Glu-1 AND *Glu-3* ALLELIC VARIABILITY OF GENUS *Triticum* – GENETIC RESOURCES IN WHEAT BREEDING

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Abstract:

SDS-PAGE was used in characterization of HMW and BLMW GS composition of 40 Triticum genotypes. Six alleles at Glu-A^{m}1 expressing four x and three y type subunits were scored. At Glu-A^{m}3 seven alleles were found. Tetraploid accessions showed three HMW GS of different electrophoretic mobility as well as a null Glu-A1 allele and ten HMW GS encoded by seven Glu-B1 alleles. Analysis of B LMW GS revealed eight specific electrophoregrams. Three to six LMW GS, encoded by Glu-A3 and Glu-B3 loci, characterized each phenotype. The obtained results represent a rich source of new variation in wheat storage proteins.

Keywords:

Wheat, glutenins, allelic variability, electrophoresis

1. INTRODUCTION

The unique properties of the wheat grain residue primarily in the gluten- forming storage proteins of its endosperm. Gliadins and glutenins are the main groups of storage proteins in wheat. Glutenin group members are classified as high- and low-molecular-weight glutenin subunits (HMW and LMW GS) according to their migration on SDS-PAGE. The synthesis of HMW and LMW GS is controlled by *Glu-1* and *Glu-3* loci, respectively. Both loci have been mapped to the homeologous group 1 chromosomes, together with the *Gli-1* loci, controlling the synthesis of major gliadin fractions. Several studies of storage proteins (5, 8, 14) have provided evidence of significant amount of genetic variation present at these loci in wild and less widely cultivated wheat genotypes.

As with most other crops, intensive breeding has lead to gene pool erosion within modern wheat cultivars. With the current availability of gene introgression methodologies (1,9), insights into the *Glu* and *Gli*

variation in wild relatives of cultivated wheats will enable the rapid transfer of genes encoding amenable glutenin and gliadin polypeptides.

The aim of the present paper was to assess the *Glu-1* and *Glu-3* allelic variability within and between different diploid and tetraploid wheat genotypes.

2. MATERIAL AND METHODS

Fifteen *T. monoccocum*, five *T. turgidum* var. *polonicum*, seven *T. turgidum* var. *dicoccum* accessions, and thirteen durum wheat cultivars (Tab. 1) originating from the Research Institute of Field and Vegetable Crops, Novi Sad (Serbia and Montenegro) and the Vavilov Institute, Sankt Petersburg, (Russia) were used in this study. Identification of individual alleles was assisted by the use of standard bread wheat cultivars (Chinese Spring, Hope, and Nizija).

Table 1. Analyzed wheat genotypes

Accession	Label	Accession	Label	Cultivar	Label
T. monococcum		*T. turgidum* var. *polonicum*		*T. turgidum* var. *durum*	
K-20984	M1	S 1302/95	P1	NSD 1	NSD 1
S 1312/95	M2	GK 330/91	P2	NSD 2	NSD 2
S 1313/95	M3	GK 331/91	P3	NSD 3	NSD 3
S 1314/95	M4	GK 332/91	P4	NSD 9	NSD 9
1315/95	M5	GK 333/91	P5	NSD 15	NSD 15
K-8555	M6	*T. turgidum* var. *dicoccum*		NSD 18	NSD 18
K-31566	M7	S 1303/95	D1	Waha	W
K-23650	M8	S 1304/95	D2	Herson	H
K-35914	M9	S 1306/95	D3	Omruf	O
K-1729	M10	S 1307/95	D4	Korifla	K
K-29603	M11	S 1308/95	D5	Stojocki	Stoj
K-20491	M12	S 1309/95	D6	Cham	C
K-14237	M13	S 1310/95	D7	Stork	S
K-23032	M14				
K-14379	M15				

Extraction and SDS-PAGE procedure for analysis of HMW GS followed the one by Vapa and Savic (12). Extraction of LMW GS followed the procedure of Gupta and MacRitchie (3). Gels were stained in Commasie Brilliant Blue R-250, or in the case of LMW GS, in silver (14).

