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Evaluation of Winter Wheat Collection in Terms of HMW- and LMW-Glutenin Subunits

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Abstract: The composition of high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits was examined in a collection of 86 Czech registered winter wheat varieties. These proteins were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. An inter-varietal polymorphism of the HMW and LMW glutenin subunits was detected. Twenty-one different patterns for HMW were identified, and eighteen for the LMW-glutenins. The different alleles encoded at the six glutenin loci were determined. Three, six, and four alleles were observed, respectively at the *Glu-A1, Glu*-B1, and *Glu-D1* loci (encoding high HMW-GS). Three, eight, and three alleles of LMW-GS were found, respectively, at the *Glu-A3, Glu- B3*, and *Glu-D3* loci. The evaluated varieties were split into four categories of baking quality, and these variety groups were analyzed for the presence of different HMW-GS and LMW-GS alleles. While the alleles *Glu-B1c* (7+9), and *Glu-D1d* (5+10) were detected exclusively in bread wheat varieties, the alleles *Glu-B1d* (6+8), *Glu-D1a* (2+12), and *Glu-A3e/f* only occurred in those varieties that are not suitable for bread-making.

Keywords: characterization; electrophoresis; genetic diversity; glutenin subunits; *Triticum aestivum* L.

Glutenin, a major class of wheat storage proteins, is polymeric and consists of both high and low molecular weight subunits. Glutenin subunits have been separated by SDS-PAGE; with HMW glutenin subunits encoded by G*lu-A1, Glu-B1,* and G*lu-D1* (Payne 1987), and LMW glutenin subunits by the G*lu-A3, Glu-B3,* and G*lu-D3* loci (Singh & Shepherd 1988; Pogna *et al.* 1990). For many years, the high molecular weight glutenin subunits (HMW-GS) have been especially important for quality screening, using the *Glu-l* scoring system. The work of Payne *et al.* (1980) provided evidence of a strong association between the presence of certain alleles coding for HMW-GS and bread-making quality. Low molecular weight glutenin subunit composition (LMW-GS) can also affect different dough properties of hexaploid wheat (Gupta & MacRitchie 1994; Eagles *et al.* 2002).

MATERIALS AND METHODS

Plant materials

For the electrophoretic analyses of HMW-GS and LMW-GS, standard (etalon) samples of kernels of 86 registered winter wheat varieties were used. Seed samples were supplied by the Central Institute for Supervising and Testing in Agriculture (CISTA-Czech Republic).

Electrophoresis

Glutenins were extracted from single crushed wheat kernels, using the procedures of Singh *et al.* (1991) and Bradová (2006). Proteins were fractionated by one-dimensional sodium dodecyl sulphate

polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli buffer system (Laemmli 1970). The acrylamide / bisacrylamide concentration (*T*), and the cross linker (*C*) were used as follows: *T* = 10% and *C* = 2.60%. Electrophoresis was performed at a constant current of 30 mA/gel, at 10°C, for the time required for the tracking marker dye to migrate off the gel. Protein in the gels were fixed for 1 hour with 10% (w/v) trichloroacetic acid solution; and subsequently stained with 0.5% (w/v) Coomassie Brilliant Blue R-250 solution, 25% (v/v) methanol, and 10% (v/v) acetic acid. De-staining was carried out with running water.

Nomenclature

The bands of HMW-GS were read, using the nomenclature described by Payne and Lawrence (1983). The nomenclature of Jackson *et al.* (1996) and Branlard *et al.* (2003) was used for the LMW-GS.

RESULTS AND DISCUSSION

Polymorphism of HMW-GS and LMW-GS

In the studied set of 86 wheat varieties twenty-one different patterns for HMW-glutenins, and eighteen for LMW-glutenins were identified; a total of 50 different patterns were obtained at the *Glu-1* and *Glu-3* loci in this collection. Three, six, and four HMW-GS alleles were identified at the *Glu-A1, Glu-B1,* and *Glu-D1* loci, respectively, as shown in Table 1. Fourteen alleles encoding LMW-GS were observed in the collection. Three, eight, and three alleles corresponded to the *Glu-A3, Glu-B3,* and *Glu-D3* loci. The highest allelic variability of HMW-GS and LMW-GS was determined to be at the *Glu-B1* locus.

