [c]Penko SPETSOV
[a]Dobroudja Agricultural Institute – General Toshevo, Bulgaria
[b]Technical University – Varna, Bulgaria
[c]Konstantin Preslavsky University of Shumen, College–Dobrich, Bulgaria
*Corresponding author: sonya_doneva@yahoo.com

Abstract

Seven hexaploid synthetic wheats (2n=42, BBA^{u}A^{u}DD), derived by crossing accessions and hybrids from *T. turgidum* (2n=28, BBA^{u}A^u) to diploid *Aegilops tauschii* (2n=14, DD), following by colchicine treatment for chromosome doubling, were subjected for seed protein analysis. Amphidiploids № 531 and № 107 revealed x-type subunit 1 at the *Glu-A1* locus, but four other synthetics (№ 32, 106, 530, 532) expressed subunit 1Ax1.1, uncommon for bread wheat, that could be an example of expanding allelic diversity for HMW-GS along with the D-genome derived genes. At the *Glu-B1* locus, five x-type subunits, viz., 7, 13, 17, 14 and 22, and four y-type subunits viz., 8, 16, 18 and 15, and their five combinations were detected. Diversity at the *Glu-D1* was found to be greater than the variety of genes contained at *Glu-A1* and *Glu-B1* loci. In synthetics, the subunit 1Dx1.5+1Dy10 was predominantly observed, which is different from the *T. aestivum* genome and affect the wheat quality to great extent. Other detected D-genome subunits were 1Dx2+1Dy11 and 1Dx4+1Dy10.1. LMW-GS discovered two different lines in each of № 32, 530 and 532 (with b and c alleles) that was further supported by the gliadin compositions. D-genome synthetic hexaploid wheats seemed to be excellent sources for selecting different glutenin compositions in wheat breeding, as well for gliadins, revealing diverse ω- and γ genes.

Key words: Synthetic wheat - HMW-GS – LMW-GS-SDS-PAGE – Gliadins – A-PAGE.

Introduction

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 wheats are good example as resulting products of wide hybridization to develop novel genetic lines and cultivated crops with resistance to different biotic and abiotic stresses (Trethowan and Ginkel, 2009; Plamenov and Spetsov, 2011), and for improving the storage proteins in wheat.

Wheat gluten proteins, called prolamins, are usually divided into two fractions, the glutenins and gliadins (Branlard and Dardevet, 1985; Rasheed et al., 2012). They confer elasticity and extensibility essential for bread making. The polymeric glutenins are subdivided into high- and low-molecular weight glutenin subunits (HMW-GS and LMW-GS) which mainly determine elasticity and viscosity of wheat dough. HMW-glutenins are encoded by two tightly linked genes at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci (x- and y-type) localized on the long arms of group 1 chromosomes, whereas LMW-GS are governed by genes at the Glu-3 loci (*Glu-A3*, *Glu-B3*, *Glu-D3*) on the short arms of group 1 chromosomes. The loci are highly polymorphic and serve as reliable genetic markers for exploring genetic variability in common wheat. New allelic variation occurs due to recombination of different HMW glutenin subunits, which usually receive *Glu-1* quality score as a potential estimate for bread-making properties (Payne et al., 1984). Although, that HMW-GS constitute about 5-10 % of the total

grain proteins, around 67 % of the variation for quality properties among wheat varieties is due to differences in their HMW-GS compositions (Payne et al., 1981, 1984).

The gliadins are monomeric and are grouped into four structural types: α-, β-, γ- and ω-gliadins. The last two are encoded by genes in *Gli–A1, Gli–B1, Gli–D1* on the short arms of group 1 chromosomes, very close to genes responsible for the expression of LMW-GS. Genes controlling α- and β-gliadins are localized on short arms of group 6 chromosomes (*Gli-A2, Gli-B2, Gli-D2*). Fractions encoded by these gliadins are linked and inherited as separate blocks. Gliadins differ in their functional properties with the glutenins contributing to unique biomechanical properties of the wheat gluten (Todorov, 2006). The gliadin and glutenin proteins interact in dough to form a network that confers cohesive viscoelastic properties allowing the detention of carbon dioxide during dough fermentation.

