COMPARISON OF THE STANDARD METHOD OF GLIADIN PROTEIN MARKERS ELECTROPHORESIS (PAGE) ACCORDING TO ISTA WITH THE METHOD OF STARCH ELECTROPHORESIS (SGE)

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Two procedures of electrophoretic gliadin separation, i.e. official methodology ISTA (1984) polyacrylamide electrophoresis (PAGE) and electrophoresis in starch gel (AGE) after Šašek and Sýkorová (1989) were compared. For parallel electrophoresis of gliadins standard seed samples of 14 common wheat varieties, supplied by the Department of Seeds and Planting Material of the State Testing Institute for Agricultural Supervision and Testing, Brno, were used. Both compared methods showed the same sensitivity of determination gliadin polymorphism. The acquired value of *t*-test 1.15 manifests (at $P = 0.05$ and critical value $t = 0.26$) insignificance of difference in the number of spectra of a model set, gained by both methods. Moreover, SGE method makes possible a genetic interpretation of electrophoretic gliadin spectra.

electrophoresis; SGE; PAGE; gliadins; polymorphism; common wheat

INTRODUCTION

To manifest signal gliadin genes various methods of separation of these prolamin wheat proteins can be used. In the Czech Republic two methods of electrophoretic gliadin separation are mostly used: official methodology ISTA (1984) of gliadin separation in polyacrylamide gel (PAGE) and gliadin separation in starch gel (Šašek, Sýkorová, 1989).

The ISTA methodology, i.e. PAGE, has an official character, that is why it is respected by member-countries of this international organization of seed testing. Its advantage consists in the use of polyacrylamide as a carrier medium which by its homogeneity provides a high repeatability of the results of separation. A disadvantage of this method is hitherto existing absence of genetic interpretation of gliadin electrophoretic spectra acquired. The proce-

I. Degree of gliadin polymorphism of standard seed samples of model set of common wheat varieties, found out by procedures PAGE (ISTA) and SGE

N.	Variety	Variant SGE									Variant PAGE		
		line	*n*	%	spectrum – allelic blocks						line	*n*	%
					1–1A	2–1A	1B	1D	6A	6B	6D			
	I Winter wheat													
1	HANA	A	35	97.2	3	1	4	9	2	1	N1	A	15	93.7
		B	1	2.8	2	0	4	(9)	2	1	N1	B	1	6.3
2	SELEKTA	A	33	91.7	2	0	3	1	2	1	1	A	15	93.7
		B	1	2.8	3	2+3	4	1	2	1	N1	B	1	6.3
		C	1	2.8	2	0	4	1	2	1	1			
		D	1	2.8	2	0	3	(2)	3	1	1			
3	SENTA	A	32	88.9	3	(3)	3	1	3	1	1	A	16	100.0
		(A)	2	5.5	3	2+3	3	1	3	1	1			
		B	1	2.8	2	0	3	1	N1	1	2			
		H	1	2.8										
4	SIDA	A	35	97.2	2	0	3	2	3	1	1	A	15	93.7
		H	1	2.8								A^x	1	6.3
5	SIMONA	A	21	58.3	9	2+3	4	1	2	1	2	A	12	100.0
		(A)	8	22.2	9	2+(3)	4	1	2	1	2			
		((A))	2	5.5	9	2+0	4	1	2	1	2			
		B	1	2.8	10	2	4	1	3	1	2			
		C	3	8.3	9	2+3	3	1	2	1	1			
		H	1	2.8										
6	SOFIA	A	30	83.3	3	2	3	1	2	1	1	A	7	58.3
		(A)	4	11.1	3	2	3	1	2	1	1	B	5	41.7

