

EVALUATION OF THE EFFECT OF THE NEW FRACTION PAIR 1Dx1.5+1Dy10 INHERITED FROM *Glu-D^t* LOCUS OF *Ae. Tauschii* (D^tD^t, 2n=14) ON SOME QUALITATIVE INDICES IN THE PROGENIES OF SYNTHETIC WHEAT VARIETIES (BBA^uA^uD^tD^t, 2n=42)**Sonya Doneva¹, Nadia Daskalova², Penko Spetsov², Emil Penchev¹**¹Dobrudzha Agricultural Institute – General Toshevo, Bulgaria²Technical University – Varna, BulgariaCorresponding author: sonya_doneva@yahoo.com**Abstract**

The effect of the new fraction pair 1.5+10, coded by *Glu-D1* locus, on the qualitative indices protein, lysine and sedimentation value in synthetic hexaploid wheat varieties was investigated in comparison to common winter wheat standards. For this purpose, in two successive cropping seasons (2013 and 2014), grains from plants of the progenies of the synthetic haploid wheat genotypes 106, 107 and 32 were analyzed; preliminary SDS-PAGE analysis determined that they were carriers of the new allele *ah* inherited from the *Glu-D^t1* locus of *Ae. tauschii*. Data were analyzed with the help of descriptive statistics, t-criterion, correlation and regression analyses, which were at the basis of the conclusions made. The obtained results allow the assumption that in the synthetic hexaploid wheat genotypes the new allele *ah*, coding for the expression of the new fraction pair 1.5+10 in D-genome, has positive effect on the qualitative indices protein, lysine and sedimentation in contrast to the standard common wheat cultivars, which do not carry this subunit.

Keywords: protein, lysine, sedimentation, HMW-GS.**Introduction**

Common winter wheat *Triticum aestivum* L. (2n=6x=42, AABBDD) is distributed on large areas in many parts of the world due to its high plasticity to the growing environments and the universal usage of the wheat plant. After the 1960's, the efforts of the researchers have been directed toward intensive wheat breeding. The goal has been to induce genetic changes in *Triticum aestivum* in order to ensure more food of higher quality for the increasing global population. In the following years, a number of high-yielding cultivars with good adaptability to biotic and abiotic stress were developed as a result from the improved growing technology. Wheat production in China, India, Pakistan, Egypt, Canada, etc., increased considerably giving sustenance to millions of people. However, the intensive breeding of bread wheat based on a limited set of cultivars lead to loss of valuable genes in the new crosses and to a standstill in the yield potential of the crop. The major challenge to contemporary breeding is to preserve the valuable traits and genes from the wild species by applying the distant hybridization method, and to further transfer them in new bread wheat lines with a view of their use as initial breeding material in breeding (Spetsov, 2004; Jauhar and Peterson, 2006; Jauhar et al., 2009; Plamenov and Spetsov, 2011; Khalid, 2013; Gurmani et al., 2014; Daskalova, 2015). Thus the resistance to fungal diseases and insect pests can be successfully increased, as well as the productivity and quality of the cultivated wheat. In this relation, hexaploid wheat forms have been developed, which can be considered a bridge between the wild and the cultural forms (Carderini and Ortiz-Monasterio, 2003; Rana et al., 2013). They are carriers of valuable prolamin alleles (glutenin and gliadin) inherited from the parental forms *Ae. tauschii* and *T. turgidum* ssp. *durum* (Wieser et al., 2003; Yang et al., 2009; Lage et al., 2006; Hu et al., 2013), many of which have positive effect on the end-use technological indices of flour (Peña et al., 1995; Hsam et al., 2001; Wieser et al., 2003; Lage et al., 2006; Tang et al., 2008; Tang et al., 2010). The main aim of this investigation was to evaluate the effect of the new fraction pair 1.5+10, coded by locus *Glu-*

D1, on some qualitative indices (protein, lysine and sedimentation) in synthetic amphidiploids developed with the participation of the diploid species *Ae. tauschii*.