This study aimed to investigate the storage seed proteins in seven D-genome synthetic hexaploid wheats (SHW), produced by crossing several accessions of *Aegilops tauschii* and *T. dicoccum*, including some F_1 and F_2 hybrids as female parents. The genetics, biochemistry and structure of protein compositions in these synthetics are important as new breeding sources for potential transfer of unique glutenin and gliadin variants to durum and common wheat breeding.

Materials and Methods

Materials

SHW (№ 32, 106, 107, 530, 531, 532 and 83/27) are presented in Table 1. Varieties Bezostaya 1 and Chinese Spring were used as checks in protein analyses.

Table 1. Pedigree of the SHW employed in the study

Breeding №	P e d i g r e e
32	F_1 (44961 x Загорка[1] x 45432) x *Ae. tauschii* № 19089
106	F_2 (44961 x Загорка x 45432) x *Ae. tauschii* № 22744
107	45398 x *Ae. tauschii* № 22744
530	510F_1 (45390 x 45398) x *Ae. tauschii* № 19088
531	510F_1 (45390 x 45398) x *Ae. tauschii* № 30422
532	510F_1 (45390 x 45398) x *Ae. tauschii* № 22744
83/27	*T.dicoccum* Khapli-III x *Ae. tauschii*

[1], Bulgarian *T. durum* variety

T. dicoccum accessions № 44961, 45390, 45398 and 45432 were obtained from the ICARDA, Syria, and *Aegilops tauschii* accessions – from the IPGR – Sadovo.

Protein extraction and SDS-PAGE

Five plants from a synthetic were grown and 50 grains per line were used to reveal the homogeneity of each SHW. Seeds were crushed and ground to powder. Gliadins were first extracted in 70% ethanol and protein fractions were separated by A-PAGE using 8% polyacrylamide gel under constant 10°C (Khan et al., 1983).

Extraction of HMW- and LMW- glutenins was performed in four stages (Singh et al., 1991). The electrophoresis was run on vertical apparatus in two ways (Laemmli, 1970): a) classical one-dimensional 12% polyacrylamide gel; б) one-dimensional 12% polyacrylamide gel SDS – PAGE with addition of 4M urea (Lafiandra et al., 1993).

Protein fractions were investigated and designated through the use of the following techniques:

- Universal system for arrangement and numbering of HMW-GS in wheat (Payne and Lawrence, 1983);
- LMW-GS nomenclature in wheat (Gupta and Shepherd, 1988, 1990);
- Combined method for LMW-GS and gliadins identification (Jackson et al., 1996);
- Catalogue for gliadins identification in common wheat (Metakovsky, 1991);
- Method for *Glu-1* score assessment as a criterion for wheat quality (Payne, 1987).

Table 2. Allelic frequencies of HMW-GS at *Glu-1* loci of 9 synthetic hexaploid wheats (including two lines in № 530 and 532)

Locus	Allele	Subunit	Number of lines	Frequency %
Glu-A1	a	1	2	22.2
	x	1.1	6	66.6
	c	null	1	11.1
Glu-B1	b	7+8	4	44.4
	f	13+16	1	11.1
	i	17+18	1	11.1
	h	14+15	2	22.2
	k	22	1	11.1
Glu-D^{t}1	ah	1.5+10	5	55.5
	-	2+11	2	22.2
	-	4+10.1	2	22.2

Results

Allelic variation and composition of HMW-GS

Eleven allelic variants of HMW-GS were detected in the synthetics (Table 2 and Fig.1). Three of them 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, 66.6%, genotypes, followed by *a* allele (22.2%) and *c* allele (11.1%). The *1Ax* subunit 1.1, which is uncommon for the A-genome of common wheat, may be considered as valuable gene for wheat improvement.

