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POLYMORPHISM OF ENDOSPERM PROTEINS IN AMPHIDIPLOIDS WITH THE G GENOME OF *Triticum timopheevii* (Zhuk.)

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Abstract
During evolution in *Triticum* the diversity of genes in *T. aestivum* L. was greatly reduced compared to its ancestors. This tendency restricted further improvement of productivity and quality in common wheat and narrowed the plant resistance to biotic and abiotic stresses. Wide hybridization resulted in synthetic genotypes that offered opportunities for introduction of new genes for useful traits in breeding. The objects of this study were two amphidiploids with G-genome inherited from tetraploid wheat relative *T. timopheevii* (2n=28, GGA^uA^u). Glutenin and gliadin allelic composition of the synthetic wheats H-68/44 and H-69/36 were analysed by SDS-PAGE and A-PAGE electrophoretic methods. New allelic variants in Glu-G1 loci, which are not characteristics for the spectrum of *T. aestivum*, were identified. In contrast to the high polymorphism of amphidiploids for high-molecular weight proteins, variation in the low-molecular glutenins was much less. More gliadin alleles in synthetic lines were found than in hexaploid wheat, due to the parent polymorphism. The results of this survey showed that synthetics with *T. timopheevii* genome might serve as an important sources of increased genetic variation for endosperm proteins in common wheat.

Keywords: synthetic wheats, *T. timopheevii*, glutenins, SDS-PAGE, gliadins, A-PAGE.

Introduction

Many cultivated and wild species from *Aegilops – Triticum* group possessed various and useful genes for wheat improvement (Monneveux *et al*., 2000; Zaharieva *et al*., 2003; Mujeeb-Kazi, 2005; Spetsov *et al*. 2006). Various synthetic and translocated genotypes were developed from *Triticum* x *Aegilops* crosses and used as bridges to transfer different breeding traits to common wheat (Jauhar and Peterson, 2006; Plamenov and Spetsov, 2011).

Wheat grain quality depends on gluten, which is the complex endosperm protein. It consists of two prolamin groups–glutenins and gliadins. Glutenins include high molecular and low molecular proteins, shortly named as HMW-GS and LMW-GS, respectively. HMW-GS are coded by two genes (x- and y-), which are localized in three loci (Glu-A1, Glu-B1, Glu-D1) on the long arms of the homoeologous group 1. LMW-GS are classified in three groups (B, C и D) due to their molecular weight and isoelectrical points (Jackson *et al*., 1983). Genes, responsible for them, are localized in the short arms of the homoeologous group 1 (Glu-A3, Glu-B3 and Glu-D3 loci). Gliadins are monomeric proteins and electrophoretically separated in α-, β-, γ-, ω- gliadins. Two of them (α- and β-) are coded by Gli-2 loci in the short arms of chromosomes 6A, 6B и 6D, and others (γ- and ω-) by Gli-1 loci in the short arms of chromosomes 1A, 1B and 1D (Masci *et al*., 2002). There is a strong connection between Glu-3 loci, responsible for LMW-GS and Gli-1 loci, conducting the output of gliadins (Singh and Shepherd, 1988).

Some investigations showed that the variation of Glu-1, Glu-3 и Gli-1 loci in bread wheat was limited (Gianibelli *et al*., 2001; Li *et al*., 2007). Related species to wheat as *Ae. tauschii* (Yan *et al*., 2003), *T. turgidum* (Li *et al*., 2006) and *T. monococcum* (Ciaffi *et al*., 1998) expressed a large diversity of glutenin and

gliadin alleles. New HMW-GS have been detected in the A genome of diploid *T. urartu* and some tetraploid species, which are potential sources for genes in wheat improvement.