Material and methods

The materials (Table 1) subjected to this investigation were progenies of three synthetic hexaploid wheat forms (SHW) – No 106, 107 and 32; in two successive harvest years (2013 and 2014) grains from selected elite synthetic plants were analyzed for content of raw protein (%), lysine (mg/100 g absolute dry matter) and sedimentation (ml). By preliminary SDS-PAGE, it was found that the grains carried the new allele coding for the subunits pair 1.5+10 inherited from the *Glu-D^t* locus of accessions from the diploid species *Ae. tauschii* - No 22744 and No 19089. The qualitative indices (protein, lysine and sedimentation) of common wheat cultivars used as standards were also analyzed for comparison (Table 1). The progenies from SHW No 106, 107 and 32 were grown under field conditions during cropping seasons 2013 and 2014 together with standard common wheat cultivars. Planting was done manually in mid-October in plots of 1.5 m rows, and interspacing 5 cm between and plants and 20 cm between the rows. Eighty seeds, the progenies from four plants of SHW 106 were sown in two replications, as well as 140 seeds from 7 plants of SHW 107, 160 seeds from 8 plants of SHW 32 and bread wheat cultivars used as standards (Sadovska ranozreika-4, M. amber, No 301, ussalka, San Pastore, Bezostaya-1 and Mironovskaya-808). The previous crop was common bean without nitrogen fertilization.

Table 1. Harvested SHW No 106, 107 and 32 and standards for analysis of qualitative indices

Breeding No	Cross No	Grown progenies plants	
107	45398 x <i>Ae. tauschii</i> 22744	7	112
106	F ₂ (44961x Zagorka x 45432) x <i>Ae. tauschii</i> 22744	4	72
32	F ₁ (44961x Zagorka x 45432) x <i>Ae. tauschii</i> 19089	8	136
<i>T. aestivum</i> : San Pastore, M. amber, cultivar No 301, Russalka, Sadovska Ranozreika-4, Mironovskaya - 808, Bezostaya-1		2 rows per cultivars in two replications	

The extraction of the high molecular weight glutenins was carried out by the method of Singh et al. (1991). The electrophoresis was run in a vertical apparatus as standard monomeric polyacrylamide gel electrophoresis on 12 % separating gel (Laemmli, 1970) at constant electric current of 20 mA per plate and room temperature, for 10-20 hrs. The gels were stained in 1 % solution of Coomassie Brilliant Blue (CBB) R 250 in acetic acid, methanol and water at ratio (1:5:4) overnight, and destained in a solution of acetic acid, methanol and distilled water (1:2:7). The quality indices protein (%), lysine (mg/100 g abs. dry matter) and sedimentation (ml) were determined by: the classical Kjeldahl method for determination of total nitrogen using Keltec equipment (Cohen, 1910); the ninhydrin method for determination of lysine content of Museiko and Sysoev (1970), and the method of Pumpyanskyi (1971) for determination of the sedimentation value. To estimate the effect of the new fraction pair 1.5+10 from *Glu-D1* locus on the qualitative indices (protein, lysine and sedimentation), the statistical packages SPSS 17.0 and Biostat 7.0 (Penchev, 1998) were used. The methods of descriptive statistics, the t-criterion (criterion of Student), correlation and linear regression analyses were applied. Based on the data from the regression analysis, regression equations of the type $Y = b \cdot X + a$ (a – regression constant; b – regression coefficient) were composed, which represented the functional relation between the two variables – protein (X) and sedimentation (Y). This relation was represented also through regression lines, which allowed determining the sedimentation values positioned on the ordinate through the values of the protein positioned on the abscissa.

Results and discussion

In two successive cropping seasons, 2013 and 2014, grains from selected plants of progenies of the synthetic aphidiploids No 106, 107 and 32 were analyzed through SDS-PAGE electrophoresis. It was found that all three synthetic forms carried the new allele *ah* coding for fraction pair 1.5+10, which was inherited from locus *Glu-D^t* of the diploid species *Aegilops tauschii* No 22744 (SHW No 107 and 106) and *Aegilops tauschii* No 19089 (SHW No 32) (Figure 1).

