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DIRECT SOMATIC EMBRYOGENESIS AND PLANT REGENERATION FROM CULTURED EMBRYOS IN THE GENUS *TRIFOLIUM*

J. Řepková

ŘEPKOVÁ, J. (Research and Breeding Institute for Fodder Crops, Troubsko): *Direct Somatic Embryogenesis and Plant Regeneration from Cultured Embryos in the Genus Trifolium.* Genet. a Šlecht., *25*, 1989 (3) : 173-180

Direct somatic embryogenesis on cultured embryos with subsequent plant regeneration was achieved in a number of *Trifolium* species: *T. repens* f. *hollandicum* and f. *giganteum*, *T. pratense* cvs. Start and Kvarta, *T. alpestre*, *T. ambiguum* cv. Summit, *T. medium*, *T. pannonicum*, *T. rubens* and *T. vesiculosum* cv. Yuchi. The most suitable stage of zygotic embryos for embryoid initiation was the torpedo stage. Somatic embryo production was initiated on the basal medium EC 6 with 0.22 μM and 0.44 μM BAP and 1.0 g/l YE. Hormone free medium L2 stimulated plantlet formation. This method is suitable for rapid clonal propagation of desired genotypes and the raising of rare interspecific hybrids.

Trifolium species; embryo culture; direct somatic embryogenesis; clover breeding

In vitro methods of explant culture are applied for the improvement of certain clover species, mainly those important as forage legumes, among which red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) are the leading crops. Another group of clovers consists of the wild species with good prospects for agricultural use as donors of some useful traits (*T. alpestre, T. ambiguum, T. medium, T. sarosiense*).

Somatic embryogenesis *in vitro* is the development of embryo-like structures from somatic cells into plants through characteristic embryological stages without fusion of gametes. This process occurs either indirectly from cells, which have subsequently been redetermined for embryogenic development after their differentiation (callus or suspension culture) or directly from the cells of an organized structure, known as predetermined embryogenic cells.

The process of somatic embryogenesis in the genus *Trifolium* has been described in a few species only. Phillips and Collins (1980) first successfully initiated embryoid formation from the callus derived from a cell suspension culture of red clover. Their method was later applied by Keyes et al. (1980) in *T. pratense* too and in *T. rubens* by Parrott and Collins (1982). White (1984) described plant regeneration from suspension cultures of white clover via somatic embryogenesis. Somatic embryos were formed callus cultures derived from hypocotyl sections of *T. incarnatum, T. vesiculosum, T. ambiguum* and *T. repens* (Pederson, 1986). But mature plants were only obtained in *T. incarnatum*.

The first report on direct somatic embryogenesis on immature zygotic embryos was presented by Maheswaran and Williams (1984) in *T. repens*. They extended this procedure to further *Trifolium* species (*T. pratense, T. resupinatum* and *T. subterraneum*) in the next paper (Maheswaran, Williams, 1986).

The first step of this work was to improve the method of direct somatic embryogenesis in zygotic embryos for a number of *Trifolium* species involved in our breeding programmes. In addition, this method was used in special situations such as plant regeneration from hybrid embryos with the aim of obtaining new interspecific hybrids.

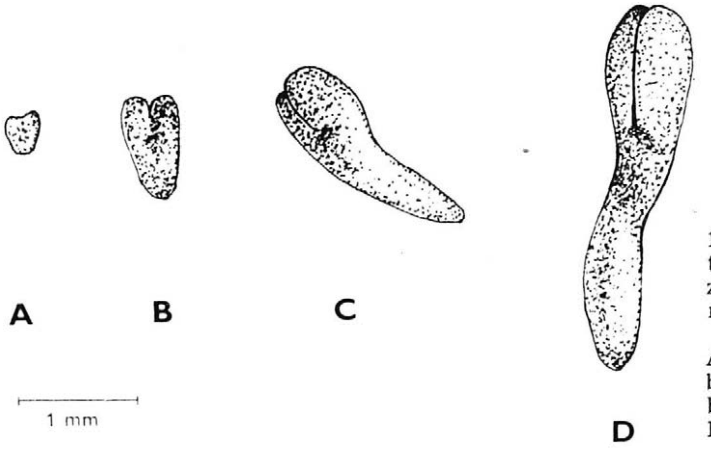
MATERIALS AND METHODS

Several *Trifolium* species were involved in the experiments: *T. pratense* L. cvs. Start (2n) and Kvarta (4n), *T. repens* L. f. *hollandicum* and f. *giganteum*, *T. alpestre* L., *T. ambiguum* M. B. cv. Summit, *T. medium* L., *T. pannonicum* Jacq., *T. rubens* L. and *T. vesiculosum* Savi cv. Yuchi.

Plants for zygotic embryo excision were grown under greenhouse conditions. Flowers were cross-pollinated by hand or with the aid of bumble-bees. Flowers with developing ovaries were harvested 6 to 10 days after pollination and surface sterilized 3 minutes in 70 per cent alcohol and 15 minutes in 5 per cent chloramine, followed by sterile water washing. Embryos in various stages of development (heart-shaped, torpedo and mature embryos, Fig. 1) were excised in sterile conditions under a stereomicroscope. Embryos were cultivated on EC 6 basal nutrient medium (Maheswaran, Williams, 1984) which supports the *in vitro* growth of immature embryos of *T. repens*. This medium was supplemented by benzylaminopurine (BAP) (0.11 μM, 0.22 μM and 0.44 μM) and 1.0 g/l yeast extract (YE). After several weeks' cultivation the cultures were transferred to hormone free medium L2 (Phillips, Collins, 1979) for plantlet formation.

RESULTS AND DISCUSSION

From the point of view of somatic embryo initiation and plant regeneration the most suitable stage of zygotic embryos was the torpedo stage for all species (Tab. I). The highest number of cultivated embryos was stimulated to embryoid production in *T. repens* f. *hollandicum* and



1. Schematic representation of the stages in zygotic embryogenesis in red clover

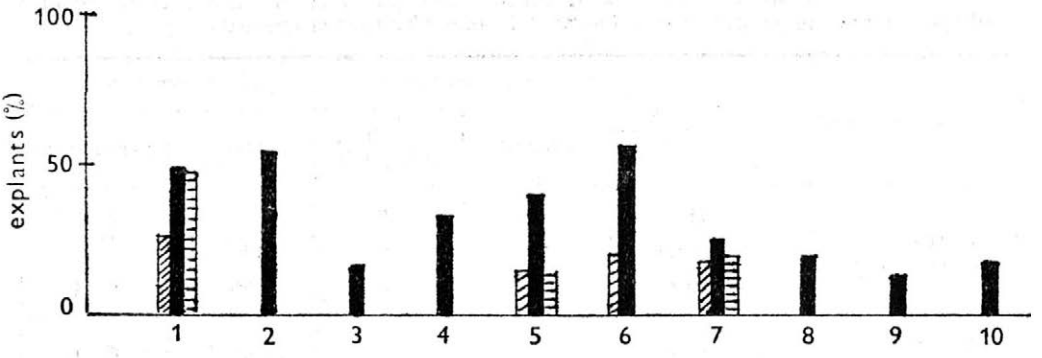
A — heart-shaped embryo, B — torpedo embryo, C — late torpedo D — mature embryo

I. Comparison of somatic embryo initiation and plant regeneration from zygotic embryos of various stages of development in eight *Trifolium* species

Clover plant	Stage of development	No. of embryos		No. of regenerants	
		excised	with embryoids	total	per embryo
T. repens L. f. *hollandicum*	heart	19	5	15	2–5
	torpedo	51	25	170	3–9
	mature	21	10	55	5–15
T. repens L. f. *giganteum*	torpedo	11	6	49	6–12
T. pratense L. cv. Start	heart	6	0	0	0
	torpedo	30	5	66	7–25
	mature	18	0	0	0
T. pratense L. cv. Kvarta	heart	11	0	0	0
	torpedo	12	4	33	4–10
	mature	15	0	0	0
T. ambiguum M. B.	heart	13	2	3	1–2
	torpedo	25	10	86	6–17
	mature	15	2	10	3–7
T. medium L.	heart	5	1	5	5
	torpedo	14	8	57	4–12
	mature	8	0	0	0
T. rubens L.	heart	11	2	10	5
	torpedo	13	3	30	5–14
	mature	5	1	3	3
T. pannonicum Jacq.	torpedo	10	2	8	3–5
T. alpestre L.	torpedo	10	1	7	7
T. vesiculosum Savi	torpedo	6	1	12	12

f. *giganteum*, *T. ambiguum* and *T. medium*. In *T. repens* f. *hollandicum*, *T. ambiguum* and *T. rubens* all three stages of zygotic embryo development gave rise to embryoid and plant regeneration (Fig. 2). Embryoids arose on the hypocotyl part of zygotic embryos without callus initiation (Fig. 3). In several cases the embryoids formed on the cotyledones of mature embryos.

Somatic embryo production was initiated on the media with YE and at two higher BAP concentrations within 5 — 7 days. The suppresion of main zygotic embryo axis was observed. After about three weeks' cultivation the cultures were transferred to basal medium L2 which stimulated

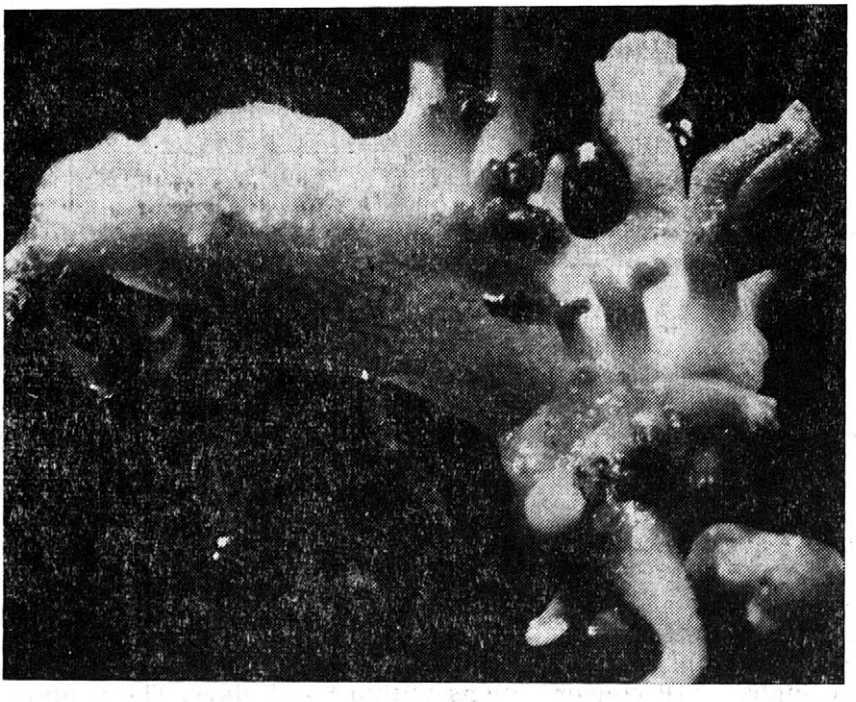


2. Regeneration frequency of explants with embryoid initiation in dependence on development stage of zygote embryo in eight *Trifolium species*

1 — *T. repens* f. *hollandicum;* 2 — *T. repens* f. *giganteum;* 3 — *T. pratense* cv. Start; 4 — *T. pratense* cv. Kvarta; 5 — *T. ambiguum;* 6 — *T. medium;* 7 — *T. rubens;* 8 — *T.pannonicum;* 9 — *T. alpestre;* 10 — *T. vesiculosum*

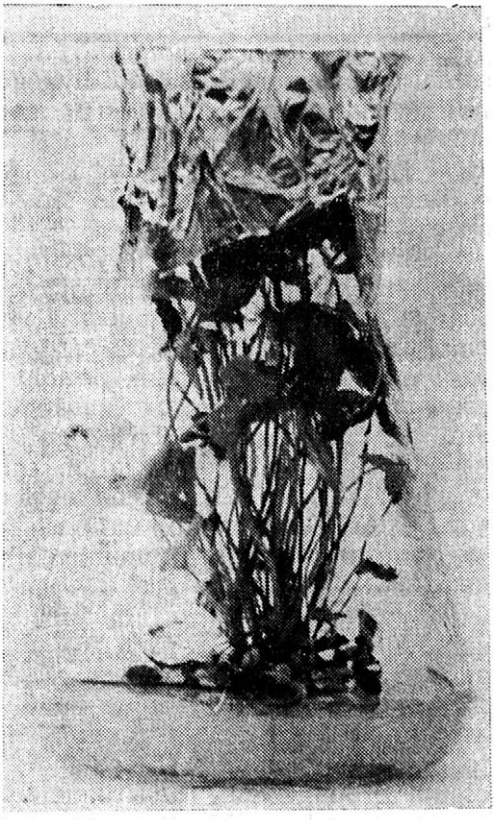
Stages of zygotic embryo: ▨ heart-shaped, ■ torpedo, ▤ mature

plantlet formation. The *in vitro* formed plants (Fig. 4) exhibited normal development when transferred into the soil (Fig. 5). The shoots and roots of regenerants evolved simultaneously. Total cultivation from embryo excision to differentiated plant regeneration lasted 5 — 6 weeks.



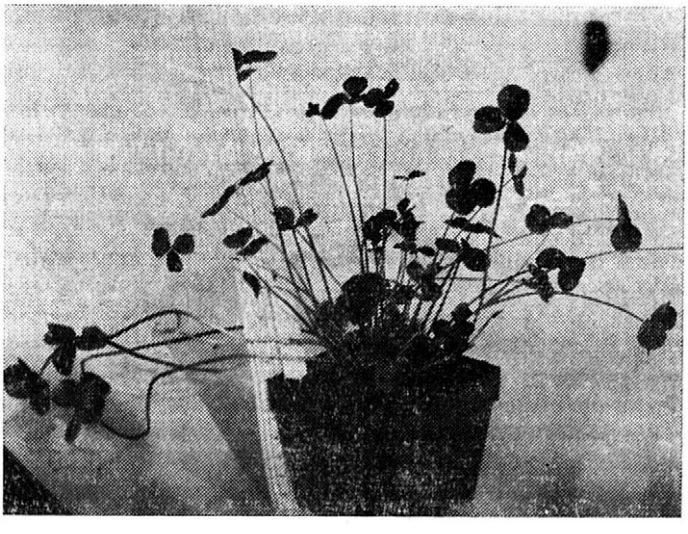
3. Embryoids developing on the hypocotyl of a torpedo zygotic embryo of *Trifolium repens* cultured on medium with BAP and YE (x 16,6)

4. A clone of plants obtained via direct somatic embryogenesis on a zygotic embryo of *Trifolium repens* after 5 weeks' cultivation



Various clover species differed in the number of plants regenerated from one zygotic embryo. The total number of plants regenerated from one embryo in its different stages is shown in Table I.

We succeeded in direct somatic embryogenesis initiation and plant regeneration in all *Trifolium* species tested; *T. repens* f. *hollandicum*



5. Regenerated plant of *Trifolium repens* transferred to soil

and f. *giganteum, T. pratense* cvs. Start and Kvarta, *T. alpestre, T. ambiguum, T. medium, T. pannonicum, T. rubens* and *T. vesiculosum.* Indirect somatic embryogenesis from hypocotyl and other seedling segments was not so successful. Parrott and Collins (1982) succeeded in somatic embryogenesis only in *T. rubens* from the callus in four *Trifolium* species from cell suspensions. Pederson (1986) also obtained regenerated plants from the only species (*T. incarnatum*) within four species tested.

Immature zygotic embryos in the genus *Trifolium* proved to be the most suitable explants for plant regeneration. We also succeeded in plant regeneration via organogenesis from the calli derived from the immature embryos of several *Trifolium* species (Řepková, in press). Other explants (hypocotyl, stem, petiole and leaf) was not suitable for plant regeneration. Embryonic tissues show enhanced potential for both direct and indirect regeneration. Zygotic embryo cultures also gave a good account of plant regeneration in other crops, particularly in cereal crops (Vasil, 1987). The age of explant (embryo) is decisive for successful *in vitro* manipulation.

