UTILIZATION OF CLINICAL ALPHA-AMYLASE KIT FOR THE INCREASING OF PRE-HARVEST SPROUTING RESISTANCE IN RYE

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A high temperature and air humidity together with rainfalls in the pre-harvest period cause grain sprouting in ears in most grown rye varieties (*Secale cereale* L.) and may reduce or even damage their end-use quality. There are only a few reports concerning genetic variation in pre-harvest sprouting resistance of rye. Among the collection of rye genetic resources, the Swedish variety Otello exhibits the highest sprouting resistance, but only medium yielding potential. The more productive variety Baro with high sprouting resistance was bred in Germany. This variety was used as a male parent in the crossing together with some female rye varieties. Values of alpha-amylase activity were obtained using the clinical alpha-amylase kit of the Lachema Firm. For calculating "sprouting indices" the method modified according to Weilenmann (1980) was used. Using this method, new rye genotypes with increased parameters of productivity and pre-harvest sprouting resistance were bred during the period of 1987 to 1992.

winter rye; varieties; grain quality; alpha-amylase kit; pre-harvest sprouting resistance

INTRODUCTION

Breeding rye for pre-harvest sprouting resistance is one of major goals because it directly affects standard quality of grain production. Basic elements influencing rye sprouting are alpha-amylase (AA) content and activity. AA is controlled by hormones (gibberellins) which affect a new synthesis in germinating grains. Sprouting is a varietal character which is influenced by climate. It can be apparent and hidden (invisible). Immature grains exhibit high AA activity which is the highest 22 to 30 days after anthesis (Buschbeck, Wilp, 1982) and which falls down to about a half at maturing (MacGregor, 1983). Using electrophoresis AA enzyme can be divided into the so-called green AA, occurring in maturing grains, and germinative AA present in germinating grains. The synthesis of green AA in rye is con-

trolled by the alpha-amy 2 locus on the long arm of the 7th chromosome, germinative AA are encoded on the 6R chromosome (Kruger, 1980; Gale et al., 1983; Ainsworth et al., 1987; Masojc et al., 1991).

Belderok and Branderburger (1970) elaborated a method for winter rye in the Netherlands enabling to predict sprouting which is based on temperature sums from milk to waxy maturity of the grain. A period of post-harvest maturing shortened with the increasing air temperature. However, that was not confirmed in Germany (Grahl, Schrödter, 1980). Based on extent investigations there is a hypothesis that winter rye kernel is ready to germinate all the time provided content of gibberellic acid inducing the AA synthesis and content of its antagonist, abscisic acid, are about the same, or abscisic acid content is lower. Prediction methods can be based on seeking rye forms showing content of abscisic acid higher than that of gibberellic acid at maturing. Weipert (1992) considers the AA activity the primary and content of total and soluble pentosans as the secondary factors of quality. Pentosans show high ability to absorb water, which protects starch granules from their degradation by AA. Also, results reported by other authors suggest that the most important factors influencing pre-harvest sprouting are amylases, production of gibberellic acid, thiol and disulfide substances, and permeability of seed coats. Genetic bases of sprouting show inheritance as a consequence of overall physiological influence on a higher gene number.

A number of physical and chemical methods for the evaluation of sprouting have been developed in the world. After the prior provocation test of ears in growth chambers, the viscosity test method (Falling Number) according to Hagberg and Perten (Perten, 1964) has been widely used. The method is suitable for field conditions where hidden sprouting was observed and varieties showed different resistance.

In years when sprouting does not occur, it is necessary to use provocation tests in breeding tests. Based on assessing Falling Number in relation to variety resistance Weilenmann (1976) elaborated a new complex evaluating system. This system, partly modified, was tested at our Institute and showed positive results (Hýža, Hubík, 1988).