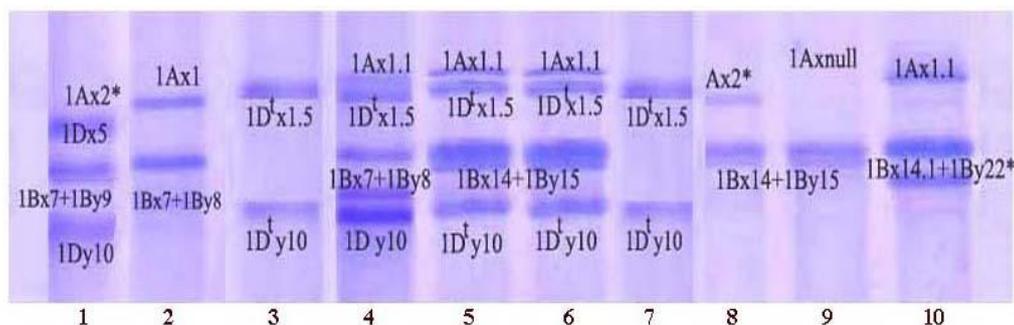


Figure 1. High molecular glutenin allelic composition of SHW: 1. Bezostaya1 (standard), 2. No 45398 (*T.dicoccum*, parent), 3. No 2744 (*Ae. tauschii*, parent), 4. SHW No 107, 5. SHW No 106, 6. SHW No 32, 7. No 19089 (*Ae. tauschii*, parent) 8. Zagorka (*T.durum*, parent), 9. No 44961 (*T.dicoccum*, parent), 10. 45432 (*T.dicoccum*, parent)

Tables 2 and 3 present the statistical parameters of the qualitative indices obtained from the analysis of the grain from the three synthetic wheat forms and from the standard common wheat cultivars. The mean value of the index protein in the synthetic wheat forms significantly exceeded the standards in both years of the investigation. This is a confirmation of the findings of Peña et al. (1995) that the lines which carry fraction pair 1.5+10 in *Glu-D1* locus have higher protein content in the flour. Concerning the indices lysine and sedimentation, the tendency toward higher mean values of the SHW forms in comparison to the standard bread wheat cultivars was present in both harvest years; this was yet another proof of the positive effect of the new allele *ah*, inherited from the *Ae. tauschii* accessions in the synthetic forms. The obtained estimations about the variation coefficients (VC%; Tables 2-3) revealed stability with regard to protein, lysine and sedimentation in the aphidiploids. In contrast, a more significant variation of the separate parameters was observed in the standard cultivars, of sedimentation in particular. These results allow the assumption that the new fraction pair 1.5+10 has a positive effect on the analyzed qualitative indices. T-test was applied for examination of the differences between the mean values of protein, lysine and sedimentation in SHW and common winter wheat. The results are given in Table 4. The difference determined between the mean values of the index protein in the amphidiploids and the standards during the two harvest years was statistically significant at the highest level – 11.28*** (harvest year 2013) and 13.66*** (harvest year 2014). The same tendency was registered also for the index lysine – 12.23*** (harvest year 2013) and 12.64*** (harvest year 2014). For sedimentation, the difference between the means of the synthetic forms and the common wheat cultivars (2.69* - harvest year 2013; 2.80* - harvest year 2014) was less significant, at level P = 0.05 of the alternative hypothesis. The obtained results lead to the conclusion that the investigated allele (*ah*) in the amphidiploids was the main reason for the existing differences. To examine this hypothesis, the correlation coefficients between the qualitative indices of SHW and the standards were calculated (Table 5).

Table 2. Statistical parameters of protein, lysine and sedimentation in SHW and standard common wheat cultivars during harvest year 2013

Trait	SHW			Standards		
	2013					
	Xmean ¹	MS ²	VC% ³	Xmean ¹	MS ²	VC% ³
Raw protein, %	14.41±0.11	1.19	3.31	11.81±0.20	0.54	4.56
Lysine, mg/100g a.d.m.	305.31±2.14	9.31	3.05	247.00±4.26	11.28	4.56
Sedimentation, ml	63.68±0.84	3.67	5.76	54.57±3.29	8.70	15.93

¹ Means; ² Mean square deviation; ³ Variation coefficient

Table 3. Statistical parameters of protein, lysine and sedimentation in SHW and standard common wheat cultivars during harvest year 2014

Trait	SHW			Standards		
	2014					
	Xmean ¹	MS ²	VC% ³	Xmean ¹	MS ²	VC% ³
Raw protein, %	15.75±0.06	0.27	1.74	12.32±0.24	0.64	5.23
Lysine, mg/100g a.d.m.	337.42±1.98	8.64	2.56	261.42±5.67	15.01	5.74
Sedimentation, ml	65.84±0.86	3.76	5.71	57.57±4.04	10.69	18.57

¹ Means; ² Mean square deviation; ³ Variation coefficient

Table 4. Estimation of the differences between the means of the qualitative indices in SHW and the standards during harvest years 2013 and 2014 through t-test