3. RESULTS AND DISCUSSION

Analysis of *T. monococcum* seed storage proteins revealed six *Glu-A^m1* alleles, assigned as *Glu-A^m1*a to *Glu-A^m1*f (Fig.1). Expression of both x and y type of glutenin subunits was observed in all except accessions

M13 and M14 where only a single x or y subunit was scored, respectively. The mobility of x subunits was within or bellow the range of subunit 5 encoded by *Glu-D1* locus of the reference cultivar Hope (Fig. 1). Three products of active *Glu-A^{m}1*-2 genes exhibited minor size differences and were all slightly slower than the subunit 7 of the reference cultivar Nizija. Variability of *Glu-A1* locus in bread wheat is significantly lower when compared to the variation of *Glu-B1* and *Glu-D1* loci. In addition, only x-type *Glu-A1* genes were active (7, 13). The results reported here provide further evidence that *T. monococcum* possesses novel allelic forms at *Glu-A^{m}1* locus (Tab. 2) and that the silencing of *Glu-A^{m}1* genes encoding y type subunits is not a common feature of *Glu-1* genes in diploid relatives of bread wheat.

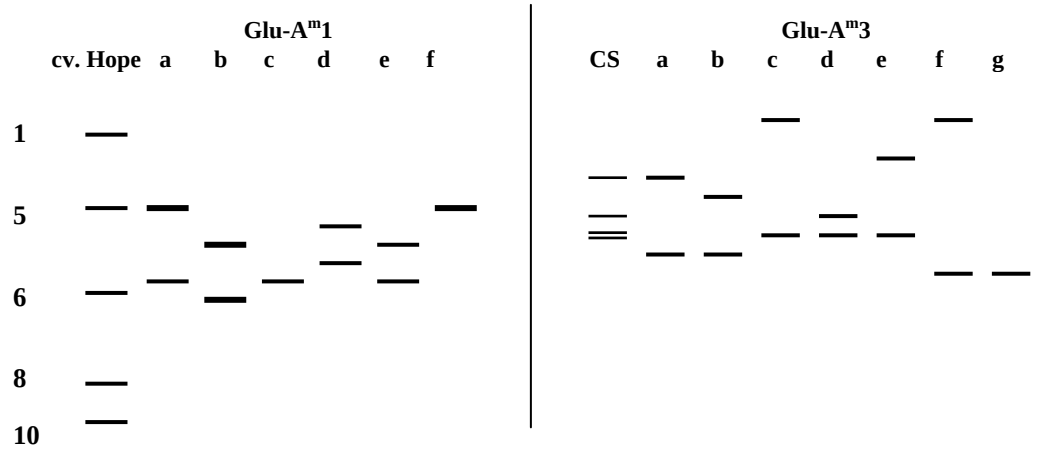

Figure 1. Diagrammatic representation of HMW and B LMW GS from T. monococcum accessions separated by SDS-PAGE. Control cultivars were Hope and Chinese Spring

SDS-PAGE analysis revealed seven different electrophoregrams of B LMW GS in *T. monococcum* all presumably coded by *Glu-3* alleles (Fig. 1). Presence of two subunits was observed in all except for M11 accession. Lee et al. (4) reported 60 different B LMW GS patterns among einkorn wheats accessions. In spite of a significantly smaller number of accessions analyzed, our results confirm a large extent of variation present at *Glu-3* locus of *T. monococcum.*

SDS-PAGE analysis of HMW GS in tetraploid wheats revealed three subunits of different electrophoretic mobility encoded by genes at *Glu-A1* as well as a null *Glu-A1* allele and ten HMW GS encoded by seven *Glu-B1* alleles (Fig. 2). The subunit 1 (*Glu-A1a*), has been found in majority of bread and durum wheat cultivars (2). In contrast, subunits 1' and 1'' of slightly higher electrophoretic mobility have been scored only in accession D1 and NSD3, as well as D6 and D7, respectively. These subunits represent novel allelic forms at *Glu-A1* locus.

Out of ten HMW GS encoded by *Glu-B1* alleles, the most frequent was the subunit 20, the product of *Glu-B1*e allele (Tab. 3). Another *Glu-B1* allele, *Glu-B1d*, present in worldwide durum wheat has been found in the

analyzed accessions, solely among the cultivated ones. The *Glu-B1* encoded HMW GS found in wild tetraploid accessions (subunits 8, 7', 14'+15' and 20') represent rare and presumably novel HMW GS (Fig 2).