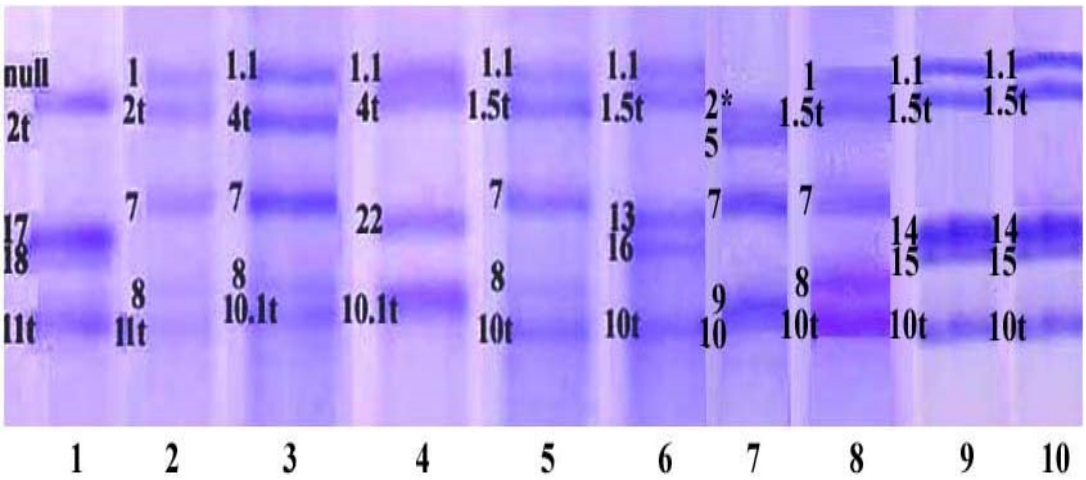

Figure 1. HMW-GS profile at 12% SDS-PAGE. 1: 83/27, 2: 531, 3: 530-1, 4:530-2, 5: 532-1, 6: 532-2, 7:Bezostaya1 (check), 8: 107, 9: 106, 10: 32

The subunit *1Ax 1* positively affected the bread quality as compared to *null* allele, which is estimated to have low Glu-1 score. No y-type subunits at the *Glu-A1* locus were found, although some authors reported their associations with good bread-making quality in wheat (Ciaffi et al., 1995). Species of *T. urartu*, *T. monococcum* and *T. boeoticum* exerted y-type subunits, that could be transferred to SHW (Rasheed et al., 2012).

The HMW-GS located in the B-genome are numerous, less frequent and don't increase the variation in quality parameters (Todorov, 2006). Two of the sevent analyzed synthetic hexaploid wheats – 530 and 532 are haracterized by two biotypes at the *Glu-B1.* Five allelic variants were detected at this locus. Three alleles, *f* , *i* and *κ*, encoded for subunits 13+16, 17+18 and 22 were less frequent (11.1%). The most frequent was *b* allele, controlled 7+8 in four (44.4%) genotypes, followed by *h* allele, responsible for subunit 14+15 in 2 synthetics (22.2%, Fig. 1). It is reported that subunit 7+8, evaluating to have score 3, assured good elasticity and extensibility essential for bread making (Payne, 1987). There were different estimates for the score of subunit 14+15, varying from Glu-1 score 2 to 3 (Payne, 1987). This subunit is rarely occurred in the *Glu-B1* locus and have unproved effect on the gluten strength. It was reported, that subunits 13+16 and 17+18 possessed high score (Glu 1 quality score 3), responsible for good bread making (Todorov, 2006).