	Protein				Lysine				Sedimentation			
	SHW/standards				SHW/standards				SHW/standards			
	texp ¹	t _{5%} ²	t _{1%} ²	df ³	t exp ¹	t _{5%} ²	t _{1%} ²	df ³	t exp ¹	t _{5%} ²	t _{1%} ²	df ³
1	11.28***	1.81	2.23	10	12.23***	1.83	2.26	9	2.69	1.89	2.36	7
2	13.66***	1.89	2.36	7	12.64***	1.86	2.31	8	2.80	1.89	2.36	7

¹ t-criterion; ² t-table; ³ degrees of freedom; 1-2013; 2-2014

*, **, *** at $P \leq 0.05; 0.01; 0.001$, respectively

In harvest year 2013, a high positive correlation was found in SHW and the standards between the qualitative indices protein and lysine – 0.903*** (SHW) and 0.999*** (standards). At a lower level of statistical significance, positive correlations were established between lysine and sedimentation (0.564**, $P=0.01$) and between protein and sedimentation (0.375*, $P=0.05$) in the synthetic forms, while in the standard cultivars the correlations between protein and sedimentation (-0.051) and between lysine and sedimentation (-0.074) were low and negative (Table 5).

Table 5. Correlations between the qualitative indices in SHW and the standards

Year	Synthetic hexaploid wheat				Standards	
	Lysine	Sedimentation		Lysine	Sedimentation	
2013	Protein	0.903***	0.375*	Protein	0.999***	
	Lysine		0.564**	Lysine	-0.051	
2014	Lysine			Lysine	-0.074	
	Protein	0.740***	0.385*	Protein	0.965***	
	Lysine		0.161	Lysine	0.476*	
					0.416*	

In harvest year 2014, the same tendency was observed toward high positive correlations between protein and lysine – 0.740*** (SHW) and 0.965*** (standards). In the synthetic forms, the correlations between raw protein and sedimentation (0.385*) and lysine and sedimentation (0.161) were less expressed than in 2013, although remaining stable and positive. In the standards, in contrast to 2013, there were positive correlations significant at a lower level between protein and sedimentation (0.476*) and lysine and sedimentation (0.416*) (Table 5). The estimation of the correlation coefficients revealed that they were largely dependant on the climatic conditions, i.e. the effect of the phenotype in the different cropping seasons on the correlation between the qualitative indices in SHW was much better expressed in comparison to the effect of the new fraction pair 1.5+10. In order to specify the investigated correlations, linear regression analysis was applied, in which sedimentation was the dependent variable (Y), and protein was the independent one (X). The established type of relationship between the two qualitative indices in both investigated cropping seasons showed that the trends in SHW and the standards were the same, i.e. the effect of the agro meteorological conditions was much stronger than the effect of the new alleles. The calculated regression equations were an evidence for this, as well as the graphic and analytical models of the amphidiploid and the common wheat cultivars in cropping seasons 2013 and 2014 (Figure 2A-B; Figure 3A-B).

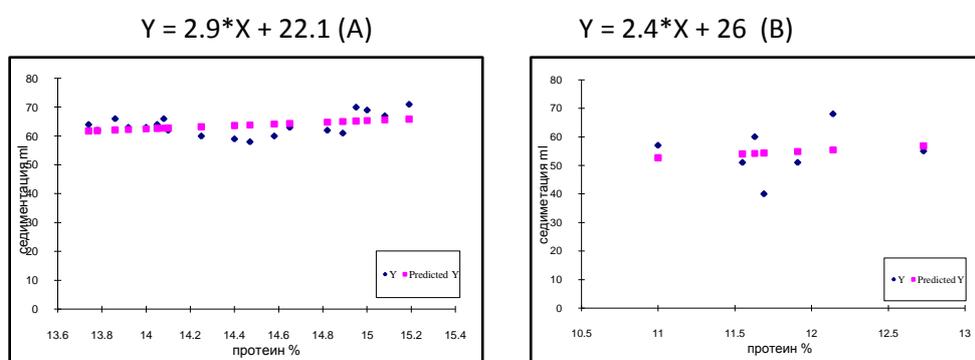


Figure 2. Graphic model sedimentation/protein in SHW (A) and standard (B), harvest year 2013

