MONITORING OF DIHAPLOID COMMON WHEAT BREEDING USING ELECTROPHORESIS OF PROTEIN GENETIC MARKERS

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The convenience of gliadin and HMW glutenin (of high molecular weight) marker-genes for monitoring dihaploid common wheat breeding was evaluated. In field microtrial conducted for performance 84 DH lines of A3 and A2 generations were assessed. Using correlation test and *t*-test the convenience of marker-genes for selection of parents providing transgression in baking quality and for diversification of DH lines into food and fodder lines.

common wheat; dihaploidy; monitoring; marker-genes; gliadins; HMW glutenins

INTRODUCTION

The paper was aimed at verification of suitability of marker-gliadin (GLD) and HMW glutenin (GLU) genes for monitoring the process of dihaploid winter wheat breeding.

When electrophoretic separation of gliadin (GLD) and HMW glutenin (GLU) proteins of caryopsis endosperm is used, some economically important wheat properties, such as baking quality, can be marked and transgression in marked properties in hybrid progeny can be predicted. By means of marker-Gld and Glu (HMW) it is also possible to select transgression individuals in homozygotic condition.

The paper was aimed at verification of suitability of marker-Gld and HMW Glu genes in production of dihaploid lines of food wheat from selection of parent genotypes and prediction of transgression in baking quality to selection of transgression regeneration dihaploid lines in homozygotic condition.

MATERIAL AND METHODS

The trial was established in 1992 by induction of dihaploidy in hybrid F1 generations by combination of FLORIDA x BUSSARD, FLORIDA x HANA, FLORIDA x ILONA, FLORIDA x JANTAR 50 and FLORIDA x ST 950-89. In 1993 the trial continued with F1 generations by combinations HANA x SIRIA and SAMANTA x APOLLO. The seed of F1 generation of the above combinations was supplied by SELGEN – the Plant Breeding Station at Stupice.

The protocol for production of haploids using anther cultures *in vitro* supplied by the Research Institute of Crop Production in Prague-Ruzyně was used to produce dihaploid lines. The medium C 17 with maltose of concentration 120 g/l, 1.5 mg 2.4 D and 0.5 mg/l kinetin was used to induce androgenesis *in vitro*. 3 g/l gelrit was used as a stiffening agent. Recovered green plants were vernalized and afterwards precultivated in the glasshouse and planted in the field nursery garden. The cytology of ploidy grade was controlled in recovered plants. Haploid plants found were colchicined. Electrophoretic analysis of gliadins in columns of starch gel (SGE) after Šašek, Sýkorová (1989) and electrophoresis of HMW glutenin subunits by the PAGE method after Laemmli (1970) were carried out to predict the baking quality of parent forms of F1 hybrids and to select transgression DH lines.

The catalogues published (Sobko, Poperelja, 1986; Payne, Lawrence, 1983) were used to detect allelic gliadin and glutenin blocks.

The scoring value of the baking prediction of GLD allelic blocks was determined under authors' own data (Šašek et al., 1989).

To detect prediction point value of allelic zones or blocks of HMW GLU zones the pieces of knowledge published (Payne et al., 1987; Lukow et al., 1989; Hammer et al., 1912) were applied.

Two spikes from each DH line of the A3 or A2 generations were sampled for electrophoretic analysis of GLD and HMW GLU. Total 461 spikes of DH lines of the A3 and A4 generations were analyzed. A concordance between prediction of baking quality of DH lines of the A3 or A2 generations and their baking quality alone, expressed by SDS sedimentation value, was determined by correlation analysis and *t*-test of different means of sedimentation values of four grades of baking quality prediction, settled after summary point values of GLD and HMW GLU of allelic blocks of different DH lines of A3 and A2 generations. To diversify DH lines of the A4 or A3 generations, field microtest of DH lines performance was established by seeding of 178 spike progenies at the Suchdol locality and 211 spike progenies at the Stupice locality (0.80 m^2 each in one replication).