From all HMW-GS variants, the D-genome alleles are essential for wheat dough and flour properties. The *Glu-D^{t}1* locus among the SHW was contributed by three alleles with the combination of three x-type and three y-type subunits (Table 2). The x-type subunits included 1.5, 2 and 4, whereas the y-type subunits exerted 10, 10.1 and 11. The most frequent subunit 1Dx1.5+1Dy10 encoded by allele *Glu-D1ah* was observed in five synthetic lines (55.5%). This subunit was discovered in SHW by William et al. (1993) and proved to have significant influence on bread-making traits (Peña et al., 1995, Tang et al., 2008). It was established for wheats with subunits 1.5+10 in combination with 7+8 at the *Glu-B1* and subunit 1 – at the *Glu-A1* locus to be superior in quality (Peña et al, 1995, Tang et al., 2008) than lines having allele d at the *Glu-D^{t}1* locus, responsible for the displaying of subunit 5t+10t. Li et al. (2009) considered this subunit as highly valuable for quality as compared to others, controlled by D-genome alleles. The importance and role of subunits 2+11 and 4+10.1 on the technological and baking properties have not well still clarified, may be because of their limited frequency in wheat genotypes and/or the different interactions between D-genome alleles and allelic variants occurred at the *Glu–A1, Glu-B1* and *Glu-B3* loci (Peña et al., 1995).

Allelic distribution and composition of LMW-GS and gliadins

LMW-GS and gliadin alleles in SHW expressed additive gene combinations as a result of their parental genotypes in crosses (Doneva and Spetsov, 2013). The variation in LMW-GS was very low as compared to variation in HMW-GS. Three alleles at the *Glu-A3* locus (b, c and d) were identified, among which allele b was the most distributed (Fig. 2). At the *Glu-D^{t}3* locus, two alleles (*a* and *c*) were identified, and the latter one expressed larger frequency (Fig. 3). SDS-PAGE analysis with 4M urea showed heterogeneity in SHW 530 and 532 at the *Glu-A3* locus, and in SHW 32 – at the *Glu-A3* and *Glu-D^{t}3* loci.

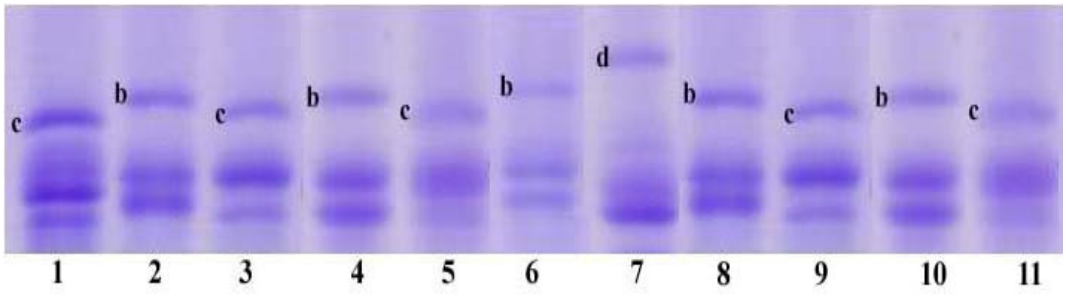

Figure 2. LMW-GS profile at *Glu-A3* locus at 12% SDS-PAGE with 4M urea, 1: Bezostaya1 (check), 2: 530-1, 3: 530-2, 4: 532-1, 5: 532-2, 6: 531, 7: 83/27 , 8: 107, 9: 106, 10: 32-1, 11: 32-2.