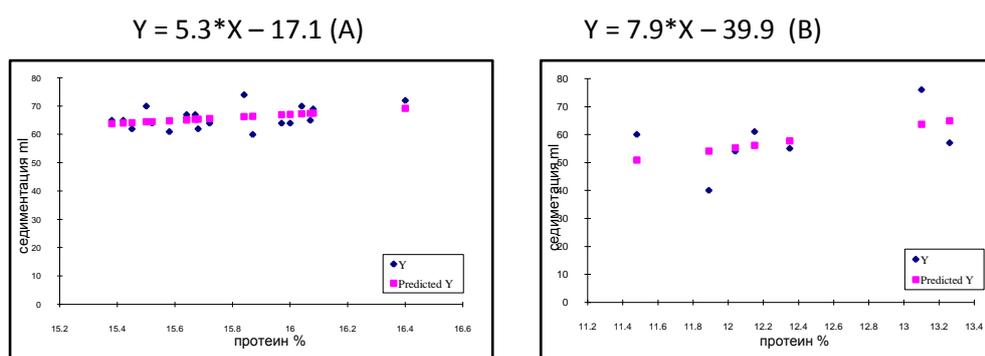


Figure 3. Graphic model sedimentation/protein in SHW (A) and standards (B), harvest year 2014

The evaluations in this investigation of the effect of the new allele *ah* from *Glu-D1* locus on the qualitative indices protein, lysine and sedimentation in the progenies of synthetic hexaploid wheat forms No 106, 107 and 32 were confirmed by the results from similar studies of other authors, as well. Ali et al. (2013) investigated the glutenin composition and the main qualitative parameters: raw protein content (%) and SDS-sedimentation volume of 24 conventional common wheat cultivars and 26 hexaploid wheat forms developed with the participation of synthetic forms; they found out that the synthetic hexaploid derivatives were characterized with considerable genetic variability in loci *Glu-A1* (0.38) and *Glu-D1* (0.59), and with higher protein content (13.8 ± 0.9) and SDS-sedimentation

volume (3.5±0.3) in comparison to the bread wheat cultivars. Analyzing 202 SHW lines, Rasheed et al. (2012^a) identified a number of new *Glu-D1* alleles (*h, n, x, ae, ah, ai, aj*) coding for subunits 5+12, 2.1+10, 2+12.2, 2.1+12.2, 1.5+10, 2.1+10.5 and 1.5+12. When investigating their effect on the end breadmaking properties, they found out that the synthetic derivatives which possessed fraction pairs 2.1+10 and 1.5+10 had higher protein content in comparison to those possessing 2.1+12.2. On the other hand, the lines with fraction pairs 1.5+10, 2.1+12.2, 5+12 and 3+10 had higher bread volume and higher sedimentation. Wieser et al. (2003) investigated the correlations between the amount of the gluten proteins and the technological indices of synthetic hexaploid forms and found out that neither the raw protein content nor the total amount of gluten protein was in correlation with any of the studied quality parameters, although the protein content in flour is a very important factor in bread making. On the other hand, they obtained data on existing positive correlations between the high and low molecular weight glutenin subunits and the SDS-sedimentation volume, gluten index, maximum resistance of dough and bread loaf, while the gliadins to glutenins ratio was in negative correlation with some of the above indices (bread volume, maximum resistance of dough). The results from the investigations show that the researches on the genetics of the qualitative indices are important for the improvement of the breeding process since these results allow determining the relationships between the composition of the storage endosperm proteins and the technological properties of grain. (Tabasum et al., 2011; Carter et al., 2012).

Conclusions

The statistical data on the effect of the new fraction pair 1.5+10 on the content of raw protein, lysine and sedimentation allow drawing the following conclusions:

1. As a result from the positive influence of the new allele inherited in synthetic hexaploid wheat forms No 106, 107 and 32 from the D-genome *Aegilops tauschii*, higher mean values of protein, lysine and sedimentation were determined in comparison to the standard common wheat cultivars.
2. The variation coefficients of the investigated qualitative indices showed higher stability of the synthetic forms due to the effect of the new allele.
3. The correlations and the regression equations determined that the relationships between protein, lysine and sedimentation in SHW were unidirectional with the relationships in the standard cultivars, i.e. the effect of the phenotype on the correlations between protein, lysine and sedimentation was much better expressed as compared to the effect of the new fraction pair from locus *Glu-D1*.

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