		B	2	5.5	3	2	3	1	2	1	2			
7	SPARTA	A	36	100.0	2	0	3	1	2	1	2	A	16	100.0
8	VLADA	A	56	93.3	14	0	1	7	2	1	1	A	15	93.7
		(A)	1	1.7	14	0	1	7	2	1	1	B	1	6.3
		B	2	3.3	4	0	1	1	2	1	1			
9	ZDAR	A	35	97.2	3	2+3	4	1	N1	1	2	A	15	93.7
		B	1	2.8	2	1	4	(1)	N1	1	2	B	1	6.3
	II Spring wheat													
1	JARA	A	45	93.7	3	3	4	9	2	1	1	A	21	52.5
		(A)	1	2.1	3	3	4	9	2	1	1	A^x	2	5.0
		B	2	4.2	12	3	4	9	2	1	1	A^{xx}	1	2.5
												B	14	35.0
												C	2	5.0
2	LINDA	A	36	100.0	5	2	1	5	N2	1	2	A	15	93.7
												B	1	6.3
3	MAJA	A	23	95.8	12	2+3	1	8	(2)	N2	1	A	13	81.2
		B	1	4.2	2+5	2+3	1	8	(2)	N2	1	A^x	2	12.6
												B	1	6.2
4	SANDRA	A	22	91.7	3	2	1	7	2	1	1	A	12	75.0
		((A))	1	4.2	(10)	0	1	7	2	1	1	A^x	1	6.2
		B	1	4.2	3	3	1	7	3	1	1	A^{xx}	2	6.2
												B	2	12.6
5	SAXANA	A	24	100.0	(5)	2	1	5	N2	1	2	A	16	100.0

Explanations: + = to example 2+3 present occurrence of two allelic blocks in heterozygotic state; H = heterozygotic state of more Gld genes; N = so far not catalogued allelic blocks; (), ((()), ^{x}, ^{xx} – modification in gliadin spectrum: brackets for SGE, asterisks for PAGE (ISTA)

dure after ISTA does not make possible, for the time being, the detection of gliadin genes, alleles, markering as individual bound features, i.e. properties, as total genetic structure of variety or new selection.

On the contrary, genetic interpretation of gliadin spectra is an advantage of gliadin separation in starch gel. Certain disadvantage of the method of starch gliadin electrophoresis is lower repeatability of analyses, conditioned by lower homogeneity of different lots of starch used for separation.

Another decisive criterion of efficiency of both compared separation methods is sensitivity of these methods, assessed according to the degree of gliadin polymorphism finding by both methods in identical set of common wheat varieties.

MATERIAL AND METHODS

Standard seed samples of 14 common wheat varieties, supplied from the harvest of 1993, at the grade S1 (Pre-Basic Seed), by the Department of Seeds and Planting Material of the State Institute for Agricultural Supervision and Testing in Brno, were used for parallel electrophoretic analysis of gliadins by the PAGE method after ISTA (1984) and SGE method after the papers of Šašek and Sýkorová (1989). Survey of varieties assessed is in Tab. I.

Standard seed samples of different evaluated varieties were randomly distributed into two variants, i.e. PAGE ISTA (variant A) and SGE (variant B). 12 to 60 randomly sampled grains were analyzed in each variant. The basic number of 12 analyzed seeds was increasing in dependence on the polymorphism ascertained in the composition of gliadins.

RESULTS AND DISCUSSION

Regarding the absence of genetic interpretation of results of separation by the PAGE method after ISTA, there are types of electrophoretic spectra found out in different evaluated varieties, marked by capital letters. Letters marked by (^{x}) or those in brackets characterize quantitatively, or qualitatively modified spectra, different in intensity of zone colouring, their mobility and number.

Electrophoretic spectra of gliadins, acquired by SGE method, are demonstrated in the form of sets of allelic gliadin blocks. In gliadin polymorphic varieties different sets of allelic blocks, corresponding to different gliadin lines, are marked by capital letters.

Gliadin homogeneity or heterogeneity of standard samples of evaluated varieties is in Tab. I.