I. Prediction of positive transgressions in scoring of baking quality after parent combinations in DH lines

	combination	maximum points of baking quality		maximum points of baking quality of DH transgr. lines	difference to maximum P		difference to minimum P	
		P1	P2		*n* (points)	%	*n* (points)	%
	Cycle I							
1	Florida x Bussard	20.5	27.5	29	1.5	5.17	8.5	41.46
2	Florida x Hana	20.5	24	28	4	14.28	7.5	36.58
3	Florida x Ilona	20.5	24.5	29	4.5	15.52	8.5	41.46
4	Florida x Siria (ST 265)	20.5	23.5	26	2.5	9.61	5.5	26.82
				(28)*	(4.5)*	16.07	(7.5)*	36.58
5	Florida x ST 950-89	20.5	23	26.5	2.5	9.43	6	* 29.27
	Cycle II							
6	Hana x Jantar	24	29.5	30.5	1	3.28	6.5	27.08
7	Samanta (ST 1393) x Apollo	23	21	28.5	4.5	15.79	4.5	21.43

* crossover constitutions

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RESULTS AND DISCUSSION

The preliminary electrophoresis of GLD and HMW GLU markers of parent forms (varieties, new selections) makes possible to acquire information on potentially transgressive combinations in baking quality. In addition, it provides a sufficient information about genetic structure of these parent forms as lines or populations of lines with eventually different genetic determination of the baking quality.

The main importance of the monitoring of DH line production consists in diversification of achieved DH lines of the A3 and A2 generations and in selection of homogenous and homozygotic DH lines of the baking or feeding type. Theoretically assumed positive transgressions in point value of the baking quality prediction by parent combinations are presented in Tab. I. In the given case average predicted positive transgression was manifested in prediction points in the value of about 3 prediction points what represents a 12% relative increase in the baking quality. For preliminary determination of concordance between prediction of baking quality via GLD and HMW GLU electrophoresis and the baking quality of DH lines alone, expressed by their sedimentation value, SDS sedimentation test of 84 DH lines of A3 or A2 generations from the field nursery of 1993/1994 at the locality of the Czech University of Agriculture in Prague-Suchdol. The concordance was determined by calculation of correlation coefficient r between point value of baking quality prediction of allelic blocks - markers of the baking quality and sedimentation value. The achieved values of correlation coefficients for $N = 82$ are presented in Tab. II. All achieved correlations between GLD and HMW GLU markers and sum of GLD and HMW GLU markers (points) and sedimentation value (ml) are highly significant, though the values of determination coefficients show lower dependence of variables.

II. Correlation analysis – linear regression: $y = a + bx$. Dependence of prediction points on the values of sedimentation test (ml)

Independent variable: sedimentation test (ml)				
Dependent variable:	absolute member a	regression coefficient b	correlation coefficient r	determination coefficient (%) r x r
GLD points (y_1)	2.031	0.22	0.315**	9.92
GLU points (y_2)	2.95	0.091	0.353**	12.46
GLD + GLU points (y_3)	5.022	0.311**	0.418**	17.47

** $P = 0.001$

T-test of different means of sedimentation values of various grades of GLD and HMW GLU markers was used as an alternative method of evaluation of the suitability of marker-Gld and Glu (HMW) genes for prediction of the baking quality of DH lines. Assessed DH lines were divided into four grades according to scoring prediction values of GLD and HMW GLU allelic blocks – markers of the baking quality.

Characteristics of grades are presented in Tabs. III and IV. Tab. V sums up the results of t-test. Grade with maximum point values of baking quality prediction of GLD as well as HMW GLU of allelic blocks are distinguished for their maximum sedimentation value in ml, significantly different from sedimentation values of remaining grades (Tab. V).