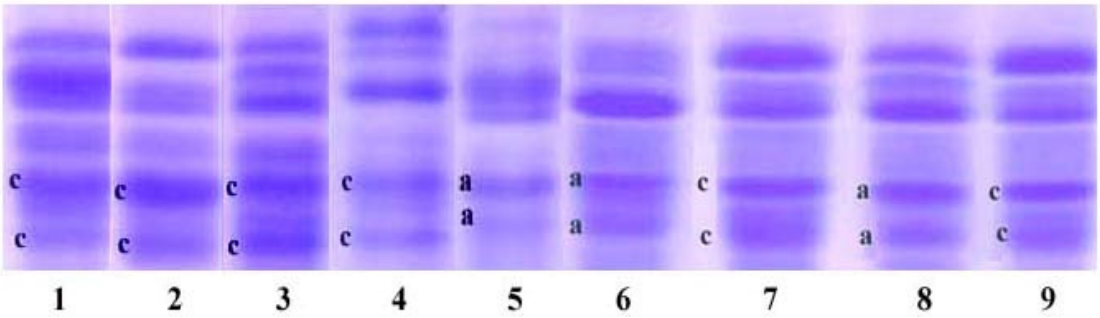

Figure 3. LMW-GS profile at *Glu-D3* locus at 12% SDS-PAGE, 1: Bezostaya1 (check), 2: 530, 3: 531, 4:532, 5: 83/27 , 6: 107, 7: 106, 8: 32-1, 9: 32-2.

A-PAGE electrophoresis for gliadins displayed that SHW 106, 107, 32, 531 and 83/27 were homogenous, while SHW 530 and 32 - heterogenous (Figs. 4 and 5). The variation at the *Gli-D*^{t} loci was due to *Aegilops tauschii* accessions from one side, and the hybrid females used in SHW breeding from the other side. The established nomenclature for gliadins in common wheat could be used in SHW to some extent (Doneva and Spetsov, 2013). The slow-moving ω-bands at the *Gli-D1* locus, characteristics for the wheat checks, were missing in SHW. The pair of ω-components (ω1 and ω2), reported firstly by Wrigley and Shepherd (1974) as typical for common wheat, was applied for identification of gliadins in hexaploid wheat accessions (Fig. 4).

Generally, *Gli-D2* blocks comprise gliadins in β- and α-regions. Most of bands in these blocks are better coloured than the areas at the *Gli-D1* locus. As observed in gliadin spectrum of *Ae. tauschii*, we found some overlapping in γ- и β- regions between bands at *Gli-D1* and *Gli-D2* in SHW. It is accepted that weaker coloured and slow-moving bands, at the beginning of β- region, are defined in the *Gli-D1* group, as compared to more intensive in colour and situated at the end of γ-region, considering to belong to *Gli-D2* locus (Lagudah and Halloran, 1987). Some β- and mostly α- gliadins of the diploid parents were not expressed in synthetics (Fig.4, Doneva and Spetsov, 2011). In this case, our results support the data published by William et al. (1993) and Yan et al. (2003). A number of gliadin blocks at the *Gli–D* locus are considered to have positive effect on gluten strength (Yan et al., 2003). A direct transfer of new gliadin alleles from SHW to wheat could enhance the variability in genes, predominantly those responsible for quality in bread wheat.

Gliadin spectrum in SHW showed that six of them (№ 32, 106, 107, 530, 531 and 532) expressed γ-*45,* which is controlled by allele at the *Gli-B1* locus (Figs. 4 and 5). This gene originated from *Triticum turgidum* accessions and was accredited to high gluten quality. SHW 83/27 exerted γ-43.5, which is commonly distributed in cultivated wheat, considering as marker for good gluten quality, too (Todorov, 2006).