MATERIALS AND METHODS

Our collection of genetic resources comprises a number of foreign varieties exhibiting increased resistance to grain sprouting in ears. The Swedish variety Otello, developed in 1971, is one of the most resistant (Persson, 1976). It has high grain quality but lower yields under our conditions. In

1990, the new high-yielding variety Baro (tested under the name SCW 1162 – Köchling, pers. comm.) was released in Germany.

Solving problems of breeding new productive rye genotypes, the male variety Baro was top-crossed to chosen rye varieties and lines such as Madar, Danko, Motto, SMH 285 and SCW 345. Beginning from the F3-generation, plant analyses for productivity and sprouting resistance were performed using the modified method according to Plarre (1980). Pair crosses were applied in breeding (Wolski, 1975).

Sprouting indices, *i*, were calculated using the method modified according to Weilenmann (1980). Values of the AA activity obtained by diffusion measurement method in agar gel containing dye of the alpha-amylase clinical kit of the Lachema Firm (which is applied to measure the AA activity in human medicine) were used (Hubík, 1987).

Agar gel composition

Solution A: 11.55 ml of concentrated acetic acid with added distilled water to 1 000 ml

Solution B: 27.2 g of sodiumacetate (NaAc.3 H_2O = trihydrate) or 16.4 g of water-free sodiumacetate (NaAc) with added distilled water to 1 000 ml

Solution C: 40 g $CaCl_2$ with distilled water to 100 ml

Acetate buffer – 100 ml – pH 4.6: 30.5 ml Solution A, 19.5 ml Solution B, 1.0 ml Solution C

Stop solution: 2 g NaOH with distilled water to 100 ml

Agar gel (the amount of Petri dishes, 8.5-cm diameter):

Solution 1 – 0.15 g of agar + 10 ml of acetate buffer

Solution 2 – 0.30 g of blue dye from labelled enzymatic substrate (Lachema clinical test) + 5 ml of acetate buffer.

Agar gel is obtained by bringing Solution 1 to mild boiling and adding Solution 2 immediately. When warm it is poured into Petri dishes and kept until stiffened. Wells in the agar plates are done using a special 2-mm-diameter puncher.

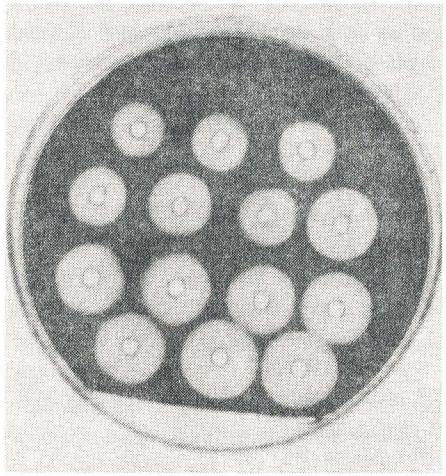
Procedure

0.4 g of coarse milling is placed in cuvettes, and 1 ml of acetate buffer is added. It is left for extraction for two hours (to stir from time to time), centrifuged and 10 µl of centrifuged extract are given in wells in agar plates. They are placed in the thermostat at 50 °C and absolute moisture for 17 hours. Then, stop solution is poured on agar plates. After 30 min they are rinsed

with distilled water and diameters of light circles are measured at 0.1-mm accuracy (Fig. 1). These diameters represent the alpha-amylase activity. Sprouting index i is calculated according to the formula:

$$i = \sqrt{\frac{A\,I}{AS\,I}\,\frac{A\,II}{AS\,II}}$$

where: A I, A II – alpha-amylase in the genotype before (A I) and after wetting (A II)
AS I, AS II – alpha-amylase in the standard Baro before (AS I) and after wetting (AS II)