T. timopheevi (GGA^uA^u) is a hulled tetraploid wheat relative (Goncharov *et al*., 2009) and still in seed production to some extent in the Caucasus (Wan *et al*., 2002). Its genomes are considered as homoeologous to wheat A- and B- genomes (Brown-Guedira *et al*., 1997). High protein content (19-22%), diversity in HMW-GS and resistance to biotic stresses are remarkable breeding traits for this tetraploid species (Obukhova *et al*. 2009). Genes from the G genome, responsible for endosperm proteins, are probably similar to those, governing the protein synthesis in hexaploid wheat. Through SDS-PAGE и PCR methods Li *et al*. (2002) found 8 allelic variants in Glu-G1, suggesting *T. timopheevi* as a valuable source for new glutenin genes in bread wheat (Li *et al*., 2007). Two genes, belonging to A- and G-genome, coding for x- and y- subunits, were isolated (Wan *et al*., 2002). It is proved that wheat lines with both types (x- and y-subunits) in Glu-A1 are better in quality than plants having only x-type subunit (Johansson *et al*., 1993).

Despite that numerous researches has been focused on seed proteins, *T. timopheevi* is deeply involved in wheat improvement as a resource of genes not only for grain quality, but also for fungi resistance – leaf rust, stem rust, powdery mildew and fusarium (McIntosh *et al*., 2008; Leonova *et al*., 2011).

Characterization of storage proteins (glutenins and gliadins) in two amphidiploids possessing the G-genome from *Triticum timopheevii*, is the main purpose of this study. The two synthetics differ with the second parent used in the cross. Analysis of synthetic lines may increase their role as important source of novel protein genes for wheat breeding.

Materials and methods

Synthetic hexaploid wheat H-68/44 was obtained from the cross between *T. timopheevii* (GGA^uA^u) and *Aegilops tauschii* (DD), and the second one, H-69/36 - between *T. turanicum* (BBA^uA^u) and *T. timopheevii* (GGA^uA^u) (Table 1). Two bread wheat varieties, Bezostaya 1 and Chinese Spring, were used as standards in biochemical analyses.

Table 1. Breeding number and genome formulae of synthetic wheats

Breeding No	C r o s s	Genome formula[1] (2n)
H-69/36	*T. turanicum* x *T. timopheevii*	$BBA^uA^uGGA^uA^u$
H-68/44	*T. timopheevii* x *Ae. tauschii*	GGA^uA^uDD

[1], Genome formulae are according Goncharov et al. (2009).

Glutenins (HMW- and LMW-GS) were extracted according to Singh *et al*. (1991). 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). The electrophoresis run on vertical apparatus in two ways: a) classical one-dimensional 12% polyacrylamide gel (Laemmli, 1970); б) one-dimensional 10% polyacrylamide gel SDS – PAGE with addition of 4M urea (Lafiandra *et al*., 1993).

Arrangement and numbering of HMW-GS in wheat was carried out according Payne and Lawrence (1983). LMW-GS nomenclature in wheat (Gupta and Shepherd, 1990) and combined method for LMW-GS and gliadin identification were adopted (Jackson *et al*., 1996). Alleles in Glu-D1 locus were described according to William *et al*. (1993), while subunits in Glu-G1 and Glu-A1 loci were marked and compared to those expressed in genomes of common wheat (Hu *et al*., 2012).

Results and discussion

Amphidiploid (AD) H-68/44 expressed the following HMW-GS subunits: 1Ax null in Glu-A1, 1Gx and 1Gy in Glu-G1, and the subunit pair 1Dx2 + 1Dy12.4 in Glu-D1 locus (Fig. 1-2). Glutenins displayed in Glu-A1 and Glu-G1 loci were inherited from the tetraploid *T. timopheevii*, and those found in Glu-D1 – from the diploid *Ae. tauschii*. These findings were supported by studies of Wan *et al*. (2002), Li *et al*. (2007) and Obukhova *et al*.

(2009) showing two HMW glutenin subunits in the G-genome, x- and y- subunit. They have lower electrophoretic mobility, but higher molecular weight than 1Bx7 in Glu-B1 of wheat varieties Bezostaya 1 and Chinese spring. The classical Laemmli system (12% SDS-PAGE) could not differentiate the subunits, expressed in *T. timopheevii*, because of their overlap. With the help of 10% SDS-PAGE with 4M urea, they were divided and identified (Todorov, 2006).