III. Division of prediction points GLD and GLU in categories

Category	GLD (points)	GLU (points)
Maximum	17–20.5	9–10
Mean	13–16.5	7–8
Minimum	7–12.5	6

IV. Statistical parameters of select sets of DH lines

Tested sets of DH lines	Number of genotypes	SDS (ml)		GLD (points)		GLU (points)		GLD + GLU (points)	
		arithmetical mean	standard deviation	arithmetical mean	standard deviation	arithmetical mean	standard deviation	arithmetical mean	standard deviation
	n	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Maximum GLD Maximum GLU	12	63.168	6.337	17.917	1.104	8.917	0.289	26.833	1.135
Minimum GLD Minimum GLU	24	44.667	4.469	10.667	1.679	6.042	0.204	16.708	1.594
Average GLD Average GLU	10	50.75	8.517	15.3	1.135	7.9	0.568	23.2	1.378
Minimum GLD Maximum GLU	7	51.143	5.699	10.321	1.663	9.143	0.378	19.464	1.906

V. Evaluation of differences in SDS test means (ml) of four groups of DH lines

DH line groups	B	C	D
A	27.288*	9.164	8.276
	2.732**	2.845	2.898
	0.001***	0.001	0.001
B		7.258	7.393
		2.732	2.756
		0.001	0.001
C			4.646
			2.947
			0.001

DH line groups	Prediction points	
	GLD	GLU
A	max.	max.
B	min.	min.
C	mean	mean
D	min.	max.

* calculated *t*-test value
** tabular *t*-test value
*** alpha significance level

The results achieved from correlation analysis and *t*-test of grades show the usability of electrophoretic analysis of gliadins and HMW glutenins as a method of prediction and selection of DH lines of food type.

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Monitorování dihaploidního šlechtění pšenice obecné pomocí elektroforézy bílkovinných genetických markerů.

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Pomocí souboru 84 dihaploidních (DH) linií generace A3 a A2 odvozených ze sedmi rodičovských kombinací F1 byla ověřována vhodnost signálních gliadinových (Gld) genů a genů podjednotek gluteninů (Glu) s VHM pro monitorování procesu dihaploidního šlechtění pšenice obecné pekařského směru.

Pro produkci dihaploidních linií byl použit protokol pro tvorbu haploidů pomocí prašníkových kultur, vypracovaný VÚRV v Praze-Ruzyni. K predikci pekařské jakosti rodičovských forem F1 hybridů a získaných DH linií generace A3, resp. A2, včetně stanovení jejich homogenity a homozygotnosti, byla uskutečněna elektroforetická analýza gliadinů (GLD SCE podle autorů Šašek a Sýkorová, 1989) a gluteninů s VHM (GLU VHM PAGE podle autora Laemmli, 1970). Alelické bloky GLD a GLU s VHM byly detekovány podle publikovaných katalogů (Sobko, Poperelja, 1986; Payne, Lawrence, 1983). Bodová hodnota predikce pekařské jakosti GLD a GLU s VHM markerů alelických bloků byla stanovena podle vlastních poznatků (Šašek et al., 1989), nebo podle publikovaných výsledků (Payne et al., 1987; Lukow et al., 1989; Hammer et al., 1992).

Pekařská jakost 84 DH linií generace A3, resp. A2 byla stanovena pomocí SDS sedimentačního testu. Konkordance mezi sedimentační hodnotou a bodovou hodnotou predikce pekařské jakosti byla hodnocena korelačním testem. Všechny získané korelační vztahy mezi sedimentační hodnotou (ml) a celkovou markerovací hodnotou GLD, GLU (VHM) a GLD + GLU (VHM) markerů jednotlivých DH linií byly vysoce významné. Hodnoty koeficientů determinace však svědčí o nižší závislosti.

Proto bylo jako alternativní metoda použito hodnocení vhodnosti Gld a Glu (VHM) signálních genů pro monitorování diploidního šlechtění pšenice obecné pot-

směru *t*-testu rozdílných průměrů sedimentačních hodnot čtyř souborů DH linií, sloučených podle bodových predikčních hodnot Gld a Glu (VHM) markerů.

Třída s maximálními bodovými hodnotami predikce pekařské jakosti se vyznačovala maximální hodnotou sedimentace, významně se lišící od sedimentačních hodnot ostatních tříd.

Získané poznatky potvrzují vhodnost signálních Gld a Glu (VHM) pro výběr rodičovských forem a F1 hybridních kombinací, poskytujících lepší pekařskou jakost, a pro diverzifikaci DH linií pekařského a krmného směru.

pšenice obecná; dihaploidie; monitorování; signální geny; gliadiny; gluteniny s VHM

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