1. Petri dish with light circles on the agar plate, their diameters represent the alpha-amylase activity

RESULTS AND DISCUSSION

The standard in selected sets was the variety Baro. Results obtained were compared to the variety Otello. Tab. I shows yearly and mean values of sprouting index i, in a set of varieties chosen among the world collection tested in the three-year period. The genotypes showing high values of sprouting indices exhibit the highest susceptibility to pre-harvest sprouting. The genotypes with indices close to 1 are highly resistant. The method was used in developing intensive genotypes with increased resistance to grain sprouting in spikes. The selection began in the F3-generation in 1989. The selection effect of the sprouting indices in successive years is given in Tab. II. The decreasing value of sprouting index i, associated with repeated selections in years is apparent from the table. Based on the analysis of variance of sprouting index (significant at $P = 0.05$) significant effects of years (70.9%)

I. Sprouting indices in the selected set of rye varieties in the period of 1990–1992

Variety	i_{1990}	i_{1991}	i_{1992}	Mean
Gepard – HY	3.51	7.52	4.93	5.32
Akkord – HY	1.42	9.40	1.13	4.02
Saratovskaja 5	–	3.50	4.38	3.94
Saratovskaja 4	–	4.70	2.80	3.75
Kelpo	1.10	5.92	–	3.51
Voschod 2	4.58	1.52	–	3.05
LPH 1 – HY	–	4.59	1.36	2.98
SMH 285	–	3.35	2.30	2.83
Madar	1.47	2.96	3.97	2.80
Pluto	0.77	3.67	3.87	2.77
Gloria	2.78	–	2.68	2.73
Dankowskie Zlote	0.94	3.21	4.00	2.72
Perola	1.12	4.29	–	2.71
Dankowskie Nowe	3.30	2.20	1.64	2.38
Talovskaja 12	1.45	3.11	–	2.28
Motto	2.21	2.44	1.32	1.99
Breno	1.25	2.39	1.99	1.88
Turbo	1.70	2.59	1.09	1.79
Dominator	–	1.87	1.50	1.69
Anna	1.37	1.58	–	1.48
Muro	0.93	1.61	1.53	1.36
Halo	1.00	1.06	0.94	1.00
Baro	0.98	0.87	0.84	0.90
Variety – mean	1,77	3.38	2.35	–

and varieties (21.99%) on total value of sprouting index were determined (Tab. III).

A highly significant effect of the year on sprouting index value can be explained by sufficient amount of free endogenous regulator, gibberellic acid, in the endosperm of matured rye grain (MacGregor, 1983) which causes immediate synthesis of alpha-amylase and consequently sprouting process under favourable, that means wet and cold, conditions. An important role in

II. Utilization of the sprouting indices in breeding new rye genotypes with a high level of sprouting resistance

Variety Hybrid	1990			1991			1992		
	n	*i*	%	*n*	*i*	%	*n*	*i*	%
All	61	1.026	–	54	1.068	–	50	1.007	–
Chosen	22	0.901	36	16	1.103	30	6	0.827	12
Otello	4	0.920	–	4	0.970	–	4	0.882	–

III. Analysis of variance of the sprouting index in the selected rye genotypes

Variation	Sums of squares	Degree of freedom	Mean squares	*F*	%
Genotype	115.41	22	5.25	3.07**	21.99
Year	33.82	2	16.91	9.88**	70.90
Error	75.35	44	1.71	–	7.20

IV. New rye genotypes with a higher level of pre-harvest sprouting resistance having the Baro variety in their pedigrees (Kroměříž, 1987–1992)

Plot No.	Genotype	Plant height (cm)	Flowering day (May)	Grain weight/plant	Sprouting index *i*
1	92–11/39,40	115	24	2.03	0.826
2	92–11/37,38	115	24	2.98	0.800
3	92–11/38,39	118	24	2.41	0.830
4	92–11/73,74	115	27	2.25	0.835
5	92–11/74,75	120	27	2.11	0.822
6	92–11/27,28	115	27	2.07	0.854
7	Baro – S 1	120	24	2.00	0.882
8	Otello – S 2	135	29	1.57	0.837
	Mean	116	25	2.31	0.828

grain sprouting is played by endogenous inhibitors of alpha-amylase. These regulatory proteins which inactivate isoenzymes of alpha-amylase in grain endosperm are contained in rye much less than compared to wheat and particularly barley (M u n d y , 1984). Using the mentioned method intensive genotypes exhibiting sprouting resistance on the level of standards are listed in Tab. IV. These genotypes have been provided to breeders.