Sensitivity of both compared methods of electrophoretic separation was judged by absolute and relative numbers of electrophoretic spectra found ascertained in evaluated varieties of both variants. *T*-test did not show statistically significant difference in the number of gliadin electrophoretic spectra acquired by both comparing methods (Tab. II).

II. *T*-test of different average numbers of gliadin electrophoretic spectra of varieties of model set, gained by SGE and PAGE (ISTA) methods

	SGE	PAGE (ISTA)
Number of observations	14	14
$\bar{x}$	2.71429	2.14286
V	2.06593	1.36264
s	1.43734	1.16732
Difference between x_1 and x_2	0.571429	
t-test value	1.1547	
Significance level	0.258716	

If the total numbers of spectra, found through the PAGE (ISTA) and SGE methods, are taken into account, both the methods manifest the same sensitivity of afflicting gliadin polymorphism.

Relative representation of different lines in gliadin-polymorphic varieties can be characterized by reliability interval. Tables of reliability interval are based upon binomial distribution of probability (Snedecor, Cochran, 1969; Wrigley, Baxter, 1974; Autran, Bourdet, 1975; Ellis, Beminster, 1977; Konarev, 1980; Šašek et al., 1983).

Šašek et al. (1983) assessed through the reliability interval minimal number of analyzed grains to determine inter-varietal gliadin polymorphism. They proved that at the number of 75 of individually analyzed grains statistically significant error of variability of results is lower than 5%.

To evaluate the sensitivity of both compared methods detection of gliadin polymorphism, the series analyses, designed for determination of electrophoretic composition of gliadins, of a wide set of varieties and new selections of domestic and foreign assortment of common wheat, were used. A lower number of grains was used for analysis (variant SGE – grain number $\bar{x} = 36$, variant PAGE ISTA – grain number $\bar{x} = 17.28$) due to technical and financial reasons.

At average three-line intravarietal polymorphism 95% reliability intervals for sets consisting of 17 grains and 36 grains are relatively close, i.e. 0 to 29 grains and 0 to 17 grains (Šašek et al., 1983).

It can be said that a fundamental difference between both the methods of gliadin electrophoresis does not consist in their sensitivity, but in possibility of genetic interpretation of gained electrophoretic gliadin spectra. The official methodology PAGE, issued by ISTA (1984), till the present time does not enable the genetic interpretation.

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Received for publication November 3, 1994

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Porovnání standardní metody elektroforézy gliadinových bílkovinných markerů (PAGE) podle ISTA s metodou škrobové elektroforézy (SGE).

Scientia Agric. Bohem., *26*, 1995 (2): 85–91.

Byly porovnávány dva postupy elektroforetické separace gliadinových bílkovin – genetických markerů pšenice, a sice postup podle mezinárodní organizace semenářské kontroly ISTA (1984), tj. elektroforéza v polyakrylovém gelu (PAGE), a postup elektroforézy ve škrobovém gelu podle autorů Šašek a Sýkorová (1989).

Účinnost obou porovnávaných postupů separace gliadinů byla posuzována podle stupně gliadinového polymorfismu modelového souboru 14 odrůd pšenice obecné. Etanolové vzorky osiv těchto odrůd ve stupni S1 dodal Odbor osiv a sadby SKZÚZ v Brně. V každé variantě (metodě) bylo analyzováno 12 až 60 náhodně odebraných zrn. Základní počet 12 analyzovaných zrn se zvyšoval v závislosti na zjištěném polymorfismu gliadinů.

Citlivost obou porovnávaných metod byla vyjádřena absolutní a relativní četností zjištěných elektroforetických spekter gliadinů celého souboru odrůd. Pomocí *t*-testu nebyl prokázán statisticky významný rozdíl v počtu spekter získaných oběma postupy (hodnota $t = 1{,}15$ při hladině významnosti $P = 0{,}05$, kritická hodnota $t = 0{,}26$).

elektroforéza; SGE; PAGE; gliadiny; polymorfismus; pšenice obecná

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