One of the most promising applications for direct somatic embryogenesis is large scale propagation. Maheswaran and Williams (1985) deal with the origin and development of somatic embryos. They conclude that in *T. repens* the young epidermal cells of embryo hypocotyl show features of proembryogenic cells. They admit both single-cell initiation and multicellular budding. Direct somatic embryogenesis is associated with greater genetic uniformity of regenerated plants owing to omission of callus stage. Therefore this method is a useful tool for clonal propagation. The frequency of somaclonal variation among regenerants is low (Maheswaran, Williams, 1987).

Another application of direct somatic embryogenesis for the improvement of red clover is its use in interspecific hybridization in the genus *Trifolium.* The embryo culture technique enables new hybrid combination raising. In the case of abnormal development of a hybrid embryo on the culture medium, no hybrid plant is obtained. This failure may be overcome by direct somatic embryogenesis or plants can be obtained from totipotent callus culture derived from zygotic embryos.

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ŘEPKOVÁ, J. (OSEVA — Výzkumný a šlechtitelský ústav pícninářský, Troubsko): *Somatická embryogeneze rodu Trifolium a její praktické využití ve šlechtění.* Genet. a Šlecht., *25*, 1989 (3) : 173-180

U několika druhů rodu *Trifolium* byla aplikována metoda přímé somatické embryogeneze ze zygotických embryí v různých fázích jejich vývoje. Tato metoda byla úspěšně aplikována u všech testovaných taxonů rodu *Trifolium: T. repens* f. *hollandicum* a f. *giganteum*, *T. pratense* cv. Start, cv. Kvarta, *T. alpestre*, *T. ambiguum*, *T. medium*, *T. pannonicum*, *T. rubens* a *T. vesiculosum*. Tvorba embryoidů byla nejefektivněji indukována u *T. repens* f. *hollandicum* a f. *giganteum*, *T. ambiguum*, *T. medium*, a to ve fázi torpéda. Tvorba embryoidů byla iniciována na bazálním médiu EC 6 a 0,22 μM a 0,44 μM benzylaminopurinu a 1,0 g kvasičného extraktu na 1 l. Následnou tvorbu rostlin podporovalo bazální médium L2 bez růstových látek.

Trifolium sp.; embryogenní kultury; přímá somatická embryogeneze; šlechtění jetele

РЖЕПКОВА, Я. (ОСЕВА — Научно-исследовательский и селекционный институт кормопроизводства, Троубско): **Соматический эмбриогенез рода *Trifolium* и его практическое использование в селекции.** Genet. a Šlecht., *25*, 1989 (3): 173-180.

У нескольких видов рода *Trifolium* применялся метод прямого соматического эмбриогенеза из зиготных зародышев в разных фазах их развития. Данный метод успешно применяли у всех таксонов рода *Trifolium*: *T. repens* f. *hollandicum* u f. *giganteum*, *T. pratense* cv. Start, cv. Kvarta, *T. alpestre*, *T. ambiguum*, *T. medium*, *T. pannonicum*, *T. rubens* и *T. vesiculosum*. Образование эмбриоидов найболее эффективно было индуцировано у *T. repens* f. *hollandicum* u f. *giganteum*, *T. ambiguum*, *T. medium*, причем в фазу торпеда. Образование эмбриоидов было инициировано на основной среде EC 6 с 0,22 μM и 0,44 μM бензиламинопурина и 1,0 г дрожжевого экстракта на 1 л. Последующее образование растений поддерживала основная среда L2 без ростовых веществ.

Trifolium sp.; культуры зародышев; прямой соматический эмбриогенез; селекция клевера

ŘEPKOVÁ, J. (OSEVA — Forschungs- und Züchtungsinstitut für Futterpflanzenbau, Troubsko): *Somatische Embryogenese der Gattung Trifolium und ihre praktische Anwendung in der Züchtung.* Genet. a Šlecht., *25*, 1989 (3) : 173-180

Bei einigen Arten der Gattung *Trifolium* wurde die Methode der direkten somatischen Embryogenese aus zygotischen Embryos in verschiedenen Phasen ihrer Entwicklung appliziert. Diese Methode wurde bei sämtlichen getesteten Taxa der Gattung *Trifolium* erfolgreich appliziert u. zw.: *T. repens* f. *hollandicum* und f. *giganteum*, *T. pratense* cv. Start, cv. Kvarta, *T. alpestre*, *T. ambiguum*, *T. medium*, *T.*

pannonicum, *T. rubens* und *T. vesiculosum*. Die Bildung der Embryoide wurde am effektivsten bei *T. repens* f. *hollandicum* und f. *giganteum*, *T. ambiguum*, *T. medium* induziert uzw. in der „Torpedo"-Phase. Die Bildung der Embryoide wurde auf dem Basalmedium EC 6 und 0,22 μM- und 0,44 μM-Benzylaminopurin und 1,0 g Hefeextrakt pro 1 l initiiert. Die nachfolgende Bildung der Pflanzen wurde durch das Basalmedium L2 ohne Wachstumssubstanzen unterstützt.

Trifolium sp.; embryogene Kulturen; direkte somatische Embryogenese; Kleezüchtung

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GENETIC ANALYSIS AND CORRELATION IN THE CONTENT OF PROTEINS AND AMINO ACIDS IN GRAIN MAIZE

B. Ryšavá

RYŠAVÁ, B. (Maize Research Institute, Trnava): *Genetic Analysis and Correlation in the Content of Proteins and Amino Acids in Grain Maize.* Genet. a Šlecht., *25*, 1989 (3) : 181-190

Following results can be obtained from an analysis of diallel crossing of the maize inbred lines with o2 gene Tva 8433 o2 and Tva 8006 o2, and their analogues Tva 8507 and Tva 8545, and from an analysis of combining ability in the content of protein and amino acids. The contents of proteins, aspartic acid, threonine, glycine, histidine, arginine, and lysine is controlled by an additive genetic system. *In case of the leucine content, both genetic systems are balanced.* Based upon GCA effects, inbred lines with o2 can be recommended for the selection use for the content of aspartic acid, threonine, histidine, lysine, and arginine. For the leucine content, inbred lines Tva 8507 and Tva 8545. Among individual amino acids, we have found in 34 cases strong correlations, in 8 cases positive dependence, in 1 case negative dependence, and in 3 cases highly significant negative dependence.

maize; amino acids; inbred lines

Ivanko (1983) states that biochemical study of plant proteins which are to contribute both to increase of the protein content and improvement of the amino acid composition, and finally, to increase their utilization by an animal organism is being deepened all over the world.

Selection for higher protein quality is influenced by negative correlation between the content of proteins and content of lysine as it has been found by many authors (Janickij, Kovalčík, 1964).

Expression of the protein content of the maize grain is influenced by an interaction of genotype X environment (Genter et al., 1956; Bressani et al., 1962; Balint et al., 1970; Decau, Pujol, 1973; Pollmer et al., 1978).

According to Zima (1979), opaque-2 gene does not influence the protein content in grain but it increases the lysine content in 76 % in inbred lines, and in 45—60 % in hybrids. Woodworth, Jugenheimer (1948) and Frey (1949) claim that the protein content is directed by a polygene genetic system. These authors have also found intermediate heredity of the protein content. Dundley et al. (1975) confirmed that it is possible to eliminate considerably the yield reduction in *o2* materials by an appropriate breeding method. With the help of recurrent selection, the grain yield of opaque-2 hybrids has increased from original 85 % to 99 % of the yield of six normal standard hybrids.

MATERIALS AND METHODS

Inbred lines Tva 8507, Tva 8545, Tva 8433 o2, and Tva 8006 o2 have been used in diallel crossing to determine genetic condition in the content of protein and 15 amino acids. After the full diallel crossing, the trial was sown in 4 replications. The trial plot had 4 rows in each replication. In the two middle rows, the seeds were produced through the mixture of pollen to determine the content of proteins and amino acids. The trial was sown with spacing 0.7 x 0.2 m, i. e. 71 428 plants per ha. There were 24 plants in one row of which 20 plants were harvested. The content of proteins and amino acids of grain has been determined from samples by analyser AAA T 339 in a laboratory in the Maize Research Institute. Method by Griffing (1956) has been used for results evaluation.

RESULTS

The content of individual amino acids can be seen in Fig. 1. Of studied indices, the significant influence of hybrids has been found only in the content of proteins and other 7 amino acids.

Results of genetic variability analyses are summed up in Tab. I. In the protein content, significant effect of GCA and SCA, and insignificant reciprocal effect has been observed. The ration of GCA : SCA (2.377 : 1) indicates the control of the protein content by an additive genetic system. The inbred lines Tva 8433 o2 (0.23) and Tva 8545 (0.34) have reached the value of GCA effects (Tab. II). The highest SCA values have been found in hybrid combinations Tva 8433 o2 X Tva 8006 o2 (0.55), Tva 8507 X Tva 8006 o2 (0.31). Negative values of SCA effects have also been found. Though a part of reciprocal effect has been found statistically insignificant in the analysis of genetic variability. relatively greater differences have been observed in the crossing direction in some hybrid combinations. The highest negative effect had the combination Tva 8006 o2 X Tva 8433 o2.

Significance of GCA, SCA and insignificance of reciprocal effect have been observed in the leucine content (Tab. I). GCA : SCA is 1.188 : : 1. It can be concluded out of this that the leucine content represents a balanced influence of an additive gene system. The highest positive GCA effects have been reached by the inbred lines without o2 gene — Tva 8507 (0.58) and Tva 8545 (0.14). negative effects have been reached by the inbred lines with *o2* gene. The highest SCA values have been observed in the hybrid combination Tva 8545 X Tva 8433 o2 (1.11), Tva 8545 X Tva 8006 o2 (0.69). Negative SCA values have also been found. The highest values of reciprocal effects have been observed in Tva 8006 o2 X Tva 8507 (0.62), and Tva 8545 X Tva 8507 (0.54).

The analysis of combining ability in the content of aspartic acid showed an expressive prevalence of GCA in the realization of this trait. The ratio of GCA : SCA (8.052 : 1) showed the prevalence of the additive gene system. According to the GCA effects, the best combining abilities could be observed in the inbred lines with *o2* gene — Tva 8433 o2 (0.12), Tva 8006 o2 (0.98). The highest SCA values have been calculated in the hybrid combinations Tva 8433 o2 X Tva 8006 o2 (0.47). Other values of SCA effects have been negative. The results of calculation of values of reciprocial effect have been both positive and negative. Occurrence of negative reciprocal effect in the hybrid combination in which the inbred line o2 was used as a mother component, only confirms the result of the

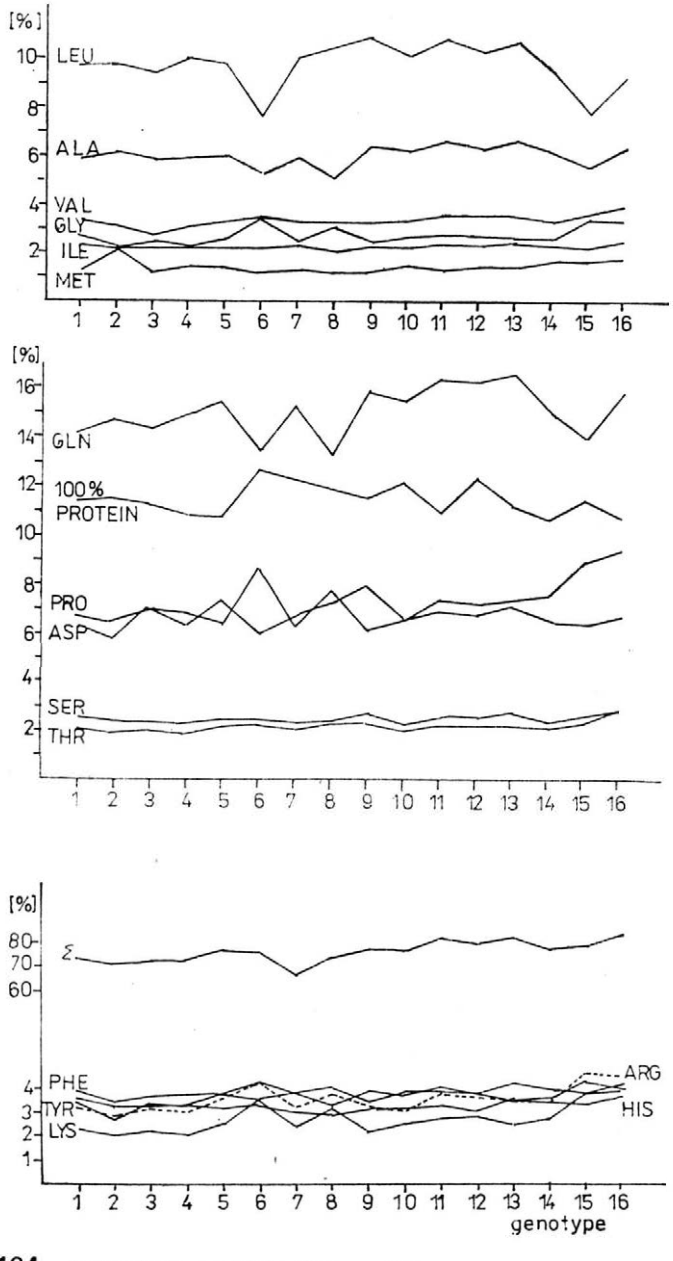
I. Analysis of variance and special combining ability and reciprocal effect in proteins and some amino acids

Character	Source of variance	Freedom degree	Mean squares	*F* values	Proportion GCA : SCA
LEU	GCA	3	2.0004	4.2551+	1.188 : 1
	SCA	6	1.6835	3.5811+	
	Recip.	6	0.5689	0.7847	
	Error		0.4701		
HIS	GCA	3	0.4604	18.3258+	12.315 : 1
	SCA	6	0.0373	1.4878	
	Recip.	6	0.0331	1.3202	
	Error		0.0251		
LYZ	GCA	3	1.2801	33.3700+	9.038 : 1
	SCA	6	0.1416	3.6926+	
	Recip.	6	0.1724	0.4496	
	Error		0.3836		
ARG	GCA	3	0.8531	10.8431+	5.844 : 1
	SCA	6	0.1459	1.8551	
	Recip.	6	0.0635	0.8078	
	Error		0.786		
Proteins	GCA	3	0.9179	8.2827+	2.377 : 1
	SCA	6	0.3861	3.4845	
	Recip.	6	0.1843	1.6635	
	Error		0.1108		
ASP	GCA	3	4.4072	13.1746+	7.052 : 1
	SCA	6	0.6252	1.8689	
	Recip.	6	0.4366	0.1305	
	Error		0.3345		
THR	GCA	3	0.1670	8.7692+	4.957 : 1
	SCA	6	0.0235	1.2366	
	Recip.	6	0.0077	0.4077	
	Error		0.0194		
GLY	GCA	3	0.5437	32.6633+	8.947 : 1
	SCA	6	0.0607	3.6505+	
	Recip.	6	0.0165	0.9971	

GCA — general combining ability
SCA — special combining ability
Recip. — reciprocal effect; + significant at $P = 0.05$

II. Effect of GCA

	Proteins	Rank	ASP	Rank	GLY	Rank	THR
Tva 8507	−0.32	4	−0.68	4	−0.30	4	−0.16
Tva 8545	0.23	2	−0.43	3	−0.12	3	−0.05
Tva 8433	0.34	1	0.12	2	0.17	2	0.04
Tva 8006	−0.25	3	0.98	1	0.25	1	0.17



1. Representation of particular amino acids in the full content of amino acids grain of four inbred lines and their hybrids

Rank	LEU	Rank	HIS	Rank	LYZ	Rank	ARG	Rank
4	0.58	1	−0.29	3	−0.46	4	−0.33	4
3	0.14	2	−0.09	4	−0.17	3	−0.18	3
2	−0.60	4	0.24	1	0.21	2	0.12	2
1	−0.13	3	0.14	2	0.42	1	0.39	1

III. Combining ability effects (SCA above the diagonal) and reciprocal effects

		Tva 8507	Tva 8545	Tva 8433o_2	Tva 8006o_2	GCA
Proteins			0.13	−0.43	0.31	−0.32
ASP			−0.11	−0.32	−0.48	−0.68
THR			−0.02	0.06	−0.08	−0.16
GLY	Tva 8507		0.04	−0.02	−0.15	−0.30
LEU			0.12	0.32	0.18	0.58
HIS			−0.02	0.02	−0.19	−0.29
LYZ			0.03	−0.28	−0.01	−0.46
ARG			0.04	−0.26	0.08	−0.33
Proteins		0.02		0.15	−0.62	0.23
ASP		0.15		−0.48	−0.32	−0.43
THR		−0.01		−0.03	−0.10	−0.05
GLY	Tva 8545	0.10		−0.19	−0.14	−0.12
LEU		0.54		1.11	0.69	0.14
HIS		0.11		−0.05	−0.03	−0.09
LYZ		0.09		−0.25	−0.28	−0.17
ARG		0.21		−0.32	−0.17	−0.18
Proteins		−0.35	0.08		0.55	0.34
ASP		0.07	−0.11		0.47	0.12
THR		0.01	9.10^{-3}		−0.03	0.04
GLY	Tva 8433 o_2	0.01	−0.08		0.20	0.17
LEU		9.10^{-3}	−0.46		−0.99	−0.60
HIS		0.09	−0.23		0.17	0.24
LYZ		0.11	−0.04		0.23	0.21
ARG		0.16	0.07		0.30	0.12
Proteins		0.02	−0.13	−0.62		−0.25
ASP		0.22	0.09	0.17		0.98
THR		0.13	−0.04	0.04		0.17
GLY	Tva 8006 o_2	0.16	−0.06	0.01		0.25
LEU		0.62	−0.64	0.08		−0.13
HIS		0.17	−0.21	−0.02		0.14
LYZ		0.11	3.10^{-3}	0.11		0.42
ARG		0.21	−0.16	0.19		0.39

IV. Correlation coefficients among particular amino acids

	ASP	THR	SER	GLU	PRO	GLY	ALA
Proteins	−0.03	0.00	−0.19	−0.28	−0.31	0.21	−0.36
ASP		0.86^{++}	0.39	−0.20	−0.26	0.90^{++}	−0.32
THR			0.52^{+}	0.03	−0.01	0.83^{++}	−0.10
SER				0.51	0.23	0.42	0.46
GLU					0.37	−0.26	0.95^{++}
PRO						−0.18	0.30
GLY							−0.41
ALA							
VAL							
MET							
ILE							
LEU							
TYR							
PHE							
HIS							
LYS							

combining ability analysis where components of reciprocal effect have been statistically insignificant.