Some LMW-GS alleles were hard to identify. Essentially, the locus *Glu-B3* can be characterized by its higher variability and the migration of some subunits associated with this locus. A similar migration of subunits was associated with Glu-*A3.* We used genotypes with a known LMW-GS allele composition (Chinese Spring: *Glu-A3a, Glu-B3a, Glu-D3a;* Gabo: *Glu-A3b, Glu-B3b, Glu-D3b;* Orca: *Glu-A3d, Glu-B3c, Glu-D3d*) to characterize wheat varieties (Branlard *et al.* 2003). The varieties Karolinum, Livia, and Rialto are characterized by the same allele at the *Glu-B3* locus; we have not, however, been successful in the identification of this allele.

Allele frequencies and baking quality

The varieties registered in the Czech Republic were grouped into four categories, according to their baking quality, by the CISTA; the allele frequencies were determined in the individual categories (Table 2).

The highest frequency in the all baking quality categories (BQC) was detected in the "null" *Glu-A1c* allele. BQCs "E", "A", and "B" were characterized by the highest occurrence alleles *Glu-B1c* (7+9) and *Glu-D1d* (5+10), which are known for their favourable effect on dough properties (Payne *et al.* 1987). On the contrary, alleles *Glu-B1d* (6+8) and *Glu-D1a* (2+12), which have a negative effect on dough strength, were only detected in the BQC variety group "C" (varieties unsuitable for yeast dough production). These alleles in this variety group were present in 54% and 71% of the varieties, respectively.

Regarding the LMW-GS alleles: *Glu-A3a*, *Glu-A3d*, and *Glu-B3g* have a positive effect on dough properties (strength and extensibility); and the allele *Glu-D3c* has been reported to have an unfavourable effect on dough properties (Branlard *et al.* 2003). Some of these alleles (*Glu-A3a*, *Glu-B3g*, and *Glu-D3c*) were found as the most frequent in our collection; occurring in 47%, 67%, and 95% of the varieties, respectively. The allele *Glu-A3d* occurred in the highest frequency in varieties of category "E" (elite quality), but this category only consisted of 6 varieties. The allele *Glu-A3e*/*f* (45%) was only present in the varieties belonging to the "C" category (varieties unsuitable for yeast dough production). This allele probably does not have any positive effect on dough properties, and consequently on the baking quality of wheat.

Analysis of glutenins is known to be a powerful tool for the evaluation of genetic resources. The glutenin characteristics of the evaluated varieties can be decisively used in wheat breeding.

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Table 1. Allele frequencies of HMW-GS and LMW-GS in wheat varieties registered in the Czech Republic

HMW-GS					LMW-GS			
Locus	allele	HMW-GS	frequency	%	locus	allele	frequency	%
Glu-A1	a	1	24	28	*Glu-A3*	a	40	47
	b	2*	1	1		d	25	29
	c	null	61	71		e/f	21	24
Glu-B1	a	7	2	2	*Glu-B3*	a	3	4
	b	7+8	15	17		c	1	1
	c	7+9	34	40		c(d)	2	2
	d	6+8	28	33		d	4	5
	e	20	1	1		f	1	1
	i	17+18	6	7		g	58	67
						j	14	16
						?	3	4
Glu-D1	a	2+12	27	32	*Glu-D3*	a	1	1
	b	3+12	1	1		b	3	4
	c	4+12	1	1		c	82	95
	d	5+10	57	66				

Table 2. The most frequent HMW- and LMW-GS alleles in baking quality categories of wheat varieties

Locus	Allele	Baking quality categories (BQC)[1]							
		E (6 varieties)		A (34 varieties)		B (22 varieties)		C (24 varieties)	
		n	%	*n*	%	*n*	%	*n*	%
Glu-A1	0	5	83	20	59	17	77	19	79
Glu-B1	c (7+9)	3	50	15	44	10	45		
	d (6+8)							13	54
Glu-D1	d (5+10)	6	100	27	79	18	82		
	a (2+12)							17	71
Glu-A3	a			19	56	12	55		
	d	4	67						
	e/f							11	45
Glu-B3	g	4	67	26	76	13	59	15	63
Glu-D3	c	5	83	32	94	22	100	23	96

[1]E – elite quality wheat, A – bread wheat, B – alternative uses, C – not suitable for bread making

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