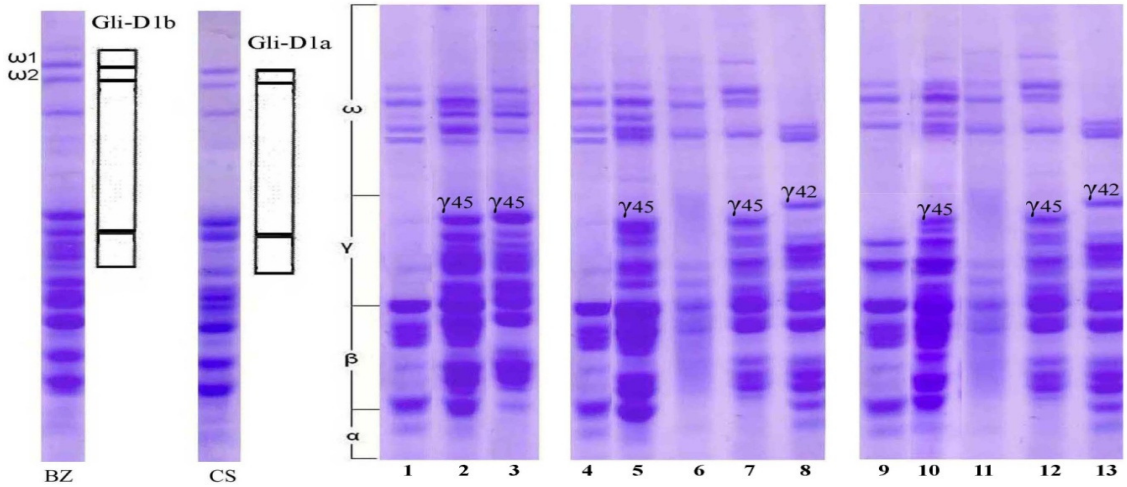

Figure 4. Gliadin profile at A-PAGE. 1: *Ae. tauschii* 22744, 2: SHW 107, 3: *T. dicoccum* 45398, 4: *Ae. tauschii* 22744, 5: SHW 106, 6: *T. dicoccum* 44961, 7: *T. dicoccum* 45432, 8: *T. durum* Zagorka, 9: *Ae. tauschii* 19089, 10: SHW 32, 11: *T. dicoccum* 44961, 12: *T. dicoccum* 45432, 13: *T. durum* Zagorka, BZ: Bezostaya1 (check), CS: Chinese Spring (check).

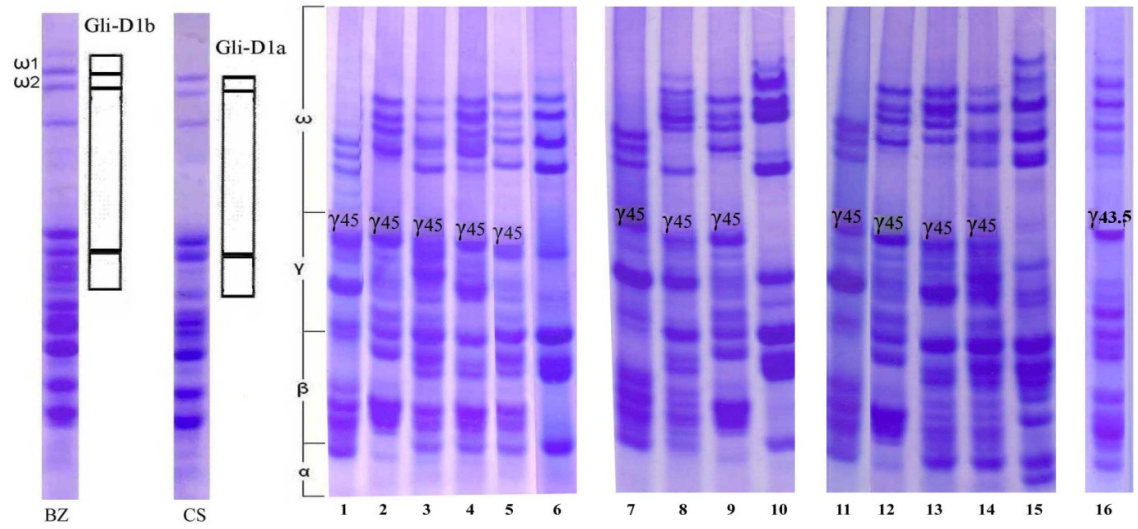

Figure 5. Gliadin profile at A-PAGE. 1: *T. dicoccum* 45390, 2: *T. dicoccum* 45398, 3: SHW 530-1, 4: SHW 530-2, 5: SHW 530-3, 6: *Ae. tauschii* 19088, 7: *T. dicoccum* 45390, 8: SHW 531, 9: *T. dicoccum* 45398, 10: *Ae. tauschii* 30422, 11: *T. dicoccum* 45390, 12: *T. dicoccum* 45398, 13: SHW 532-1, 14: SHW532-2, 15: *Ae. tauschii* 22744, 16: SHW 83/27, BZ: Bezostaya1 (check), CS: Chinese Spring (check).