Significant GCA influence, and SCA and reciprocal effect insignificance have been observed in the threonine content (Tab. I). It results from the ratio of GCA : SCA (4.85 : 1) that this trait is under control of the additive genetic system. Values of GCA have been positive in the inbred lines with *o2* gene — Tva 8006 o2 (0.17), Tva 8433 o2 (0.02), and their analogues had negative values —0.16 and —0.05. Positive values (0.06) have been obtained in the hybrid combination Tva 8507 × Tva 8433 o2. In other cases there were negative values. The hybrid combination Tva 8006 o2 × Tva 8507 (0.13) had the highest value of reciprocal effect. The direction of crossing had negligible influence in other combinations.

Analysis of genetic variability in the histidine content showed an influence of the general combining ability. It is possible to suppose from the ratio of GCA : SCA (12.315 : 1) that the histidine content is under control of the additive genetic system. The GCA effects made it their analogues had negative values —0.16 and —0.05. Positive values (0.24, 0.14) have been obtained in the inbred lines with *o2* genes, and negative values of GCA (—0.29, —0.09) in their analogues. The highest SCA values for the histidine content were in Tva 8507 × Tva 8433 o2, and Tva 8433 o2 × Tva 8006 o2 (0.17) — Tab. III.

Correlation between proteins, amido acids

Correlation between particular traits (proteins, amino acids) characterizes dependence of traits in a set. Observed correlation expressed

VAL	MET	ILE	LEU	TYR	PHE	HIS	LYZ	ARG
0.03	−0.23	−0.23	−0.26	−0.44	−0.45	0.31	0.12	0.00
0.61^{+}	0.14	0.34	-0.63^{++}	0.53	0.14	0.73^{++}	0.93^{++}	0.91^{++}
0.79^{++}	0.22	0.54^{+}	−0.41	0.47	0.28	0.77^{++}	0.91^{++}	0.87^{++}
0.73^{++}	0.18	0.75^{++}	0.21	0.53^{+}	0.66^{++}	0.24	0.40	0.55^{++}
0.38	0.18	0.72^{++}	0.85^{++}	0.29	0.73^{++}	−0.25	−0.22	−0.11
−0.06	−0.27	0.11	0.35	0.86	0.20	−0.14	−0.19	−0.12
0.72^{++}	0.04	0.30	-0.67^{++}	0.34	0.04	0.91^{++}	0.98^{++}	0.94^{++}
0.24	0.25	0.67^{++}	0.91^{++}	0.27	0.74^{++}	0.42	−0.38	−0.27
	0.26	0.77^{++}	−0.03	0.36	0.51^{+}	0.69^{++}	0.71^{++}	0.74^{++}
		0.16	0.00	−0.10	0.67	−0.10	0.13	0.10
			0.43	0.65^{++}	0.89^{++}	0.23	0.32	0.40
				0.05	0.56^{+}	-0.60^{+}	-0.66^{++}	-0.56^{+}
					0.77^{++}	0.20	0.36	0.45
						0.01	0.06	0.19
							0.88^{++}	0.83^{++}
								0.95^{++}

by the significance of a correlation coefficient expressed by the total of relationship valid in the studied diallel set. Correlation coefficients between individual amino acids are in Tab. IV. Limits of high significance were reached by coefficients between aspartic acid and threonine (0.86^{++}), glycine (0.90^{++}), histidine (0.73^{++}), lysine (0.93^{++}), arginine (0.91^{++}), between threonine and glycine (0.88^{++}), valine (0.79^{++}), histidine (0.77^{++}), lysine (0.01^{++}), and arginine (0.87^{++}). Values between serine and valine were (0.73^{++}), isoleucine (0.75^{++}), phenylalanine (0.66^{++}), between glutamic acid and alanine (0.95^{++}), isoleucine (0.72^{++}), leucine (0.85^{++}), phenylalanine (0.73^{++}). High significance was calculated between glycine and valine (0.72^{++}), histidine (0.91^{++}), lysine (0.08^{++}) and arginine (0.94^{++}), between alanine and isoleucine (0.67^{++}), leucine (0.91^{++}), phenylalanine (0.74^{++}). 0.77^{++} was calculated in valine, 0.68^{++} with isoleucine, 0.71^{++} with lysine, 0.69^{++} with histidine, and 0.74^{++} with arginine. Isoleucine reached limits of high significance with tyrosine (0.65^{++}) and phenylalanine (0.89^{++}). Tyrosine with phenylalanine reached value 0.77^{++}, histidine with lysine 0.88^{++} and with arginine 0.83^{++}, and lysine with arginine 0.95^{++}. Significant influence between individual amino acids was calculated in eight cases.

Highly significant negative correlation was observed in aspartic acid and leucine, between leucine and glycine, leucine and lysine. Correlation coefficient -0.56^{++} was calculated in arginine and leucine. In our experiment, no significance or high significance, neither positive nor negative, was observed between individual amino acids or proteins.

DISCUSSION

Comparison of by us obtained results with those obtained by other authors is not easy. Every researcher chooses for his work his own sets of genotypes which are characterized by certain traits and chooses different methods of elaboration. The next problems is that evaluation is done in different climatic-agroclimatic conditions which influence considerably the manifestation of observed traits, and also contribution of individual genetic influences. Comparison is thus possible only in general conclusions, and namely in analogical trial scheme.

Those by us chosen inbred lines have been crossed according to the diallel scheme to compare the contribution of combining abilities of particular genotypes in the content of proteins and amino acids. The method by Griffing (1956) made it possible to consider the general and special combining ability of the observed traits.

According to our results, the content of proteins, aspartic acid, threonine, glycine, arginine, and lysine is controlled by an additive gene system. Genetic additive systems prevail nonadditive genetic systems in the observed traits. In case of leucine, GCA and SCA were quite balanced.

Our results are equal with those of Zima (1979) by which *o2* gene does not influence the protein content in the grain. Correlation coefficient of 0.123 between the content of proteins and lysine has been found between the content of proteins and particular amino acids. Zima (1979) has found a significant negative correlation between the content of proteins and lysine ($r = -0.35$) in inbred lines and ($r = -0.40$) in hybrids. Adverse influence of the high protein content on the grain yield was observed by Ključko, Maksak (1969). A negative correlation coefficient ($r = -0.486$) has been found between these traits. At the same time, these authors observed that the protein content per unit of area in inbred lines depended upon both grain yield and protein content. That by us observed correlation coefficient between the grain yield and protein content was ($r = -0.026$). Jugenheimer (1976) has found a negative correlation between the grain yield and protein content ($r = -0.522$). The author observed considerable influence of the mother component.

CONCLUSION

Generally it is possible to state that the content of proteins, aspartic acid, threonine, glycine, histidine, arginine, and lysine is controlled by an additive genetic system. Both additive and non-additive genetic systems are balanced in case of the leucine content. Possitive GCA effects in inbred lines Tva 8433 o2 and Tva 8006 o2, and negative values in Tva 8507 and Tva 8545 have been calculated in all cases in the content of aspartic acid, threonine, histidine, lysine, and arginine. The situation was contrary in case of the leucine content.

Correlation between the content of proteins and that of 15 amino acids has been studied. 34 cases of strong positive dependence and 8 cases of positive dependence have been found. In one case, negative significant dependence has been calculated, and in 3 cases highly significant dependence.

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RYŠAVÁ, B. (Výskumný ústav kukurice, Trnava): *Genetická analýza a vzájomné vzťahy pri obsahu biedlkovín a aminokyselin pri kukurici na zrno.* Genet. a Šlecht., *25*, 1989 (3) : 181-190

Z analýzy dialelického kríženia samoopelených línií kurice s o2 génom Tva 8433 o2 a Tva 8006 o2 a ich analógov Tva 8507 a Tva 8545 ako aj analýzy kombinačnej schopnosti pri obsahu bielkovín a niektorých aminokyselin možno urobiť závery: Obsah bielkovín, kyseliny asparágovej, treonínu, glycínu, histidínu, arginínu, lyzínu sú pod kontrolou aditívneho genetického systému. Pri obsahu leucínu sa oba genetické systémy vyrovnávajú. Samoopelované línie s o2 genom možno na základe efektov GCA odporúčať pre šľachtiteľské využitie pre obsah kyseliny asparágovej, treonínu, histidínu, lyzínu a arginínu. Pre obsah leucínu samoopelované línie Tva 8507 a Tva 8545 bola zistená v 34 prípadoch silná závislosť medzi jednotlivými aminokyselinami a osem závislostí kladných, jeden prípad negatívnej a tri prípady vysokopreukaznej negatívnej závislosti.

kukurica; aminokyseliny; samoopelované línie

РИШАВА, Б. (Научно-исследовательский институт кукурузы, Трнава): **Генетический анализ и взаимные отношения при содержании белков, аминокислот и кукурузы на зерно.** Genet. a Šlecht., *25*, 1989 (3) : 181-190.

Из анализа диаллелического скрещивания самоопыленных линий кукурузы с o2 геном Тва 8433 o2 и Тва 8006 o2 и их аналогами Тва 8507 и Тва 8545, а также анализа способности комбинации при содержании белков и некоторых аминокислот можно провести заключение: содержание белков, аспарагиновой кислоты, треонина, глицина, гистагина, аргинина, лизина находится под контролем адиттивной генетической системы. При содержании лейцина обе генетические системы выравниваются. Самоопыленные линии с o2 на основе эффектов GCA можно рекомендовать для селекционного использования за содержание аспарагиновой кислоты, треснина, гистидина, лизина и аргинина. При содержании лейцинов самоопыленных линий Тва 8507 и Тва 8545 была установлена в 34 случаях сильная зависимость между отдельными аминокислотами и восемь положительных зависимостей, один случай отрицательный и три случая высоко достоверной отрицательной зависимости.

кукуруза; аминокислоты; самоопыленная линия

RYŠAVÁ, B. (Forschungsinstut für Maisbau, Trnava): *Genetische Analyse und gegenseitige Beziehungen der Eiweiß- und Aminosäurengehalts bei Körnermais.* Genet. a Šlecht., *25*, 1989 (3) : 181-190

Anhand von Analysen der diallelischen Kreuzung selbstbestäubter (s.) Maislinien mit den *o2*-Genen Tva 8433 o2 und Tva 8006 o2 und deren Analogen Tva 8507 und Tva 8545 sowie anhand der Analyse der Kombinationsfähigkeit in bezug auf den Gehalt an Eiweißstoffen und einigen Aminosäuren können folgenden Schlußfolgerungen gezogen werden: der Gehalt der Eiweißstoffe, der Asparaginsäure des Threonins, Glyzins, Histidins, Arginins und Lysins stehen unter Kontrolle des additiven genetischen Systems. Beim Leuzingehalt stehen beide genetische Systeme in Gleichgewicht. Die s. Linien mit o2 können aufgrund der GCA-Effekte in bezug auf den Asparaginsäure-, Threonin-, Histidin-, Lysin- und Argininingehalt für die züchterische Anwendung empfohlen werden. In bezug auf den Leuzingehalt der s. Linien Tva 8507 und Tva 8545 wurde in 34 Fällen eine starke Abhängigkeit zwischen den einzelnen Aminosäuren sowie acht positive Abhängigkeiten, ein Fall einer negativen und drei Fälle hochsignifikanter negativer Abhängigkeiten festgestellt.

Mais; Aminosäuren; selbstbestäubte Linie

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EVIDENCE FOR GENETIC POLYMORPHISM IN PROGENY OF HIGH-PROTEIN HUSKLESS MUTANTS OF BARLEY DEMONSTRATED BY HORDEI-SPECTRA

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PONERT, J. — UHLÍK, J. (Czechoslovak Biochemical Society, Praha; Agricultural Technical University, Praha): *Evidence for Genetic Polymorphism in Progeny of High-protein Huskless Mutants of Barley Demonstrated by Hordein-Spectra.* Genet. a Šlecht., *25*, 1989 (3) : 191-207.

Complex of hordeins from caryopsis of spring barley Czechoslovak cultivar Atlas and of M_{13}-progeny of its mutants has been separated by extraction and electrophoresis on polyacrylamide gel, followed by computerized densitometry. Such method seems to be more resultative for identification and semiquantitative estimation of newly named individual hordeins. For distinguishing of hordein-spectra types the hordeins of the first main group have been used as genetic markers. In such a way the genetic polymorphism in progeny of high-protein huskless mutants originated from M_{13}-individual plants has been confirmed in M_{13}-progeny. The possible reasons of such genetic polymorphism are discussed.

hordeins; high-protein huskless mutants; two-rowed spring barley; Czechoslovak barley mutants; genetic markers; genetic biochemical polymorphism; *Hordeum;* lysine negative correlation with protein; individual selection in M_2- and M_3-generations; M_{13}-generation

On the base of dependence of formation of specific proteins on genotype it is possible to use the knowledge of barley special proteins--hordeins for estimation of degree of genetic identity of individual plants of barley. Further possibilities given by estimation of protein hordeins-complexes are proposed by Sozinov et al. (1976, 1978). Identification and distinguishing of individual hordeins produced on the base of different genetic informations is nevertheless dependent on the sensitivity of the analytical method used.

Instead of formerly used starch gel electrophoresis with visual estimation we have recently used more advanced and more sensitive method, giving us the possibility of more clear distinguishing of larger number of hordeins with some possibility of quantitative estimation of relative amounts of individual hordeins. The extraction system for hordeins has been modified by Bartko (1987 — personal communication), hordeins have been then separated by electrophoresis on polyacrylamide gel, coloured by Blue R-250 in trichloracetic acid and densitometrically evaluated under the computer control (program Analyst — 1 of Bio-Rad). Such densitograms allow to us the distinguishing of fine genetic types in the progeny of mutants. For such study the advanced M_{13} — progeny of individually selected in M_3 plants is used by us.

