Session II: Biodiversity and Conservation

Poster presentation

Diversity of Gliadins and HMW Glutenin Subunits in Czech Registered Wheat Varieties

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Abstract: The composition of gliadins and HMW glutenin subunits in Czech registered wheat varieties was investigated by electrophoresis. The different alleles encoded at 6 gliadin and 3 glutenin loci were identified from a set of 80 varieties. Electrophoretic patterns of gliadins were obtained using SGE, HMW glutenin patterns using SDS PAGE. Gliadin diversity was high as 13, 5, 6, 8, 5, 10 and 9 alleles were found at *Gld-1-1A*, *Gld-2-1A*, *Gld-1B*, *Gld-1D*, *Gld-6A*, *Gld-6B* and *Gld-6D*, respectively. At *Glu-1A*, *Glu-1B* and *Glu-1D*, encoding HMW-GS, 3, 7 and 4 alleles were observed, respectively. Identification of the varieties was verified according to the composition of gliadins and HMW-GS. Genetic constitution was defined; the presence of the genetic protein markers of baking quality, frost hardiness and stem rust resistance was detected. Pedigrees of the varieties were checked so that patterns of the parent varieties and their hybrid progenies were compared.

Keywords: wheat; gliadin; glutenin; electrophoresis; variety identification

The use of registered crop varieties makes their expeditious identification important; its significance is increased by the diversity of varieties in many important traits. Each variety is characterised by a specific set of traits that determine its use. Gliadins and glutenins, i.e. storage proteins of wheat grain, are genetic markers allowing the expeditious and objective identification of a variety, determination of its genetic constitution, and determine some important characteristics and traits. The patterns of the particular components of gliadins and glutenins obtained by electrophoresis provide a description of each genotype based on the utilisation of the above-mentioned genetic markers.

MATERIAL AND METHODS

For electrophoretic analyses of gliadins and high-molecular-weight glutenin subunits (HMW-GS) we used standard (etalon) samples of seeds of 80 registered spring and winter wheat varieties received from the Central Institute for Supervising and Testing in Agriculture.

Gliadins were determined by a vertical starch gel electrophoresis (SGE) in Al-lactate buffer (Černý & Šašek 1996). Allelic gliadin blocks of zones were separated from the electrophoretic patterns according to Šašek *et al.* (2000). HMW-GS were determined by SDS-PAGE (Černý & Šašek 1996). Particular

Table 1. Diversity and frequency of gliadin and glutenin alleles in winter and spring wheat varieties

Loci	Number of alleles (*n*)			The most frequenced allele			
	spring wheat	winter wheat	spring and winter wheat	spring wheat		winter wheat	
				allele	(%)	allele	(%)
Gliadins							
1-1A	6	11	13	0	55.5	2	40.5
2-1A	4	5	5	2	55.5	0	69.0
1B	3	6	6	1	66.6	4	65.5
1D	3	7	8	7	44.4	1	70.2
6A	3	5	5	4	44.4	2	43.7
6B	4	8	10	1	77.7	1	58.8
6D	6	8	9	1	44.4	1	35.8
HMW-GS							
1A	3	2	3	1	53.4	0	77.5
1B	4	6	7	7+9	60.0	7+9	41.1,
1D	3	4	4	5+10	66.7	5+10	66.1

alleles of HMW-GS were identified according to Payne and Lawrence (1983).

RESULTS AND DISCUSSION

Allelic diversity and frequency of gliadin and glutenin alleles

In the studied set of 80 wheat varieties, 56 gliadin and 14 glutenin allelic blocks were identified (Table 1). The highest allelic variability was determined at gliadin locus *Gld 1-1A* where 13 allelic blocks were identified. The largest differences between spring and winter wheat were determined at gliadin loci *1-1A*, *1D* and *6B*. Rye translocation 1R/1B that is expressed by allelic block *GLD 1B3* (Černý & Šašek 1996) was identified in 14.5% of the studied winter varieties of wheat while this rye translocation is not present in the set of spring wheats.

Contrary to gliadins the highest variability of glutenins was expressed at locus *1B* (Table 1). Glutenin allelic blocks *GLU 1B6+8*, *GLU 1B17+18*, *GLU 1B20* were detected only in winter wheats whereas the glutenin allelic block *GLU 1B14+15* was present only in the set of spring wheats. Spring wheats were also characterised by the presence of 2* allele at 1A loci that was not identified in winter wheats. The most frequent combination of glutenin allelic blocks in winter wheats was *GLU 1A0*, *GLU 1B 7+9*, *GLU 1D 5+10*, in spring wheats *GLU 1A1*, *GLU 1B 7+9*, *GLU 1D 5+10* (Table 1).

Markers of economically important traits

Genes determining gliadins and HMW-GS, as a result of linkage with loci or blocks of polygenes, influence important traits of wheat, e.g., baking quality, frost hardiness, and resistance to rusts.

HMW glutenin alleles – markers, e.g. *GLU 1A1*, *GLU 1B7+8*, *GLU 1B7+9* and especially *GLU 1D17+18* and *GLU 1D5+10* jointly with gliadin blocks-markers, e.g. *GLD 1B1* and *GLD 1B4* are markers of higher baking quality (Šašek *et al*. 2000). The maximum accumulation of these markers was detected in the winter varieties Akteur, Batis, Caphorn, Karolinum, Rexia, Vlada, Vlasta and spring varieties Linda, Maja, Saxana, Triso and Leguan.