Synthetic H-69/36 exerted the following HMW-GS: 1Ax 2* and 1Ay in Glu-A1, 1Bx7 in Glu-B1, and 1Gx in Glu-G1 (Fig. 3-4). Allele *b*, coding 1Ax 2* in Glu-A1, was inherited from *T. turanicum.* Results of 12% SDS-PAGE and 10% SDS-PAGE with 4M urea showed the presence of only subunit x in Glu-G1. The second fraction, differing in molecular weight and electrophoretical mobility among those of 1By9 and 1Dy10 in Glu-1 of wheat checks Bezostaya 1 and Chinese Spring, was identified as subunit 1Ay, transferred to H-69/36 from the locus Glu-A1 of *T. timopheevii*. Our data are in conformity with Hu *et al*. (2012) for similarity of 1Ay with 1Bx7, 1By8, 1Dy10 and 1Dy12 in many *Triticum* species, and its exhibition even stronger than 1Dy12 in common wheat.

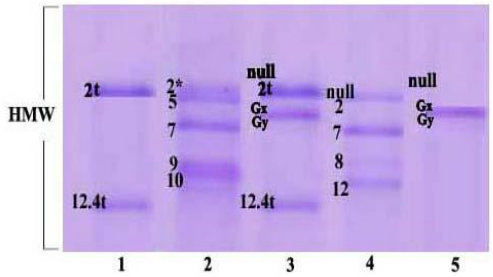

Fig. 1. 12% SDS-PAGE of HMW-GS: 1. *Ae. tauschii*, 2. Bezostaya 1; 3. AD H-68/44; 4. Chinese Spring; 5. *T. timopheevii*.

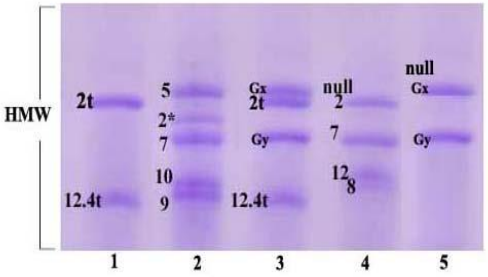

Fig. 2. 10% SDS-PAGE of HMW-GS wth urea: 1. *Ae. tauschii*; 2. Bezostaya 1; 3. AD H-68/44; 4. Chinese Spring; 5. *T. timopheevii*.

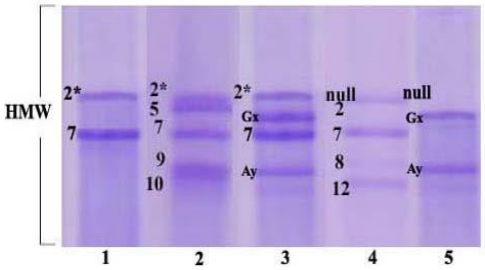

Fig. 3. 12% SDS-PAGE of HMW-GS: 1. *T. turanicum;* 2. Bezostaya 1; 3. AD H-69/36; 4. Chinese Spring; 5. *T. timopheevii*.

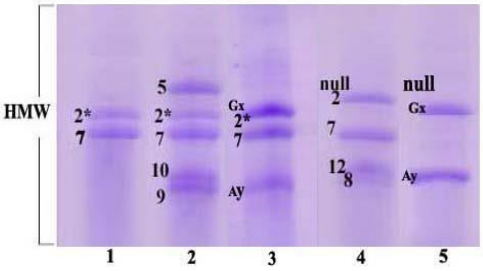

Fig. 4. 10% SDS-PAGE of HMW-GS wth urea: 1. *T. turanicum*; 2. Bezostaya 1; 3. AD H-69/36; 4. Chinese Spring; 5. *T. timopheevii*.

Synthetic H-68/44 expressed 12 fractions in LMW-GS (5 major and 7 minor), against 11 glutenin subunits for AD H-69/36 (Fig. 5-6). LMW-GS were identified in the B-zone, probably inherited from *T. timopheevii*. According to the nomenclature of Gupta and Sheppherd (1990) for the low molecular weight of glutenins in common wheat, the two synthetics displayed a subunit in Glu-A3, probably coded by *c* allele. Two subunits in Glu-D3 of H-68/44, similar to the expression of *a* allele in common wheat cv. Chinese Spring, were also found. They might originate from the diploid *Ae. tauschii*. The protein composition of Glu-B3 in AD H-69/36 was not identified.