MATERIALS AND METHODS

Starting Material

As starting material Czechoslovak two-rowed spring barley cultivar Atlas has been used. This cultivar has husky caryopsis and is originated as hybrid Diamant × mutant S 55. As Czechoslovak cultivar Diamant is a radiomutant, we may say, that Czechoslovak Atlas is the hybrid between two mutants.

Chemical Mutagenesis

Husky caryopsises of cv. Atlas has been exposed to 17 concentrations of methyl--nitro-urea in span between 1 mmol to 10 mmol for 3 hours at temperature 25 ± ± 1 °C. Washing for 4 hours and 3.5 hours drying at 40 °C followed. 12 other hours the material has been dryed at 24 °C.

Further Breeding and Testing

M_1 has been cultivated in field in 1974. In M_2-plants the huskless caryopsises have been selected and in further generations the crude protein content has been estimated in huskless progenies. Dependence among lysine per protein and protein content per dry matter of caryopsis has been studied in M_3-generation and for huskless mutant ÚJ-5 and huskless mutant ÚJ-21 — families the negative correlation between crude protein and lysin has been established (Fig 1).

Final biochemical estimation of mutants for proteins and content of individual amino acids in hydrolyzed storage — proteins has been realized in M_5 — M_8 generations (Uhlík, Burianová, 1983). Description and yield characteristics of huskless barley mutants ÚJ-5g and ÚJ-21a in generation M_6 and M_7 have been published by Uhlík, Birianová (1982), possibilities for backcross with other Czech barley cultivars are mentioned by Uhlík et al. (1986) and Uhlík, Marek (1988). The huskless mutants exceed 80 per cent of husky cultivar Atlas. In comparison with these yields it is necessary to take into account the necessary correction: the hull forms about 11 per cent of the weight of husky caryopsis.

Analytical

As sample the individual caryopsis is used in the form of meal, extracted by 1.0 ml of extractive mixture. Composition of the extractive mixture is as follows: 2-chlorethanol — 30 g; urea — 18 g; 2-mercaptoethanol — 1 g; redestiled water ad 100 ml.

The polyacrylamid gel is prepared from the following compounds: acrylamid — 80 g; bisacrylamid — 6 g; urea — 60 g; acetic acid glacial — 500 ml; ascorbic acid — 1 g; glycine — 1 g; redestiled water ad 1000 ml.

Cell: Bio-Rad Protean II 16 x 18 cm, thickness of gel 1.5 mm. Conditions for electrophoretic separation: constant current

40 mA, 10000 Vh (approximately 18 hours at appr. 580 V).

Colouring of proteins by 0.2 per cent solution of Serva Blue

R-250 in 10 per cent trichloracetic acid.

After drying the gels have been estimated by densitometer VD 620

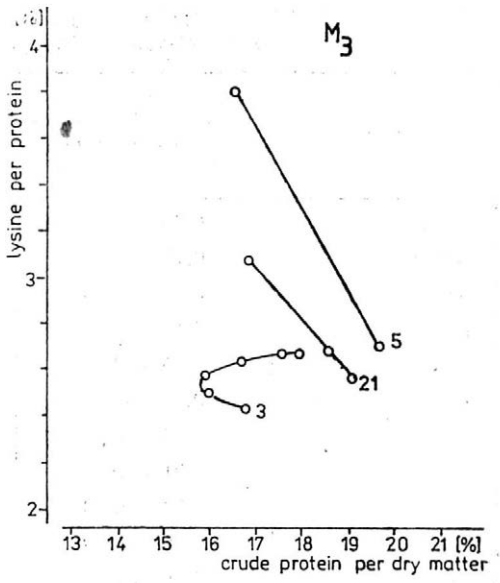
using the program (software) Analyst — 1 (Bio-Rad).

Identification and Naming of Hordeis

We are able to identify the peaks representing the individual hordeins on the base of their relative mobility, their sequence on densitograms and densitograms' „finger-prints" careful examination and comparison. After naming the main identified hordeins more precise and more convenient comparison of hordein-mixture separation-spectra is possible. Using the sequence of main hordein descendently arranged according to their relative content we are able to write short formulas characterizing different types of natural hordein composition.

As genetic markers the hordeins of the first main group have been used by us. Our first main group of hordeins probably corresponds to the „C-hordeins group" of other authors e. g. Køie et al. (1976).

1. Mutual dependence between lysine content in protein and protein content in dry matter of caryopsis in mutant families in M3-generation. In mutant ÚJ-5--family and in mutant ÚJ-21-family the negative correlation between lysine-content and protein-content is manifested, while in mutant ÚJ-3-family such negative correlation has been overcome



RESULTS AND DISCUSSION

Identification of individual hordein by means of one-dimensional vertical electrophoresis on polyacrylamide gel based on two physical chemical characters, namely on the electric charge and diameter of the protein molecule only, is difficult. Further methods for full identification, e. g. determination of the sequence of amino acids in the isolated and purified protein eventually followed by some sophisticated analysis of its tertiary structure, are time consuming and not operative for the purpose of the plant breeding and for these reasons they were not used in this study. From other methods the immunochemical methods and radio-immuno-assay might be perspective in future, although we are not sure if complex information about all the hordeins would be obtainable by them. Nevertheless, for the purpose of our study for evaluation of the genetic polymorphism we find the method used to be fully adequate.

For the genetic analysis of practical important characters with complicated heredity and manifestation as baking quality or winter resistence Sozinov (1985) has proposed the blocks of glyadins as genetic markers in wheat. Similarly the block method for hordeins is recommended in barley (Sozinov, 1985; Šašek et al., 1988). The concept of such blocks is based on the correlation between hordeins found during the hybridological analysis. Nevertheless, for the genetic analysis of mutants we do not accept such approach taking into account the possibility of point mutations. We prefer to use the individual hordeins as genetic markers especially in the case of genetic comparison of mutants.

The names given to hordeins by us serve for helping- or stipulative designation of components of the hordein fraction of storage proteins of caryopsis. Such naming will further help us in forming of so called "hordein formulas" of types of hordeins natural composition.

I. Relative mobilities of main hordeins in the analytical system used (For the beginning of gel = 0.00, for end of the plate = 1.00)

Name of hordein	Range of relative mobilities for the top of the peak on different gel plates	Name of hordein	Range of relative mobilities
ant 1	0.09 – 0.12	B0	0.30 – 0.40
sub P	0.10 – 0.15	REF	0.38 – 0.45
prop	appr. 0.15	B1	0.43 – 0.51
P0	0.14 – 0.19	B2	0.45 – 0.54
Atl 1	0.16 – 0.21	B3	–0.51 – 0.57
Atl 2	0.18 – 0.23	B4	0.49 – 0.61
a	0.20 – 0.25	B5	0.54 – 0.62
P1	0.21 – 0.26	B6	0.57 – 0.65
P2	0.23 – 0.28	B7	0.62 – 0.70
P3	0.26 – 0.29	B9	appr. 0.76
P4	0.28 – 0.31		
P5	0.29 – 0.32		

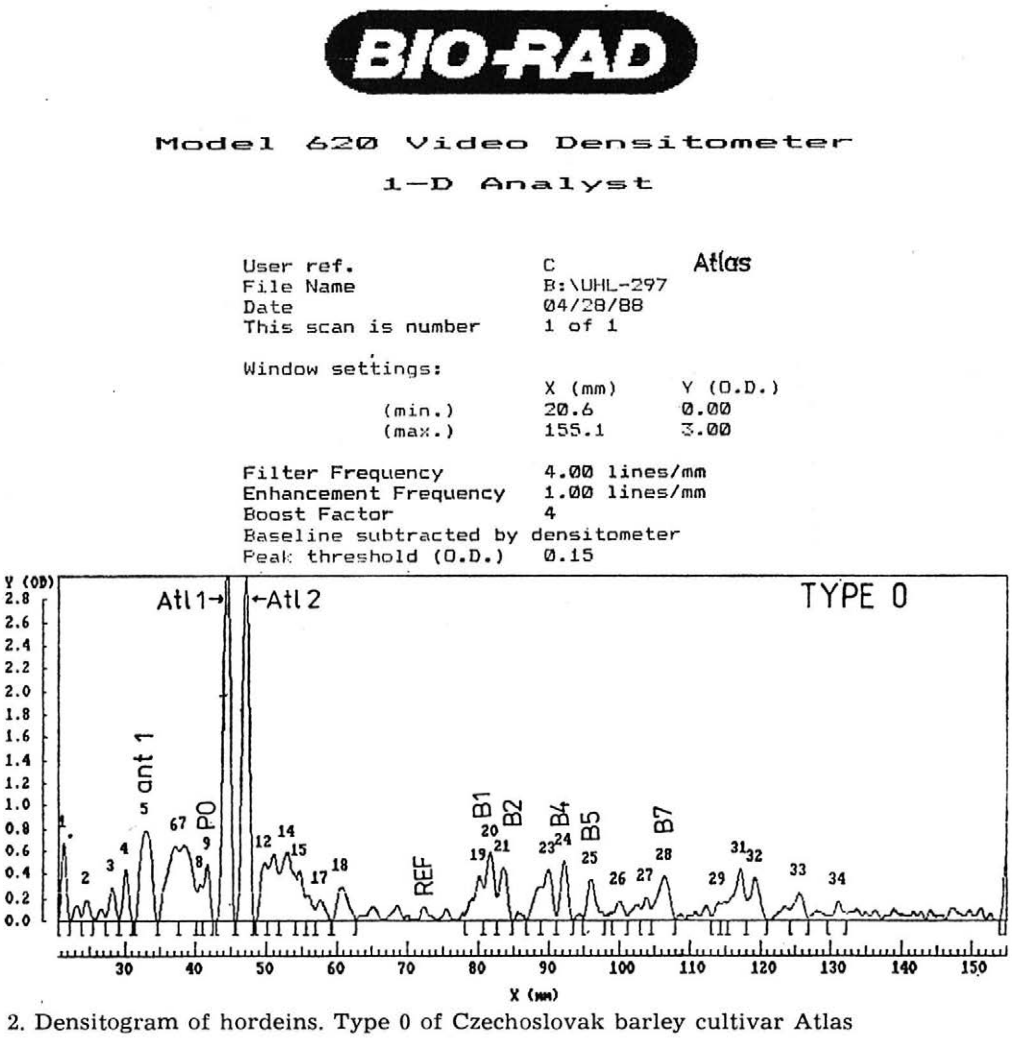
Note: hordeins fork 1, interfork, fork 2 substitute Atl 2 on some densitograms, having the relative mobility near to Atl 2

In the preceding Tab. I we can compare the relative mobilities of hordeins. The relative mobility of identified named hordein give us the good first orientation. On the other hand it is not possible to make full identification of individual hordeins on the base of its relative mobility only, because some "flowing" of the numerical values of relative mobility between different gel plates and even between different samples on the same gel plate is necessary to take into account. Approximate limits of such "flowing" of numerical values of relative mobility for the experimental conditions formerly described are given in Tab. I. So the sequence of peaks on densitogram as well as the careful examination and comparison of the whole "finger-prints" on densitograms of hordein--mixture separation-spectra are necesary for identification of individual hordeins, too.

In such a way we can overcome the slight differences between migration distances for the same individual protein from different samples, e. g. for hordein P_0. During such comparison we have to keep in mind that in various types of hordeins' natural composition the same hordein often covers the different relative area and has the different height of peak. So you can see on different densitograms for hordein P_0 in the case of ilustrated further mixture types, e. g. the following values: 52 mm/ > 2.8 OD, 42 mm/ 0.4 OD, 54 mm/ 0.6 OD, 63 mm/ 2.2 OD etc.

Hordeins from prop to P5 belong to our main group of hordeins, while the second main group of hordeins starts with BO and continues after REF through B1 further.

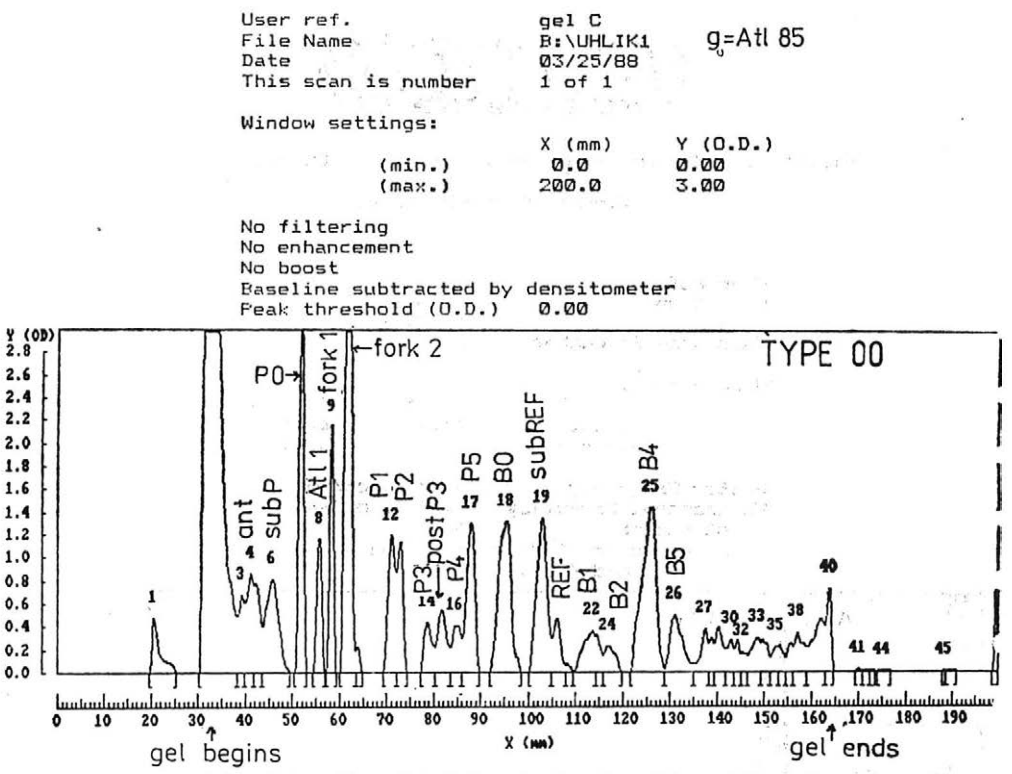
There are two approaches how to look for the relative content of main hordeins: according to the height of peaks on densitograms or



2. Densitogram of hordeins. Type 0 of Czechoslovak barley cultivar Atlas

according to the relative area. In the first case we select the characteristical hordein common four types (of hordein-mixture separation--spectra) compared or the hordein with the highest peak. To such selected peak the number 100 is given by convention. In both approaches we are able to arrange descendently the named hordeins.

Fig. 2 — Fig. 13 show us the types of natural hordeins composition distinguished in our material. In Tab. II relative area of hordein-peak on model densitogram are given according to the types. Characterization of the types of natural hordeins composition is based on hordeins of the first main group, nevertheless also the other hordeins are mentioned in Tab. III and IV, too. Both these tables show the named hordeins in descendent order, Tab. III according to their relative height of peak on densitogram, Tab. IV according to their relative area with special attention to 10 per cent- and 5 per cent-limits. We can easily see, that both approaches give to us generally similar but not fully identical results. The characteristical hordeins important for distinguishing the types are underlined in Tab. III and IV. We can see that all these differential



3. Densitogram of hordeins. Type 00 of Czechoslovak cultivar Atlas. An extraordinary densitogram without correction for the beginning and the end of the separation gel

hordeins belong to the first main group of hordeins. These differential hordeins are, nevertheless, not specific for some natural hordeins-composition type, but differ quantitatively being present in various types in different relative content. We prefer to limit us to the semiquantitative estimation only and try to find the characteristic formulas for the types (Tab. V) based on "finger-prints". Further it is possible to evaluate the types hordeins natural compositions on the base of occurrence of common or specific characters and to make the appropriate grouping of types, as shown in Tab. VI.