Secalin allelic block *GLD 1B3* (rye translocation 1B/1R) is a marker of poor baking quality (Dhaliwal & MacRitchie 1990). It was found only in winter varieties Athlet, Clarus, Karolinum, Livia, Rapsodia, Rialto, Sida and Windsor. The above-mentioned secalin allelic block *GLD 1B3* is also a marker of resistance to stem rust (Šašek *et al.* 2000) due to the translocation of a rye chromosomal segment to the genome of common wheat (1R/1B).

Some gliadin genes are markers of frost hardiness (Černý *et al.* 1999). The presence of both main markers of frost hardiness, allelic blocks *GLD 1D5* and *GLD 6A3* simultaneously, was not detected in

Table 2. Verification of the pedigree of Niagara variety

Variety	GLD allelic blocks at loci							GLU allelic blocks at loci		
	1-1A	2-1A	1B	1D	6A	6B	6D	1A	1B	1D
P_1 Danubia	3	3	3	2	2	1	1	1	7+9	5+10
P_2 Viginta	2	0	1	1	1	1	1	0	7+9	5+10
P_3 Ilona A	3	0	1	5	1	1	1	0	7+9	5+10
Ilona B	2	0	1	5	1	1	1	0	7+9	5+10
Niagara A	2	0	1	1	1	1	1	0	7+9	5+10
Niagara B	2	0	4 - N	1	1	1	1	0	7+9	5+10
Niagara C	2	0	1	1	1	1	1	0	7+9	5+10
Niagara D	2	0	4 - N	1	1	1	9 - N	0	7+9	5+10
Niagara E	2	0	1	1	1	1	9 - N	0	7+9	5+10

P – parental varieties; N – illegitimate allelic blocks

any of the evaluated varieties. Allelic block *GLD 6A3* was present in 16% of winter wheat cultivars.

Intra- and inter-varietal polymorphism

To identify wheat varieties by electrophoretic analysis of gliadins and HMW-GS it is necessary to know a potential polymorphism in the electrophoretic patterns of the above-mentioned genetic markers. In polymorphic varieties their genetic constitution is characterised by the number of gliadin and glutenin lines. Therefore varieties/populations should be verified or identified according to the number and ratios of gliadin and glutenin lines typical of the given variety/population.

Out of the total of 80 studied varieties (67 winter wheat varieties, 13 spring wheat varieties), 58 varieties (72%) appeared to be homogeneous in the electrophoretic patterns of gliadins and HMW-GS therefore they can be considered as varieties of the type pure lines, composed of a single gliadin and glutenin line.

Gliadin and glutenin polymorphism was detected in 21 varieties (26%). Twenty varieties exhibited a polymorphism in gliadin patterns (winter varieties – Astella, Bill, Globus, Hana, Ilona, Karolinum, Meritto, Mladka, Niagara, Sepstra, Siria, Solara, Šárka, Trend, Windsor; spring varieties – Aranka, Corso, Leguan, Munk, Sandra) and 1 variety (Asta) exhibited a polymorphism in HMW-GS patterns only. Simultaneous polymorphism in the electrophoretic patterns of gliadins and HMW-GS was found out only in the spring variety Sandra.

Relative ratios of the particular protein lines in heterogeneous varieties of the type populations composed of several protein lines are important for the stability of a registered variety, its maintenance and growing and for the control of its variety trueness and purity in the evaluation of seed lots and commercial seed. In some varieties/populations the mutual ratio of protein lines ensures yield stability. An example is the Windsor variety, in which lines "A" and "C" (75% in total) have *GLD 1B4*, i.e. the marker of higher baking quality, and line "B" (its ratio in the variety is 25%) possesses allele *GLD 1B3*, i.e. the marker of stem rust resistance and simultaneously the marker of poor baking quality. The combination of the three lines at a ratio of 75%:25% guarantees satisfactory resistance to stem rust (slow rusting).

In the set of the evaluated varieties 95% of them have unique, variety-specific electrophoretic patterns of gliadins and HMW-GS. Hence it is possible to identify these varieties reliably and expeditiously by electrophoresis of gliadins and HMW-GS according to variety-specific "fingerprints". The winter wheat varieties Viginta and Bruneta have fully identical electrophoretic patterns of both gliadins and HMW-GS. Among spring wheats the identical gliadin and glutenin electrophoretic patterns were detected in varieties Linda and Saxana. The identity of electrophoretic patterns of the used protein markers in the above-mentioned varieties is conditioned by a high level of the relationship of compared varieties.

Verification of the pedigree of wheat varieties

by protein genetic markers

The electrophoresis of gliadin and HMW-GS genetic markers makes it possible to compare the electrophoretic patterns of parental varieties and their hybrid progeny. If a hybrid variety is characterised by the presence of foreign protein allele or foreign allelic block that is not present in parental varieties, the declared pedigree of such a hybrid variety is not correct and legitimate.

Winter wheat Niagara which comes from declared crossing of the parental varieties (Danubia × Viginta) × Ilona is an example of the illegitimate pedigree of a variety in the present assortment of registered varieties. It represents the population composed of 5 gliadin lines. In the gliadin electrophoretic patterns of line B, line D and line E foreign allelic blocks that are not present in any of the declared parental varieties were detected (Table 2).

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