Conclusions

HMW-GS subunit 1.5+10 was expressed in four synthetics, SHW 32, 106, 107 and 532, which is controlled by *Glu-D1ah* allele. This gene originated from *Aegilops tauschii* accessions № 19089 and 22744, and seemed to be a perspective allele for bread wheat improvement. *Ae. tauschii* accessions № 19088 and 30422 donated subunits 4+10.1 and 2+11 to SHW 530 and 531, respectively. One subunit 1Ax1.1 was found in four SHW, which is unusual for the A-genome of hexaploid wheat, too.

At the *Glu-B1* locus, five x-type subunits, viz., 7, 13, 17, 14 and 22, and four y-type subunits viz., 8, 16, 18 and 15, and their five combinations were detected.

LMW-GS analysis revealed two different lines in each of SHW 32, 530 and 532 (with b and c alleles), that was further supported by the gliadin compositions. The new gliadin genes in synthetics (γ-43.5, γ-45, and alleles encoding different ω- and β - subunits) may have significance for the plant breeding to improve the gluten quality in wheat.

References

Branlard, G., Dardevet, M., 1985. Diversity of grain proteins and bread wheat quality. Correlation between high-molecular-weight subunits of glutenin and flour quality characteristics. J Cereal Sci 3: 345-354.

Ciaffi, M., Margiotta, B., Colaprico, G., De-Stafanis, E., Sgrulletta, D., Lafiandra, D., 1995. Effect of high temperatures during grain filling on the amount of insoluble proteins in durum wheat. J Genet Breed 49: 285-296.

Doneva, S., Spetsov, P., 2011. Polymorphism in glutenins and gliadins in *Aegilops tauschii* accessions (2n=14, DD). Field Crop Studies, t. VII-2: 243-254 (in Bulg.).

Doneva, S., Spetsov, P., 2013. Protein compositions in three synthetic hexaploid wheats (2n=42, BBA^UA^UDD). Annual scientific book of TU–Varna, t. 2: 23-29 (in Bulg.).

Gupta, R.B., Shepherd, K.W., 1988. Low-molecular glutenin subunits in wheat: their variation, inheritance and association with bread-making quality. Proc of 7th Int. Wheat Genet. Symposium, Cambridge, UK, 13-19 July: 943-949.

Gupta, R.B., Shepherd, K.W., 1990. Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. 1. Varation and genetic control of the subunits in hexaploid wheats. Theor Appl Genet 80: 65-74.

Jackson, E.A., Morel, M.H., Sontag-Strohm T., Branlard, G., Metakovsky, E.V., Redaelli, R., 1996. Proposal for combining the classification system of alleles of Gli-1 and Gli-3 loci in bread wheat (*Triticum aestivum* L.). J Genet & Breed 50: 321-336.

Khan, K., Mcdonald, C.E., Banasik, O.J., 1983. Polyacrylamide gel electrophoresis of gliadin proteins of wheat variety identification – procedural modifications

and observations. Cereal Chem 60: 178-181.

Laemmli, U. K., 1970. Clavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, vol. 227: 680 – 685.

Lafiandra, D., D'Ovodio, R., Porceddu, E., Margiotta, B., Colaprico, G., 1993. New data supporting high mol glutenin subunit 5 as the determinant of quality differences among the pairs 5+10 and 2+12. J Cereal Sci 18: 197 – 205.