We see that all the mutant types differ from type No 0 of starting Czechoslovak cultivars Atlas. Type No 0 has not been found in mutant ÚJ-5g or ÚJ-21a. From Tab. VI and from previous tables, too, we learn that P0 — hordein is characteristic for all mutant types but not apparent for type No 0 of Atlas. Nevertheless P0 — hordein is present in type No 00 of Atlas. Type No 00 of Atlas resembles to some extend the mutant type No 5 of mutant ÚJ-5g. But also in this case we can find differences. According the height of peaks for type No 00 the middle part of the hordeins-order is P5 > P1 ≥ P2 > post P3 > P3 > P4, while for mutant type No 5 the hordeins-order is P2 > P1 > P3 > P4 > P5 > post P3. At type No 00 the hordein interfork has not been found, while such hordein is present at type No 5.

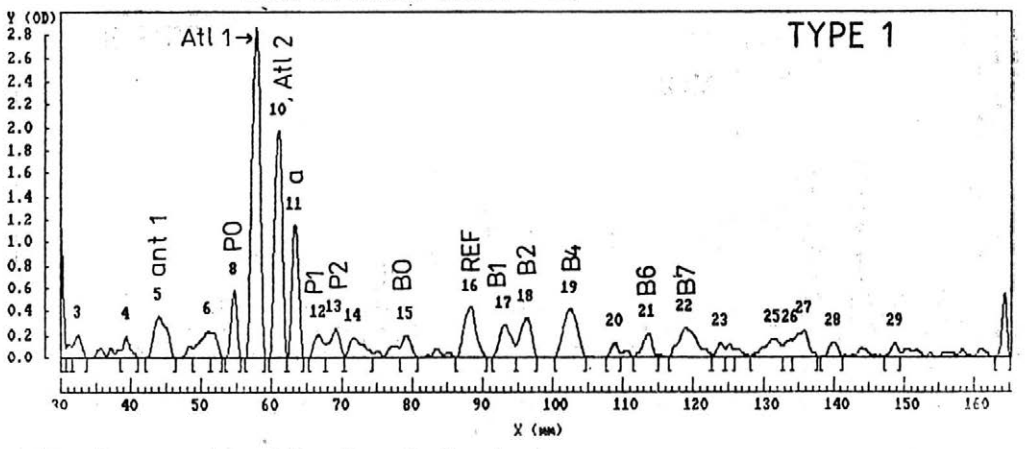
In Tab. VII the occurrence of types of hordeins natural composition is given for M_{13} generation. Types NoNo 1 and 4, according to the recent

User ref. AIII9 plucha-2 DOKSANY-21a
File Name B:\PONRT14B
Date 04/27/88
This scan is number 1 of 1

Window settings:

	X (mm)	Y (O.D.)
(min.)	30.0	0.00
(max.)	165.5	3.00

Filter Frequency 4.00 lines/mm
Enhancement Frequency 0.50 lines/mm
Boost Factor 2
Baseline subtracted by densitometer
Peak threshold (O.D.) 0.12



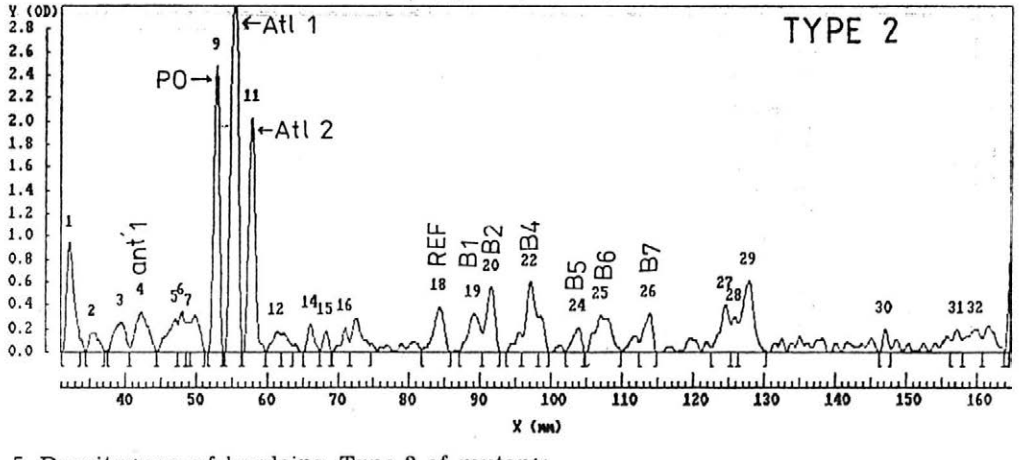
4. Densitogram of hordeins. Type 1 of mutants

User ref. D-typ 1 ÚJ-21a
File Name B:\UHL-298
Date 04/28/88
This scan is number 1 of 1

Window settings:

	X (mm)	Y (O.D.)
(min.)	30.9	0.00
(max.)	164.9	3.00

Filter Frequency 4.00 lines/mm
Enhancement Frequency 1.00 lines/mm
Boost Factor 4
Baseline subtracted by densitometer
Peak threshold (O.D.) 0.15



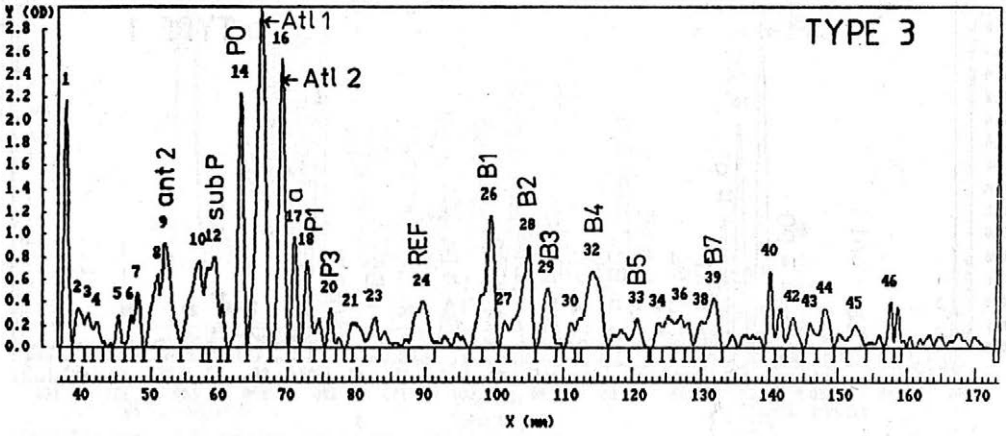
5. Densitogram of hordeins. Type 2 of mutants

User ref.	BI2 s pluchou	DUŠNÍKY-21a
File Name	B:\PONRT12B	
Date	04/27/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	36.5	0.00
(max.)	173.6	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	1.00 lines/mm
Boost Factor	5
Baseline subtracted by	densitometer
Peak threshold (O.D.)	0.20



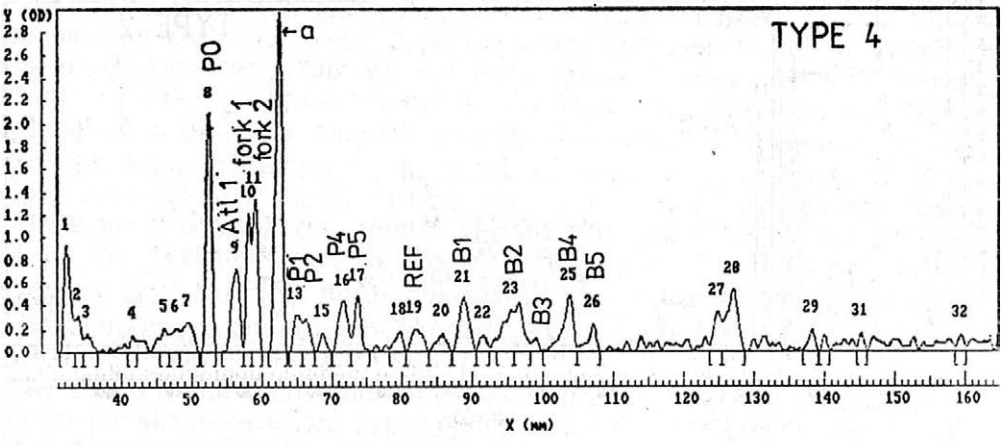
6. Densitogram of hordeins. Type 3 of mutants

User ref.	D-typ 3	ÚJ-21a
File Name	B:\UHL-298B	
Date	04/28/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	30.9	0.00
(max.)	164.9	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	1.00 lines/mm
Boost Factor	4
Baseline subtracted by	densitometer
Peak threshold (O.D.)	0.15



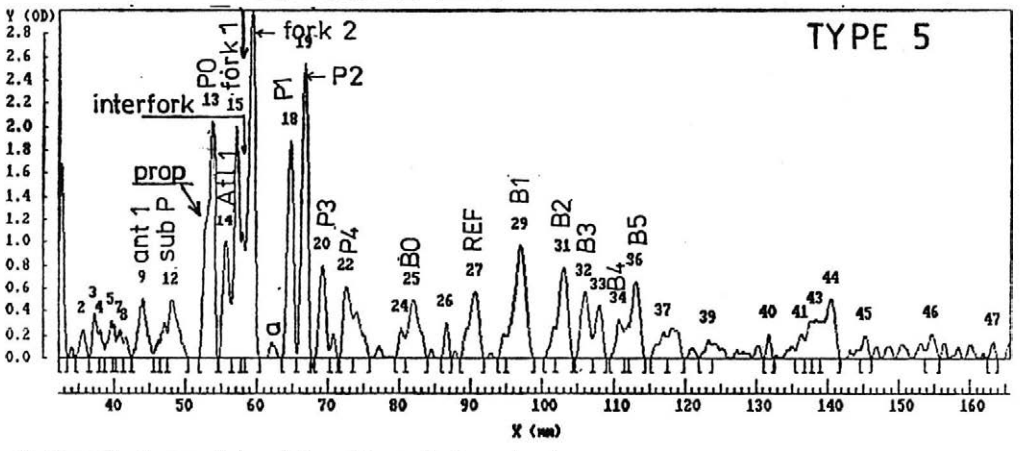
7. Densitogram of hordeins. Type 4 of mutants

User ref.	BII3 holy-1	DUŠNÍKY-5gP
File Name	B:\PONRT17	
Date	04/27/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	32.2	0.00
(max.)	165.9	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	1.00 lines/mm
Boost Factor	7
Baseline subtracted by densitometer	
Peak threshold (O.D.)	0.15



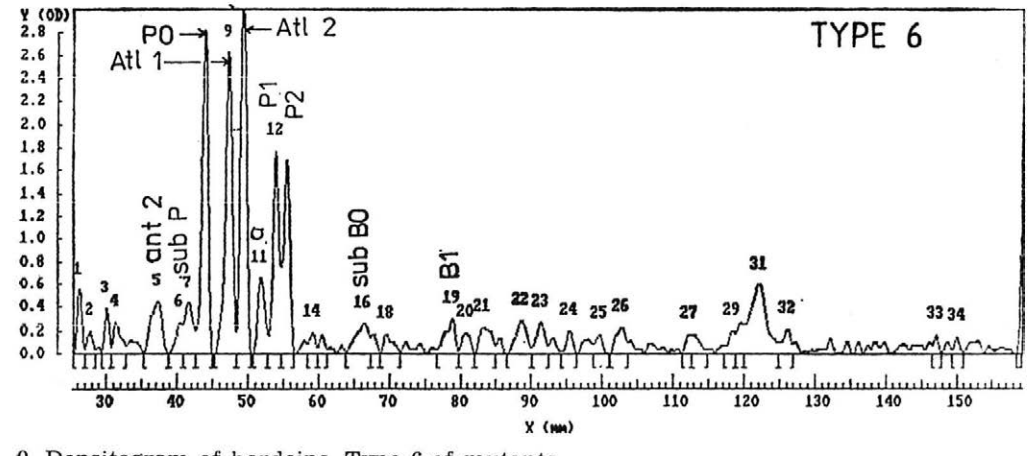
8. Densitogram of hordeins. Type 5 of mutants

User ref.	F-typ 2	ÚJ-5gP
File Name	B:\UHL-300A	
Date	04/28/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	25.4	0.00
(max.)	159.5	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	1.00 lines/mm
Boost Factor	4
Baseline subtracted by densitometer	
Peak threshold (O.D.)	0.15



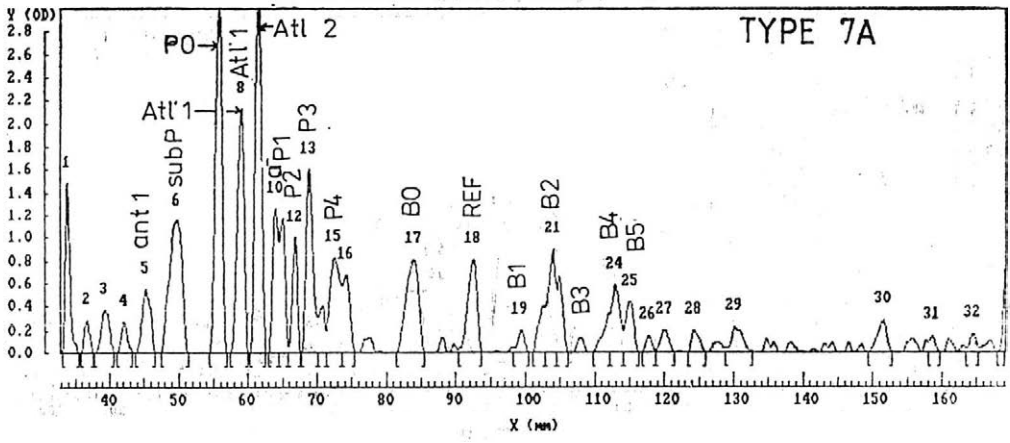
9. Densitogram of hordeins. Type 6 of mutants

User ref.	AII3 holy	DOKSANY-5gP P
File Name	B:\PONRT16	
Date	04/27/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	32.8	0.00
(max.)	169.3	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	0.50 lines/mm
Boost Factor	4
Baseline subtracted by	densitometer
Peak threshold (O.D.)	0.15



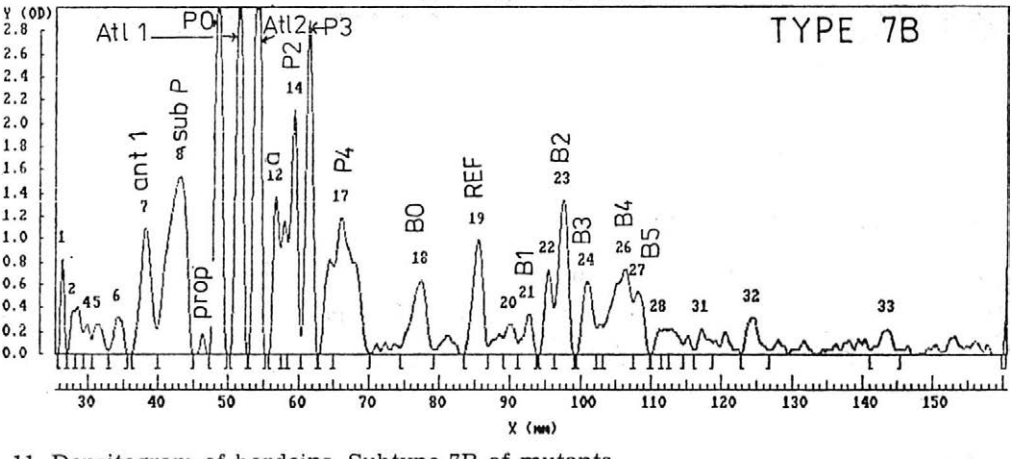
10. Densitogram of hordeins. Subtype 7A of mutants

User ref.	AI2 hola typ 1-3
File Name	B:\PONRT10
Date	04/27/88
This scan is number	3 of 3

Window settings:

	X (mm)	Y (O.D.)
(min.)	25.4	0.00
(max.)	160.7	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	0.50 lines/mm
Boost Factor	4
Baseline subtracted by	densitometer
Peak threshold (O.D.)	0.20