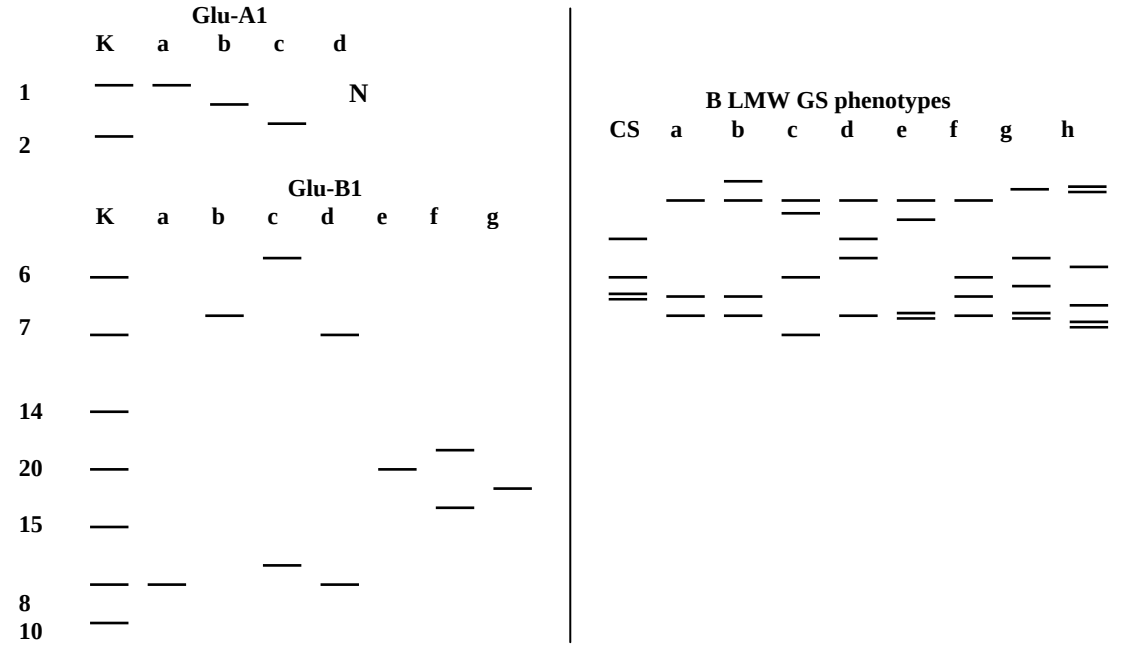

*Figure 2. Diagrammatic representation of HMW and B LMW GS from T. turgidum accessions and cultivars separated by SDS-PAGE. **K** stands for reference glutenin subunits, and **CS** stands for cv. Chinese Spring*

Table 2. HMW and B LMW GS composition of T. monococcum accessions

Locus	Allele	Accessions
Glu-A^{m}1	a	M2, M3
	b	M4, M5
	c	M14
	d	M11
	e	M1, M6, M7, M8, M9, M10, M12, M15
	f	M13
Glu-A^{m}3	a	M1
	b	M2, M3
	c	M4, M6, M7, M 8, M9, M10, M12
	d	M5
	e	M13, M15
	f	M14
	g	M11

Electrophoretic analysis of reduced and alkylated B LMW GS revealed the presence of eight specific B LMW GS electrophoregrams (Fig 2). Each

Glu-3 phenotype was characterized by three to six LMW GS subunits, encoded by *Glu-A3* and *Glu-B3* loci (Fig 2). Durum cultivars originated from Novi Sad, with the exception of NSD18, all had the same B LMW GS composition.

Table 3. HMW GS composition and B LMW phenotypes of T. turgidum genotypes

Locus	HMW GS (allele)	Accession	B LMW phenotype	Accession
Glu-A1	1 (a)	D2, D3, D4, D5, P1, NSD15	a	P2
	1' (b)	D1, NSD3	b	H, D6, D7
	1" (c)	D6, D7	c	P1, D1, D3, D4
	N (d)	P2-P5, NSD1-NSD3, NSD18, W, H, O, K, C, S, Stoj	d	C, W
Glu-B1	8 (a)	P1, D1, D3	e	NSD1, NSD2, NSD3, NSD9, NSD15
	7' (b)	D4, P3, P4	f	Stoj, S, P5, NSD18, O, K
	6'+8' (c)	D5, D2, NSD15, K	g	D2, D5
	7+8 (d)	NSD1, NSD9, W, H, C, S	h	P3, P4
	20 (e)	P5, NSD2, NSD3, NSD18, O, Stoj		
	14'+15' (f)	D6, D7		
	20' (g)	P2		

The high levels of LMW GS variation in tetraploid wheats among the accession analyzed in this paper are no exception to the findings of other authors (1, 5). Recently, five alleles at *Glu-A3* and 14 alleles at *Glu-B3* have been characterized in durum cultivars (6). Current views into the influence of different classes of seed storage proteins stress the HMW and LMW GS composition as the main factor in bread and pasta making quality (10).

4. ACKNOWLEDGEMENTS

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