CONCLUSION

This work describes one of the methods for assessment of the alpha-amylase activity which can be applied in breeding practice. Using the commercial Czech substrate called Spofa-test and calculating the sprouting index allow us to improve resistance of rye varieties and lines to pre-harvest sprouting. The method is fully compatible with the Falling Number method. Its advantage is a low amount of grain (ca 7 g) for analyses, which is very important in breeding when pair crosses are applied.

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Využití klinického alfa-amylázového testu pro zvýšení odolnosti žita proti porůstání zrna v klasech.

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Vyšší teplota a vlhkost vzduchu, spolu se srážkami v předsklizňovém období, vyvolávají v produkčních oblastech u většiny pěstovaných odrůd žita, ale i u ostatních chlebových obilovin, porůstání zrna v klasech a tím snižování až znehodnocování jejich technologické hodnoty. Porůstavost se projevuje jako „vnější", nebo „skrytá". Její projev souvisí s aktivitou alfa-amyláz v zrně.

Ze světové kolekce genetických zdrojů žita je nejodolnější odrůdou proti porůstání švédská odrůda Otello. Z ní byla v Německu vyšlechtěna produktivnější odrůda Baro, vysoce odolná proti porůstání (tab. I).

Použitím hodnot aktivity alfa-amylázy získaných metodou difuzního měření aktivit v agarovém gelu obsahujícím barvivo z klinického alfa-amylázového testu firmy Lachema byly metodou upravenou podle W e i l e n m a n n a (1980) vypočítány indexy porůstání – *i*. V práci je popsána modifikovaná metodika hodnocení a použité chemikálie.

Ve výběrových souborech byla jako standardní zvolena odrůda Baro a výsledky byly srovnávány s odrůdou Otello. Hodnota indexu porůstání *i* byla vypočítána podle vzorce:

$$i = \sqrt{\frac{A\,I \quad A\,II}{AS\,I \quad AS\,II}}$$

kde: A I, A II – aktivita alfa-amylázy genotypu před (A I) provokací a po (A II) provokaci
AS I, AS II – aktivita alfa-amylázy standardy před (AS I) provokací a po (AS II) provokaci

U rostlin z kombinací pocházejících z křížení linií se stejným rodičem byla potomstva dále vzájemně prokřižována metodou párového křížení (W o l s k i , 1975).

Od generace F_3 byla prováděna selekce také na produktivitu s aplikací modifikované metody podle P l a r r e (1980).

Po selekci potomstev získaných z generativního křížení osmi odrůd a novošlechtění s rezistentní odrůdou Baro vykázaly v konečné fázi vybrané genotypy hodnoty převyšující standardu Baro o 0,231 a odrůdu Otello o 0,143. Ze srovnání difuzní metody v agarovém gelu obsahujícím barvivo z klinického testu a metody podle Hagberga (Falling Number) je zřejmá plnohodnotná zastupitelnost. U difuzní metody je předností velmi nízká navážka vzorků pro rozbory (7 g) oproti Hagbergově metodě (20 g). Na základě statistického zhodnocení výsledků za tříleté období byly zjištěny statisticky významné podíly ročníků (71 %) a odrůd (22 %) na celkové hodnotě indexu porůstání.

Získané genotypy se zvýšenou odolností proti porůstání byly spolu s metodikou použití alfa-amylázového testu doporučeny ke šlechtitelskému využití.

ozimé žito; odrůdy; kvalita zrna; alfa-amylázový test; odolnost proti porůstání

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