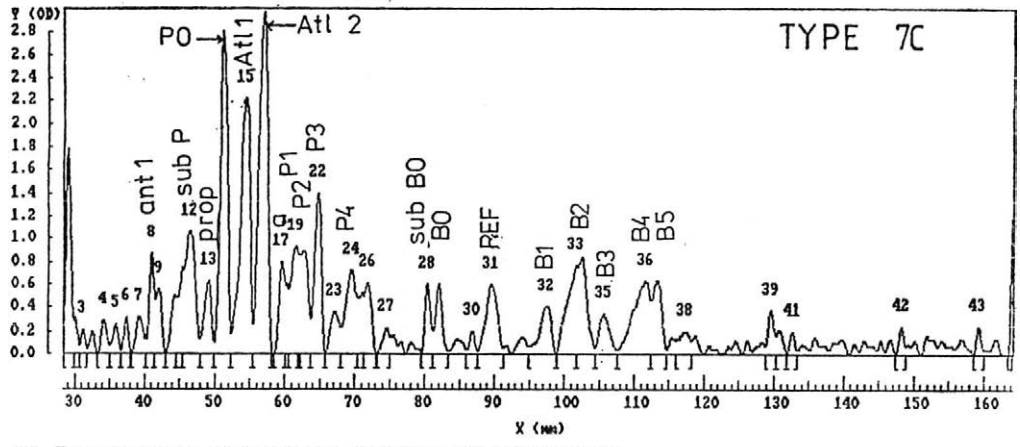
11. Densitogram of hordeins. Subtype 7B of mutants

User ref.	AII7 s pluchou	DOKSANY 21a
File Name	B:\PONRT11A	
Date	04/27/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	28.4	0.00
(max.)	164.4	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	1.00 lines/mm
Boost Factor	5
Baseline subtracted by	densitometer
Peak threshold (O.D.)	0.20



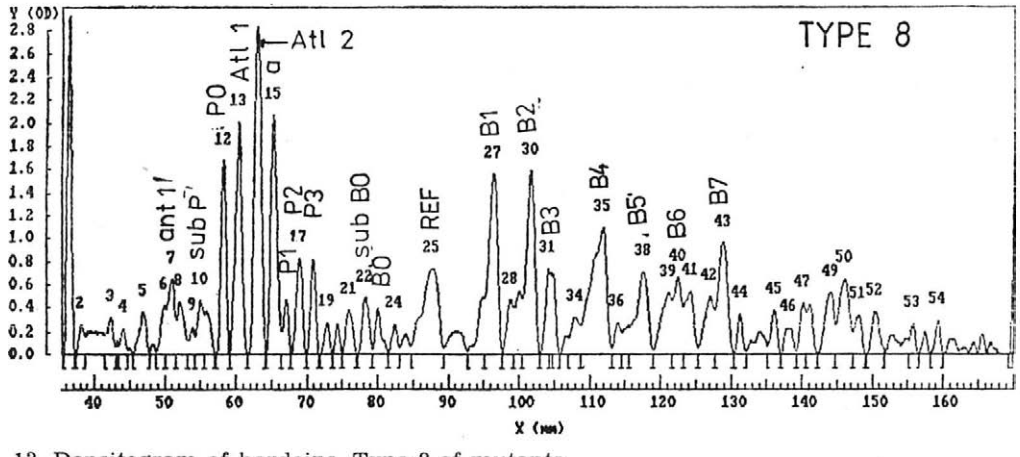
12. Densitogram of hordeins. Subtype 7C of mutants

User ref.	AI7 plucha	DOKSANY-5gP
File Name	B:\PONRT15B	
Date	04/27/88	
This scan is number	1 of 1	

Window settings:

	X (mm)	Y (O.D.)
(min.)	35.4	0.00
(max.)	170.2	3.00

Filter Frequency	4.00 lines/mm
Enhancement Frequency	1.00 lines/mm
Boost Factor	7
Baseline subtracted by	densitometer
Peak threshold (O.D.)	0.22



13. Densitogram of hordeins. Type 8 of mutants

II. Relative area (% total) of the peak on densitograms. Data for named hordeins based on model densitograms (see Fig. 2 — Fig. 13)

Hordein	Type No.										
	0	1	2	3	4	5	6	7A	7B	7C	8
ant	5.85	4.12	3.20	6.51		2.19	3.54	2.50	4.20	4.31	3.74
sub P	5.30						2.55	7.89	10.2	4.64	0.81
prop	1.04									1.97	
P0	1.76	3.05	9.94	5.99	9.78	8.25	11.6	10.7	8.19	8.84	3.22
Atl 1	14.6	20.4	17.2	12.4	5.08	3.31	12.6	7.58	7.79	9.32	4.86
Atl 2	13.9	13.0	9.62	7.37	—	—	13.2	10.8	8.34	11.4	7.66
fork 1	—	—	—	—	4.82	5.23	—	—	—	—	—
interfork	—	—	—	—		1.96	—	—	—	—	—
fork 2	—	—	—	—	6.15	10.2	—	—	—	—	—
a	2.22	6.86		2.39	17.0	.	3.26	3.67	2.97	2.11	5.05
P1	3.09			2.25	2.11	5.80	7.39	3.54	2.28	0.55	0.87
P2	4.62			0.67	1.68	7.99	7.41	2.63	5.01	3.01	1.94
P3	1.87			0.78	0.88	2.64		6.02	6.67	4.16	1.56
P4	0.94			0.38	3.54	2.50		4.67	7.66	3.09	0.54
P5	1.01				2.70	1.72		2.41		1.91	0.43
P6	2.09										
sub B0	.		1.03	0.96	.	0.73	2.35			1.43	1.26
B0	.	1.64	2.09	.	.	2.71	0.65 }	5.48 }	3.30	1.67	0.93
REF	.	4.72	2.95	2.59	1.98	3.02	.	4.00	3.49	2.99	4.65
B1	3.22	3.09	2.84	4.31	3.99	5.80	2.09	0.69	0.91	2.11	5.36
sub B2								3.60			
B2	2.37	3.46	3.41	4.83	3.00	3.57	1.12	1.78	1.09	3.25	4.99
B3	.	.	.	2.28	.	2.41	.			1.57	1.44
B4	2.52	5.31	3.91	4.83	4.30	1.16	2.21	2.55	1.95	4.48	6.56
B5	2.04		1.34	1.01	1.68	2.77	1.61	1.89	4.54	2.53	3.19
B6	.	1.83	<3.81	.	.	.	0.89	0.48	2.26	.	1.99
B7	2.99	<4.43	2.11	2.13	.	.	0.85	0.86	4.76	.	3.48
B9				1.52							

knowledge, are limited in their occurrence to the 21a — mutant family, while types No 5 and 8 to the 5g — mutant family. Type No 6 seems to be more frequent in the mutant 5g — family than in the 21a — one. Mutant types of hordeins natural composition, that is NoNo from 1 to 8, has not been found in starting Czechoslovak cultivar Atlas. Similarly, Atlas types of hordeins natural composition, e. g. types NoNo 0 and 00, has not been found in mutants ÚJ-5g and ÚJ-21a. In such a way it is proved on the base of these hordeins' genetic markers, that mutations really have taken place.

From Tab. VII we can also conclude on the base of presence of several types of hordeins' natural composition that genetic poly-

III. Characterization of types of hordeins composition. The first approach. Named hordeins descendently arranged according to the relative height of peaks

Type	Hordeins
Atlas No 0	Atl 1 > Atl 2 ⩾ ant
Atlas No 00	fork 2 > P0 > fork 1 > B4 ⩾ subREF ⩾ B0 ⩾ P5 > P1 ⩾ Atl 1 ⩾ P2
Mutants types:	
1	Atl 1 > Atl 2 > a > P0 > REF > B2 > ant
2	Atl 1 > P0 > Atl 2 > B4 > B2 > REF > ant ≐ subP
3	Atl 1 > Atl 2 > P0 > B1 > a > ant 2 > B2 > subP
4	a > P0 > fork 2 > fork 1 > Atl 1
5	fork 2 > P2 > P0 > fork 1 > P1 > Atl 1 > B1 > P3 > B2
6	Atl 2 > P0 > Atl 1 > P1 > P2 > a > ant > subP
7A	Atl 2 > P0 > Atl 1 > P3 > a > subP > P1 > P2 > B2 > P4 > B0 > > REF
7B	Atl 2 > P0 > Atl 1 > P3 > P2 > subP > a > P4 > P1 > subP > > a > B2 > P4 > P1 > ant > REF > B4 > B0
7C	Atl 2 > P0 > Atl 1 > P3 > subP > P1 > P2 > ant > a > B2 > > P4 > B4 > B5 > REF > sub B0 > B0
8	Atl 2 > a > Atl 1 > P0 > B2 > B1 > B4 > B7 > P2 > P3

Note: hordeins important for distinguishing of types are emphasized

morphism takes place in both mutant families under study. We have to remember that both mutant-families started after double individual selection from single caryopsis in M_3 generation. In M_{13} in morphologically stabilized huskless high-protein (from 123 to 129 per cent of control Atlas) mutant families we have in such a way found genetic polymorphism as to hordeins. So the progeny of the same mutant is characterized by different genotypes controling the composition of protein fraction of hordeins. It is a question if such genetic differentiation is to be elucidated only to by-mutations originated during the short-time 3 hours' mutagen influence or to retard-mutations in further M generations. Larger genetic variability in M_{13} established by us recently might be eventually a results of recombination of mutant genes in a case that some allogamy have taken place in early M generations in connection with the possible presence of some open flowers in these early M generations, although the usual autogamy generally works.

Up to now the starch-electrophoresis has been used for hordeins in Czechoslovak cultivars of barley (Hýža, Rozkošná, 1979; Hýža, Voňka, 1980; Marek, Minařík, 1981) by means of which 17 to 19 zones are visually distinguishable. Our newly used method of polyacrylamid gel electrophoresis with computerized evaluation of densitograms is more sensitive, more objective and enables more detailed analysis of hordeins and in such a way more detailed genetic analysis. Special extraction mixture proposed by Bartko and used in our study is more selective for extraction of hordeins than extraction systems used by formerly mentioned authors, in papers of them some

IV. Characterization of types of hordeins composition. The second approach. Named hordeins descendently arranged according to the relative area of their peaks. 10 per cent and 5 per cent levels taken into account. The parts of obtained sequences and characteristic hordeins with diagnostic value for distinguishing of types are emphasized

Type	Relative area (per cent of total)		
	more than 10 %	between 5 and 10 %	less than 5 %
Atlas No 0	Atl 1 > Atl 2 > ant >	subP >	P2 > B1 > P1 > B7 > > B4 > B2 > a > P6
Atlas No 00		fork 2 > B4 > B0 > > P0 >	sub REF > P5 > > subP > fork 1 > P1
Mutants types:			
1	Atl 1 > Atl 2 >	a > B4	REF > ant
2	Atl 1 >	P0 > Atl 2 >	B4
3	Atl 1 >	Atl 2 > P0 >	B1
4	a >	P0 > fork 2 > > Atl 1 >	fork 1
5	fork 2 >	P0 > P2 > P1 ≐ ≐ B1 > fork 1	
6	Atl 2 ≥ Atl 1 ≥ P0 > P2 ≐ P1		
7A	Atl 2 ≐ P0 >	subP > Atl 1 > > P3 > B0 >	P4 > REF > a ≥ ≥ subB 2 ≥ P1
7B	subP >	Atl 2 ≥ P0 ≥ ≥ Atl 1 > P4 > > P3 > P2	
7C	Atl 2 >	Atl 1 ≥ P0 >	subP+ > B4+ > P3 > > B2 > P4 ≐ P2 ≐ ≐ REF
8		Atl 2 > B4+ > B1 > > a ≥	B2 ≥ Atl 1 > > REF+ > B7 > P0

* — hordein not clearly separated, so the real value of relative area of its own peak ought to be less and position of hordein more to the right than given in the table

risk for presence of other protein non-hordein zones remains. For this reason and generally for the large difference between their starch gel electrophoresis and our polyacrylamid gel electrophoresis it is not possible to compare their zones and our hordein-peaks.

Possible range of hereditarily dependent changes induced by mutagenesis is very interesting from point of view of biochemical genetics. Uhlík, Burianová (1983), Uhlík, Marek (1986) have described the changes in binded amino acids composition of storage proteins in barley caryopsis achieved by mutagenesis and they have shown the large possible extent of such changes of amino acid composition of crude protein. Recently we are able to emphasize also the changes in specific barley prolamines, that is hordeins, achieved by mutagenesis.

V. Formulas of hordein composition types based on "finger-prints" of the first main group of proteins (hordeins)

Type	Hordeins formula
Atlas type O	Atl 1 > Atl 2
Atlas type 00	fork 2 > P0 > fork 1 > P5
Mutants types:	
1	Atl 1 > Atl 2 > a > P0
2	Atl 1 > P0 > Atl 2
3	Atl 1 > Atl 2 > P0
4	a > P0 > fork 2 > fork 1 > Atl 1
5	fork 2 > P2 > P0 > fork 1 > P1 > Atl 1
6	Atl 2 > P0 > Atl 1 > P1 > P2
7A	Atl 2 > P0 > Atl 1 > P3 > a
7B	Atl 2 > P0 > Atl 1 > P3 > P2
7C	Atl 2 > P0 > Atl 1 > P3 > subP > P1
8	Atl 2 > a > Atl 1 > P0

VI. Evaluation of types of natural hordein composition on the base of occurrence of common and specific semiquantitative character inside the first main group of hordein- peaks from densitograms. Comparison with 'Atlas' type 0.

	Atl 1 remains, Atl 2 reduced			Atl 1 reduced, Atl 2 substituted by two hordeins fork 2, fork 1			Atl 2 remains, Atl 1 slightly reduced		
Characteristic hordein prevailing				a	fork 2	fork 2			
Characteristic hordein apparent, but not prevailing (in descendent order)	a P0	P0	P0	P0 fork 2 fork 1	P2 P0 fork 1 P1	P0 fork 1 P5	P0 P1	P0 P3	a P0
Natural hordein composition type No.	1	2	3	4	5	00	6	7	8

VII. Occurrence of natural hordein composition types in M_{13} generation

Mutant	Natural hordein composition type No.									
	1	2	3	4	5	6	7A	7B	7C	8
ÚJ - 5 g		+	+++		+	++	+		+	+
ÚJ - 21 a	+	+	+++	+		+	+	+	+	

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PONERT, J. — UHLÍK, J. (Československá biochemická společnost, Praha; Vysoká škola zemědělská, Praha): *Průkaz genetického polymorfismu v potomstvu vysokobílkovinných bezpluchých mutantů ječmene pomocí hordeinových spekter.* Genet. a Šlecht., *25*, 1989 (3) : 191-207

Směs hordeinů z obilek československé odrůdy jarního ječmene Atlas a M_{13} generace potomstva jeho mutantů byla oddělena extrakcí a dělena elektroforézou na polyakrylamidovém gelu, kvantitativně hodnocena počítačově řízenou a vyhodnocovanou densitometrií. Tato metoda je zřejmě účinnější pro semikvantitativní hodnocení nově pojmenovaných individuálních hordeinů než dosud prováděné subjektivně hodnocené metody škrobové elektroforézy. Pro rozlišení typů hordeinových spekter byly jako genetické markery užity hordeiny první hlavní skupiny. Takto byl prokázán genetický polymorfismus v potomstvu (v pokročilé M_{13} generaci) vysokobílkovinných bezpluchých morfologicky stabilizovaných mutantů pocházejících z individuálních rostlin M_3 generace. Jako možné příčiny tohoto genetického polymorfismu jsou uvažovány nejen vícenásobné současné i následné mutace, ale i výskyt otevřených květů v časných generacích mutantů, který by eventuálně mohl ohrozit jinak běžnou autogamii.

hordeiny; vysokobílkovinní bezpluší mutanti; dvouřadý jarní ječmen; československí mutanti ječmene; genetické markery; genetický polymorfismus biochemických znaků; *Hordeum;* negativní korelace relativního obsahu lyzínu v bílkovinách a hrubých bílkovin v sušině; individuální výběr v M_2 a M_3 generacích; potomstvo mutantů M_{13}

ПОНЭРТ, Й. — УГЛИК, Я. (Чехословацкое биохимическое общество, Прага, Сельскохозяйственный институт, Прага): **Генетический полиморфизм в потомстве высокобелковых голозерновковых мутантов ячменя установленный с помощью анализа гордеинов.** Genet. a Šlecht., *25*, 1989 (3) : 191-207.