Lagudah, E. S., MacRitchie, F., Halloran, G.M., 1987. The influence of high – molecular weight subunits of glutenin from *T. tauschii* on flour quality of synthetic hexaploid wheat. J Cereal Sci 5: 129 – 138.

Li, Y., Chengyan, H., Xinxia, S., Qiunqi, F., Genying, L., Xiusheng, C., 2009. Genetic variation of wheat glutenin subunits between landraces and varieties and their contributions to wheat quality improvement in China. Euphytica 169: 159-168.

Metakovsky, E.V., 1991. Gliadin allele identification in common wheat. II. Catalogue of gliadin alleles in common wheat. J Genet & Breed 45: 325-344.

Payne, P.I., 1987. Genetics of wheat storage proteins and the effect of allelic variations on bread – making quality. Ann. Rev. Plant Physiol. 38: 141 – 153.

Payne, P.I., Holt, M., Jackson, E.A., Law, C.N., 1984. Wheat storage proteins: their genetics and potential for manipulation by plant breeding. Philos Trans R Soc Lond B 304: 359-371.

Payne, P.I., Lawrence, G.J., 1983. Catalogue of alleles for the complex gene loci Glu-A1, Glu–B1 and Glu–D1 which code for high–molecular weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11: 29-35.

Payne, P.I., Holt, L.M., Law, C.N., 1981. Structural and genetical studies of the high–molecular weight subunits of wheat glutenin. 1. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum)*. Theor Appl Genet 60: 229 – 236.

Peña, R.J., Zarco–Hernandez, J., Mujeeb–Kazi, A., 1995. Compositions and bread–making quality characteristics of synthetic hexaploid wheats derived from *Triticum turgidum* x *Triticum tauschii* (Coss.) Schmal. crosses. J Cereal Sci 21: 15 – 23.

Plamenov, D., Spetsov, P., 2011. Synthetic hexaploid lines are valuable resources for biotic stress resistance in wheat improvement. J Plant Pathol 93 (2): 251-262.

Rasheed, A., Safrad, T., Gul-Kazi, A., Mahmood, T., Akram, Z., 2012. Characterization of HMW-GS and evalution of their diversity in morphologically elite synthetic hexaploid wheats. Breed Sci 62: 365-372.

Singh, N.K., Shepherd, K.W., Cornish, G.B., 1991. A simplified SDS–PAGE procedure for separating LMW subunits of glutenins. J Cereal Sci 14: 203 – 208.

Tang, Y., Yang, W., Tian, J., Li, J., Chen, F., 2008. Effect of HMW-GS 6+8 and 1.5+10 from synthetic hexaploid wheat on wheat quality traits. Agricultural Science of China 7: 1161-1171.

Todorov, I., 2006. Study on seed proteins and their application as genetic markers in wheat breeding. D. Sci. dissertation, NCAN, Sofia.

Trethowan, R.M., van Ginkel, M., 2009. Synthetic wheat – an emerging genetic resource. In: Carver B. (ed.). Wheat - Science and Trade: 369-386. Wiley-Blackwell, Ames, IO, USA.

Wrigley, C.W., Shepherd, K.W., 1974. Identification of Australian wheat cultivars by laboratory procedures. Examination of pure samples of grain. Aust J Exp Agric Anim Husb 14: 796.

William, M.D.H., Peña, R.J., Mujeeb–Kazi, A., 1993. Variation of seed proteins and isozymes in the *T. tauschii* (*Ae. squarrosa* 2n = 2x = 14, DD). Theor Appl Genet 87: 257 – 263.

Yan, Y.M., Hsam, S.L., Yu, J.Z., Jiang, Y., Zeller, F.J., 2003. Genetic polymorphisms at Gli–Dt gliadin loci in *Ae. tauschii* as revealed by acid polyacrylamide gel and capillary electrophoresis. Plant Breed 122: 120-124.