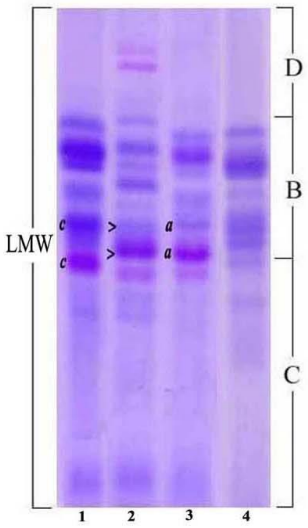

Fig. 5. 12% SDS-PAGE of LMW–GS in Glu-D3: 1. Bezostaya 1; 2. AD H-68/44; 3. Chinese Spring; 4. AD H-69/36; arrows indicate Glu-D3 subunits in H-68/44 on the *a* allele in Chinese Spring; Bezostaya 1 has *c* allele in Glu-D3.

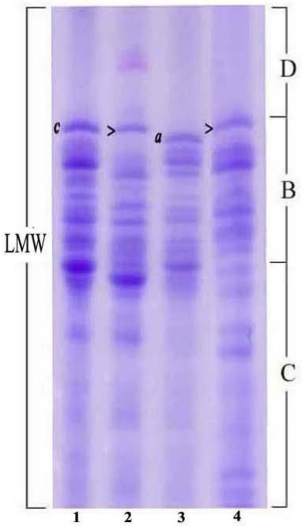

Fig. 6. 10% SDS-PAGE with 4M урея in Glu-A3: 1. Bezostaya 1; 2. AD H-68/44; 3. Chinese Spring; 4. AD H-69/36; arrows indicate Glu-A3 subunits on the level of *c* allele in Bezostaya 1; Chinese Spring has *a* allele in Glu-A3.

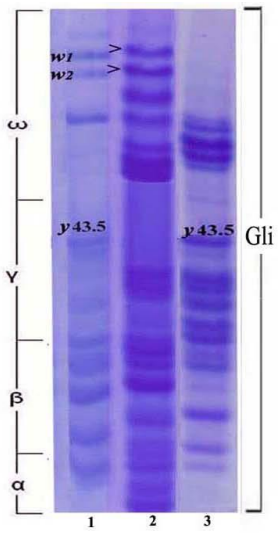

Fig. 7. A-PAGE in gliadins: 1. Bezostaya 1; 2. AD H-68/44; 3. AD H-69/36; arrows indicate Gli subunits corresponded to w_1 and w_2 from Bezostaya 1; $\gamma_{43.5}$ is marked in H-69/36 and Bezostaya1.

Each synthetic line was characterized by a distinct spectrum of gliadins (Fig. 7). AD H-68/44 exerted 22 bands (eight are ω-, five-γ-, six-β- and three-α-gliadins). Slow moving pair of ω-fractions, characteristic for Gli-D1 of wheat check Bezostaya 1 and for any hexaploid wheat variety, was also identified. Six ω-, six γ-, five β- and two α-gliadins were visualized in synthetic H-69/36. The typical ω_1- and ω_2- subunits for wheat cultivars were not expressed in this synthetic. One γ-gliadin *43.5*, coded by a gene in Gli-B1 locus, was indicated. This subunit is characteristic for common wheat, corresponding to good gluten quality (Todorov, 2006).

Conclusions

1. Expression of subunit 1Gx at Glu-G1 in two synthetic wheat lines involving the G genome of *Triticum timopheevii*, was recorded.
2. Synthetic H-69/36 exerted 1Ay subunit, coded by a gene in Glu-A1 locus. A gliadin for good gluten quality (γ- 43.5) was only registered in this amphidiploid.
3. Synthetic line H-68/44 displayed HMW-GS 1Dx2 and 1Dy12.4 subunits and different LMW-GS in B- and D-zones, which were absent in check wheat cultivars Bezostaya 1 and Chinese Spring. Additionally, both synthetics showed a lot of gliadin alleles and could be of great interest as sources of genes for improved grain quality in wheat.

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