Анализ гордеинов из зерновок ярового ячменя чехословацкого культивара Atlas и поколения M_{13} его мутантов осуществлен с помощью селективной экстракции и электрофореза на полиакрыламидном геле. После скрашивания проводилась автоматизированная дензитометрия с помощью ЭВМ. Такой метод более эффективен и надежен для идентификации и полуколичественной оценки вновь обозначаемых индивидуальных гордеинов. Для характеризации типов гордеиновых спектров применены гордеины первой основной группы. Таким образом выявлен генетический полиморфизм в мутантных семьях поколения M_{13} выведенных из индивидуальных растений M_3 поколения. Следует обратить внимание, что эти мутантные семьи гомогенны с точки зрения морфологических признаков и повышенного содержания грубого белка, но полиморфны с точки зрения типов состава естественных смесей гордеинов. Обсуждаются возможные причины генетического полиморфизма в потомстве мутантов.

гордеины; высокобелковые голозерновковые мутанты; двурядный яровой ячмень; чехословацкие мутанты ячменя; генетические маркеры; генетический биохимический полиморфизм; *Hordeum*; отрицательная корреляция содержания лизина и общего содержания белков в зерновке и случай преодоления этой отрицательной корреляции; индивидуальная селекция в M_2 и M_3 генерациях; M_{13} генерация

PONERT, J. — UHLÍK, J. (Tschechoslowakische biochemische Gesellschaft, Praha; Landwirtschaftliche Hochschule, Praha): *Nachweis des genetischen Polymorphismus in der Nachkommenschaft der spelzenlosen Hochprotein-Gerstenmutanten mit Hilfe von Hordeinspektren.* Genet. a Šlecht., *25*, 1989 (3) : 191-207

Ein Gemisch von Hordeinen aus Karyopsen der tschechoslowakischen Sommergerstensorte Atlas und des M_{13}-Generation der Nachkommenschaft ihrer Mutanten wurde auf dem Wege der Extraktion getrennt und mit Hilfe der Elektrophorese auf Polyakrylamidgel geteilt und mittels der rechnergestützten und -ausgewerteten Densitometrie auch quantitativ bewertet. Diese Methode scheint wirksamer und günstiger für die semiquantitative Bewertung der neu benannten individuellen Hordeine als die bisher angewendeten und subjektiv bewerteten Methoden der Stärkeelektrophorese zu sein. Zur Unterscheidung der Typen der Hordeinspektren wurden als genetische Tracer Hordeine der ersten Hauptgruppe herangezogen. Auf diese Art und Weise konnte der genetische Polymorphismus in der Nachkommenschaft (in der fortgeschrittenen M_{13}-Generation) der spelzenlosen morphologisch stabilisierten Hochproteinmutanten aus individuellen Pflanzen der M_{13}-Generation nachgewiesen werden. Als mögliche Ursachen für den genetischen Polymorphismus kommen nicht nur die mehrfachen gleichzeitigen und nachfolgenden Mutationen aber auch das Auftreten der geöffneten Blüten in frühen Generationen der Mutanten, das die sonst übliche Autogamie gefährden könnte, in Frage.

Hordeine; spelzenlose Hochproteinmutanten; zweireihige Sommergerste; tschechoslowakische Gerstenmutanten; genetische Tracer; genetischer Polymorphismus der biochemischen Merkmale; *Hordeum;* negative Korrelation des relativen Lysingehaltes in Proteinen und Rohproteinen in der Trockensubstanz; individuelle Auswahl in den M_2- und M_3-Generationen; Nachkommenschaft der M_{13}-Mutanten

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We call the attention of our readers to Number 4/1989 of the periodical

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which will bring the below-mentioned papers. These papers will be published in Czech language with Russian, English and German summaries.

Šašek A., Kubánek J., Černý J., Hauser L., Krautová E.: Electrophoretic Characteristics of Gliadins and HMW Subunits of Glutenins of New Common Wheat Varieties Certified in 1988

Marek V., Němec Z., Hýža V., Mogileva V. I., Jelínek L.: SDS Sedimentation/Proteins Ratio as an Indirect Indicator of Feeding Value of Wheat and Triticale Grain in Early Generations of Breeding

Kábrt B.: Genetics of the Development Type of Hexaploid Wheat

Foltýn J., Škorpík M., Táborská J.: Stem Structure of Wheat Varieties in relation to the Dwarfness Donors Nordin 10 and Tom Thumb

Ponert J., Mrštíková H.: The Intensity of Callus Formation in Flower Buds of Sugar Beet *(Beta vlugaris)* in *in vitro* Axenic Culture

Stuchlíková E., Bartoš P., Neuhäuslová Z.: Genetics of Resistance to Leaf Rust of Wheat [*Puccinia persistens* Plow. var. *tritici-cina* (ERIKS) URBAN et MARKOVÁ] in Czechoslovak Varieties of Wheat with *1B/1R* Translocation

Šimurková J., Žák Š., Lomjanský S.: Selection of a Paternal Component of the Sugar Beet Intera Variety

Cagaš B.: Breeding Meadow Fescue for Resistance to Crown Rust *(Puccinia coronata* Corda var. *coronata)*

Chloupek O., Babinec J.: The Influence of Seed Provenance and Synthetic Generation on Forage and Seed Yields in Synthetic Populations of Lucerne

TRANSFER OF A NEW GENE FOR STEM RUST RESISTANCE FROM *TRITICUM MONOCOCCUM* L. TO HEXAPLOID WHEAT, *T. AESTIVUM* L.

J. Valkoun, D. Kučerová, P. Bartoš

VALKOUN, J. — KUČEROVÁ, D. — BARTOŠ, P. (Research Institute of Crop Production, Praha-Ruzyně): *Transfer of a New Gene for Stem Rust Resistance from Triticum monococcum L. to Hexaploid Wheat, T. aestivum L.* Genet. a Šlecht., *24*, 1989 (3) : 209-214

A gene for wheat stem rust resistance, designated tentatively as *Sr Tm2*, has been transferred from diploid wheat *T. monococcum*, accession 1509/2, to hexaploid wheat *T. aestivum* cv. 'Zlatka'. A simplified indirect method, involving a single bridging cross with tetraploid wheat *T. durum* 3310, a single backcross to *T. aestivum*, and a limited number of generations with cytological or/and disease tests, was employed in the gene transfer. *Sr Tm2* gives only partial protection against the Czechoslovak population of *P. graminis* f. sp. *tritici*, because it is ineffective to isolates of physiological race 11 but the gene may be valuable in some combinations with other resistance genes.

Triticum monococcum L.; *Triticum aestivum* L.; *Puccinia graminis*; stem rust resistance; resistance gene transfer; wide crosses

High resistance of cultivated einkorn wheat, *Triticum monococcum* L., to main foliar diseases of wheat has been reported by a number of authors. Vavilov suggested to use this diploid species in resistance breeding of hexaploid common wheat, *Triticum aestivum* L. already in the year 1913 (Vavilov, 1913 — cit. Vavilov, 1964). The use of the direct hybrids of *T. aestivum* × *T. monococcum* in gene transfer has not been successful owing to lethality or severe growth and developmental defects of the F_1 hybrids (The, Baker, 1975). This difficulty can be overcome by indirect "bridging" method where *T. monococcum* is first crossed to tetraploid wheat and the resultant autosterile triploid F_1 hybrid is either pollinated with *T. aestivum* directly or backcrossed to the tetraploid parent and the derivatives of the backcross are then pollinated with hexaploid wheat. In the last two decades both methods were successfully employed in transference of resistance genes from the diploid donor to hexaploid wheat (Kerber, Dyck, 1973; The, 1973; Mc Intosh et al., 1984; Valkoun et al., 1985; 1986a, b). Three genes for resistance to wheat stem rust have been transferred — *Sr 21* (The, 1973), *Sr 22* (Kerber, Dyck, 1973; The, 1973) and *Sr 35* (Mc Intosh et al., 1984; Valkoun et al., 1985, 1986b). Valkoun and Bartoš (1981) distinguished two additional stem rust resistance genes in diploid wheats according to reactions to several isolates of the pathogen. Genetic analysis at the diploid level has revealed that one of the genes, tentatively designated as *Sr Tm2* is present in *T. monococcum* accession 1509 and segregates as a single

dominant Mendelian factor in a cross with the susceptible *T. monococcum* accession 1557 (Valkoun et al., 1983).

The present paper describes the transfer of this gene from diploid donor to hexaploid wheat.

MATERIALS AND METHODS

Selfed ear progeny 1509/2, which had uniform reaction to all the stem rust isolates tested, served as a donor of *Sr Tm*$_2$ in the initial cross with susceptible tetraploid wheat, *Triticum durum* Def. accesion 3310. Both *T. monococcum* 1509 and *T. durum* 3310 were obtained from wheat collection of the Zentralinstitut für Genetic und Kulturpflanzenforschung, Gatersleben, GDR, by courtesy of Dr. Ch. Lehmann.

The triploid F_1 hybrid "bridge" was crossed to the susceptible Czechoslovak spring wheat cultivar Zlatka (*Triticum aestivum* L.). After a single backcross to the hexaploid parent cv. Zlatka, resistant plants were selected and selfed. The most fertile and homozygous resistant plants were identified in the progeny on the basis of spike fertility and plant progeny testing. Further individual selection was applied to the non-segregating offsprings. The most important selection criteria were the spike fertility and phenotypical resemblance to *T. aestivum*. Isolate G 702 belonging to physiological race 14 of wheat stem rust, *Puccinia graminis* f. sp. *tritici* Eriks. et Henn., was used for disease resistance tests from the initial steps of the wide hybridization programme up to the selfed progeny of the first backcross to *T. aestivum* (S_1BC_1 generation). The disease tests were carried out in the greenhouse at the seedling stage by a method described earlier for wheat leaf rust (Valkoun et al., 1986a).

After several generations grown in the field, morphologically uniform hexaploid *T. aestivum* lines were selected, resistant to several isolates of stem rust. Comparison of reactions to a set of stem rust isolates (Valkoun, Bartoš, 1981) was employed for postulation of the genetic basis in the wheat lines tester.

Somatic chromosome counts were made by a standard procedure (Valkoun et al., 1986a).

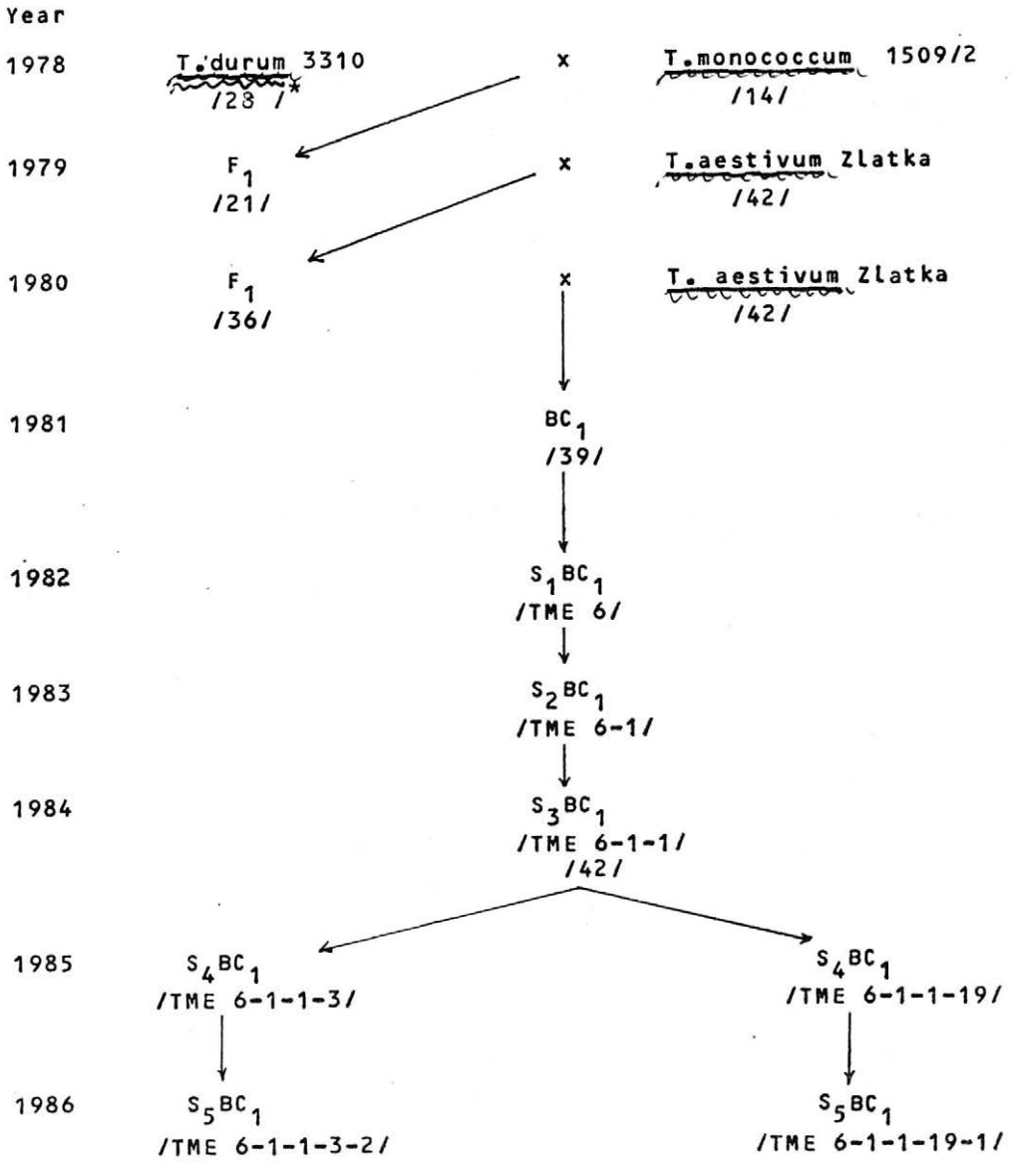
RESULTS AND DISCUSSION

The course of the transfer of stem rust resistance from diploid donor *T. monococcum* 1509/2 to the hexaploid level is presented in Fig. 1. The scheme of crossing and the generation sequence including resistance and cytological testes differ from those involved in the transfers of resistance genes *Lr Tm*$_1$ and *Sr 35* (Valkoun et al., 1986a, b) in several aspects:

a) the triploid F_1 hybrid is pollinated directly with *T. aestivum*
b) there is only a single backcross with *T. aestivum*
c) resistance tests are limited to initial generations up to S_1BC_1
d) cytological control is made only in F_1, BC_1 and S_3BC_1.

At the beginning of the year 1987, reactions of six S_5BC_1 lines and a single S_4BC_1 line were analyzed by means of six tester isolates of *P. graminis* which had been used earlier for identification of stem rust resistance genes in diploid wheats (Valkoun, Bartoš, 1981). The results are shown in Tab. I.

Line TME-6-1-1-3 is heterogenous in its reaction to stem rust isolates G69, G702 and G605 owing to segregation in a single dominant resistance gene. Its descendant, line TME 6-1-1-3-2, and the related line TME 6-1-1-19-1, display uniform reaction to all the isolates, i. e. they are homozygous in the transferred resistance. Susceptibility of these lines to isolate G425 indicates that they do not posses *Sr 21,* a gene for resistance to stem rust in diploid wheat Einkorn 1,3 (The, 1973).



* Chromosome numbers or/and line designations in brackets

1. Crossing and generation sequence involved in the transfer of stem rust resistance

Gene *Sr 21* is most probably also present in *T. monococcum* 1509/2, in addition to *Sr Tm2* (Valkoun et al., 1983).

From comparison of the disease reaction pattern of the hexaploid lines TME 6-1-1-3-2 and TME 6-1-1-19-1 with reactions characteristic for particular *Sr* genes in diploid wheats (Tab. II) it is obvious, that both the lines carry a single *T. monococcum* resistance gene, *Sr Tm2*, derived from the diploid donor *T. monococcum* 1509/20 without any

I. Reaction of *T. monococcum* Einkorn 1,3 and hexaploid lines derived from *T. monococcum* to six isolates of *P. graminis*

Line	Isolate					
	G69	G702	G530	G425	G802	G705
TME 6-1-1-3	;1/3	;1/3	3	3	3	;1/3
TME 6-1-1-3-2	;1	;1	3	3	3	;1
TME 6-1-1-19-1	;1	;1	3	3	3	;1
TME 6-1-1-23-1	;1/3	;1/3	3	3	3	;1/3
TME 8-2-1-1-1	;1/3	;1/3	3	3	3	;1/3
Einkorn 1.3	;1-2	3	3	;1-2	3	3

II. Comparison of reactions of two TME lines with reaction patterns characteristic of the particular *Sr* gene in diploid wheats*

Genotype	Isolate					
	G69	G702	G530	G425	G802	G605
TME 6-1-1-3-2	R	R	S	S	S	R
TME 6-1-1-19-1	R	R	S	S	S	R
Sr 21	R	S	S	R	S	S
Sr 22	R	R	R	S	S	R
Sr 35 (= *Sr* Tm_1)	R	R	R	R	R	S
Sr Tm_2	R	R	S	S	S	R
Sr Tm_3	R	S	S	R	R	R
0	S	S	S	S	S	S

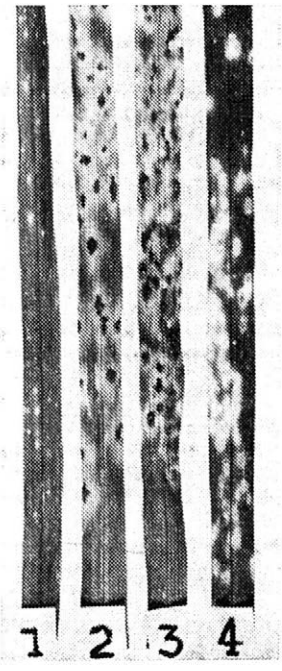
* the characteristic reaction patterns were derived from earlier results (Valkoun und Bartoš, 1981)

R = resistant reaction S = susceptible reaction

combination with other genes for stem rust resistance derived from diploid wheats.

It may be concluded that a fourth gene for resistance to *P. graminis* f. sp. *tritici,* designated tentatively as *Sr* Tm_2, has been successfully transferred from diploid wheats to hexaploid wheat, *T. aestivum* cv. Zlatka. Results of preliminary yield trials demonstrate a comparatively good agronomic performance of TME 6-1-1, the parental line of both the hexaploid derivatives with *Sr* Tm_2. This line even exceeds in yield its hexaploid progenitor cv. Zlatka.

However, the gene *Sr* Tm_2 ineffective to some races which are common in the Czechoslovak population of wheat stem rust, especially the isolates belonging to physiological race 11. If this gene is to be used in the Czechoslovak wheat breeding programmes, it should be combined with gene(s) that confers resistance to those virulent races. For example, combination with another *T. monococcum* gene, *Sr 35,* would protect against all Czechoslovak stem rust isolates. Effectiveness of particular resistance genes may be different in various geographical



2. Seedling reactions to isolate G702 of *P. graminis* f. sp. *tritici*
1 — *T. monococcum* 1509/2, $2n = 14$
2 — *T. durum* 3310, $2n = 28$
3 — *T. aestivum* cv. Zlatka, $2n = 42$
4 — TME 6-1-1, resistant derivative of the cv. Zlatka, $2n = 42$ (S_3BC_1 generation)

regions, e. g. Mc Intosh et al. (1984) found a low effectiveness of *Sr 35* on the Australian population of the pathogen. However, the same gene is very valuable for Czechoslovakia, giving protection against all contemporary isolates of *P. graminis* f. sp. *tritici* (Valkoun et al., 1986b). In the case of *Sr Tm*$_2$ the situation may be just opposite.

The simplified method employed in the transfer of *Sr Tm*$_2$ from *T. monococcum* to hexaploid wheat proved to be as efficient as those used for genetic transfer of other *T. monococcum* genes, *Lr Tm*$_1$ and *Sr 35* (Valkoun et al., 1986a, b), but the latter were more laborious.

The level of resistance expressed by infection types decreased slightly in hexaploid derivatives in comparison to the diploid donor of *Sr Tm*$_2$ (Fig. 2). A similar effect was observed in other diploid wheat genes, *Sr 22* and *Lr Tm*$_1$, transferred into hexaploid genetic background (Kerber, Dyck, 1973; Valkoun et al., 1986a). The four genes for resistance to wheat stem rust, *Sr 21, Sr 22, Sr 35,* and *Sr Tm*$_2$, derived from *T. monococcum* contribute to the enrichment of *T. aestivum* gene pool. Thus, in agreement with Vavilov's suggestion, the cultivated einkorn wheat is becoming an important source of genes which may be directly exploited in disease resistance breeding of common wheat.

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VALKOUN, J. — KUČEROVÁ, D. — BARTOŠ, P. (Výzkumný ústav rostlinné výroby, Praha-Ruzyně): *Přenos nového genu odolnosti ke rzi travní z Triticum monococcum L. do hexpoidní pšenice, T. aestivum L.* Genet. a Šlecht., *25*, 1989 (3) : *: 209-214*

Byl přenesen gen odolnosti ke rzi travní, předběžně označený jako *Sr Tm*$_2$, z diploidní pšenice *T. monococcum* 1509/2 do hexaploidní pšenice *T. aestivum* odrůdy 'Zlatka'. Pro přenos tohoto genu bylo použito zjednodušené metody — nepřímé, která zahrnovala jedno „můstkové" křížení s tetraploidní pšenicí *T. durum*, jedno zpětné křížení s *T. aestivum* a omezený počet generací s cytologickou kontrolou a nebo infekčními testy. Gen *Sr Tm*$_2$ poskytuje pouze částečnou ochranu k československé populaci *P. graminis* f. sp. *tritici*, protože je neúčinný k izolátům fyziologické rasy 11, ale může být cenný v některých kombinacích s jinými geny rezistence.

Triticum monococcum L.; *Triticum aestivum* L.; *Puccinia graminis*; odolnost ke rzi travní; přenos rezistence; vzdálená hybridizace

ВАЛКОУН, Я. — КУЧЕРОВА, Д. — БАРТОШ, П. (Научно-исследовательский институт растениеводства, Прага-Рузыне): **Пересадка нового гена устойчивости к линейной ржавчине из** *Triticum monococcum* L. **на гексаплоидную пшеницу,** *T. aestivum* L. Genet. a Šlecht., *25*, 1989 (3) : 209-214.

Упомянутый ген, обозначенный как *Sr Tm*$_2$ пересаживали с диплоидной пшеницы *T. monococcum* 1509/2 на гексаплоидную *T. aestivum* сорта 'Златка'. Пользовались упрощенным методом — косвенным, который включал одно «мостковое» скрещивание с тетраплоидной *T. durum*, одно обратное с *T. aestivum* несколько поколений с цитоконтролем или инфекционными тестами. Ген *Sr Tm*$_2$ обеспечивает лишь частичную защиту чсл. популяции *P. graminis* f. sp. *tritici*, т. к. не воздействует на изоляты физиол. расы 11. Однако он может быть ценным в некоторых комбинациях с другими генами устойчивости.

Triticum monococcum L.; *Triticum aestivum* L.; *Puccinia graminis*; устойчивость к линейной ржавчине; перенос устойчивости; отдаленная гибридизация

VALKOUN, J. — KUČEROVÁ, D. — BARTOŠ, P. (Forschungsinstitut für Pflanzenproduktion, Praha-Ruzyně): *Übertragung des neuen Schwarzrostresistenzgens vom Triticum monococcum L. in den hexaploiden Weizen T. aestivum L.* Genet. a Šlecht., *25*, 1989 (3) : 209-214

Es wurde das Schwarzrostresistenzgen *Sr Tm*$_2$ vom diploiden Weizen *T. monococcum* 1509/2 in den hexaploiden Weizen *T. aestivum* der Sorte Zlatka übertragen. Zur Übertragung dieses Gens wurde eine vereinfachte, indirekte Methode herangezogen, die eine „Brückenkreuzung" mit dem tetraploiden Weizen *T. durum*, eine Rückkreuzung mit dem *T. aestivum* und eine begrenzte Zahl der Generationen mit Zytologischer Kontrolle oder mit Infektionstests umfasst. Das Gen *Sr Tm*$_2$ bietet nur einen teilweisen Schutz gegen die tschechoslowakische Population von *P. graminis* f. sp. *tritici*, da es gegenüber den Isolaten der physiologischen Rasse 11 unwirksam ist. In einigen Kombinationen mit anderen Resistenzgenen kann es aber wertvoll sein.

Triticum monococcum L.; *Triticum aestivum* L.; *Puccinia graminis*; Schwarzrostresistenz; Resistenzübertragung; entfernte Hybridisation

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EFFECT OF DIFFERENT GROWTH SUBSTANCES ON CALLUS INDUCTION AND MORPHOGENESIS OF SUGAR BEET *IN VITRO*

J. Farago, I. Seman

FARAGO, J. — SEMAN, I. (Research and Breeding Institute for Seed Root Crops and Technical Crops, Bučany): *Effect of Different Growth Substances on Callus Induction and Morphogenesis of Sugar Beet in vitro.* Genet. a Šlecht, *25*, 1989 (3) : 215-222.

Experiments were carried out to study the influence of seven growth substances on sugar beet explants grown *in vitro*. Effects of these substances on callus induction, rhizogenesis and organogenesis in two clones of sugar beet and their dependence on the explant type were evaluated. Callus induction was most intensive on the Murashige-Skoog medium with 2,4-D, added at a concentration of 0.1 — 0.3 mg/l; the best results in rhizogenesis were observed on media with IAA in concentrations of 0.1 — 10.0 mg/l, and on NAA and IBA concentration of 3.0 — 10.0 mg/l. Shoot formation was low. 7 — 29 % explants regenerated on a medium with BAP; all concentrations used were effective (0.1 — — 10.0 mg/l).

sugar beet; *in vitro* culture; callus induction; rhizogenesis; organogenesis

Sugar beet belongs to important agricultural cultures. Owing to the great size of its plants and some biological peculiarities, it does not belong to typical model species. That is why not much information dealing with cultivation of tissues and organs of sugar beet *in vitro* can be found in literature. Available results are often inconsistent, sometimes even contradictory.

In our research we concentrated on the study of reaction of different explant types of sugar beet on growth substances most commonly used in explant cultures. The following types of processes were studied: callus induction, rhizogenesis and organogenesis.

MATERIALS AND METHODS

Two genetically different clones of sugar beet grown *in vitro* for 5 years, designated DA/3 and L 4/1 were used. Clone DA/3 descends from diploid pollen-fertile polygerm plant, clone L 4/1 was derived from a diploid pollen-sterile monogerm plant. Three sorts of explants were chosen: shoot tip, segments of leaf blade, and segments of leaf stalk, 5 mm each. Basic medium by Murashige and Skoog (1962) was used for cultivation and it was supplemented with one of the following growth substances: 6-benzylaminopurine (BAP), kinetin (KIN), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), α-naphtaleneacetic acid (NAA), 2,4-dichlorphenoxyacetic acid (2,4-D) and gibberelic acid (GA_3). Each growth substance was used in five concentrations (mg/l): 0.1, 0.3, 1.0, 3.0 and 10.0. Explants were cultivated in the dark at a temperature of 20 ± 2 °C. Experiments were evaluated after 25, 50 and 100

I. Dependence of frequency and intensity of morphogenetic reaction and callus formation of sugar beet explants on the concentration of growth substances in medium

Clone	Growth substance	Number of explants	Number of necroses	Type of structure	Percentages of explants forming callus (C), roots (R) or shoots (S)						Intensity of morphogenetic reaction				
					concentration of growth substance					average	concentration of growth substance				
					0.1	0.3	1.0	3.0	10.0		0.1	0.3	1.0	3.0	10.0
DA/3	BAP	70	13	C	29	71	50	93	57	60.0	+	+	+	+	+
				R	79	79	36	21	0	42.8	+	+	+	+	−
				S	7	21	14	29	14	17.1	++	++	+++	+++	++
	KIN	70	13	C	0	14	29	36	43	24.3	−	+	+	+	+
				R	57	57	50	43	0	41.4	++	+	+	+	−
				S	0	0	7	7	14	5.7	−	−	+	+	+
	NAA	70	4	C	0	21	14	0	0	7.1	−	+	+	−	−
				R	21	64	86	79	86	67.1	+	++	++	++	+++
				S	0	0	0	0	0	0	−	−	−	−	−
	IAA	70	1	C	7	0	21	36	43	21.3	+	−	+	+	++
				R	100	86	100	93	100	95.7	++	++	+++	+++	+++
				S	0	7	0	7	0	2.8	−	+	−	+	−
	IBA	70	4	C	14	0	0	7	14	7.1	+	−	−	+	+
				R	64	71	93	100	100	85.7	++	++	+++	+++	+++
				S	0	0	0	0	0	0	−	−	−	−	−
	2,4-D	70	2	C	100	93	93	86	71	88.6	+++	+++	++	++	+
				R	100	14	0	0	0	22.8	+	+	−	−	−
				S	0	0	0	0	0	0	−	−	−	−	−
	GA_3	70	12	C	21	0	7	0	0	5.7	+	−	+	−	−
				R	64	57	71	57	64	62.8	+	+	++	++	++
				S	14	0	0	7	0	4.3	++	−	−	+	−

				C	57	50	86	86	79	71.4	+	+	+	+	+
	BAP	70	18	R	7	14	7	0	0	5.7	+	+	+	−	−
				S	7	0	14	21	14	11.4	++	−	+++	++	++
				C	29	21	0	7	0	11.4	+	+	−	+	−
	KIN	70	30	R	7	21	21	14	36	20.0	+	+	+	+	+
				S	0	0	0	14	14	5.7	−	−	−	++	++
				C	36	14	21	29	29	25.7	+	+	+	+	+
	NAA	70	33	R	21	14	36	43	29	28.6	+	+	+	++	++
				S	0	0	0	0	0	0	−	−	−	−	−
				C	14	0	36	43	50	28.6	+	−	+	++	++
L 4/1	IAA	70	24	R	50	50	79	64	57	60.0	++	++	++	++	+++
				S	0	0	0	0	0	0	−	−	−	−	−
				C	21	50	21	79	64	47.1	+	+	+	++	++
	IBA	70	32	R	7	14	7	43	64	27.1	+	++	++	+++	+++
				S	0	0	0	0	0	0	−	−	−	−	−
				C	64	71	71	57	57	64.3	++	++	+	+	+
	2,4-D	70	19	R	43	7	0	0	0	10.0	+	+	−	−	−
				S	0	0	0	0	0	0	−	−	−	−	−
				C	29	0	0	7	0	7.1	+	−	−	+	−
	GA_3	70	50	R	29	7	7	0	0	8.6	+	+	+	−	−
				S	14	0	0	0	0	2.8	++	−	−	−	−

days of cultivation. Occurrence and intensity of callus induction and two types of morphogenesis — rhizogenesis and organogenesis — were evaluated in each explant. Intensity of each type of morphogenesis and callus induction was visually evaluated as follows: — no response; + low intensity; ++ medium intensity; +++ high intensity.

Relative frequency of occurrence of individual types of morphogenesis and callus induction was calculated out of the total number of explants in each treatment.

RESULTS

The results of our experiments are summarized in two tables and one figure. In total, 504 explants were planted (including 14 explants of the control treatment) from each clone. Great differences were found in reaction of explants of these clones to cultivation conditions created in the experiments. After 100 days of cultivation, clone L 4/1 had higher values concerning explants necrosis — 41.3% (DA/3 —9.9%), and callus forming explant rate — 35.3% (DA/3 — 30.1%). On the other hand, lower values were obtained in root formation — 22.2%, and in regeneration of shoots — 3.0% in clone L 4/1 (58.5, and 4.8% in clone DA/3).

Dependence of frequency and intensity of callus induction and morphogenetic reaction of sugar beet explants on the concentration of individual growth substances in the medium can be found in Tab. I. The highest callus formation with the most intensive callus growth was found on media with 2,4-D. The most intensive formation and growth of callus was observed on media with a low 2,4-D concentration (0.1 and 0.3 mg/l). Callus was homogeneous, friable, white to yellowish. Relatively high callus formation was observed also on media with BAP, especially in clone L 4/1. But in this case, as also in other variants, the majority of calluses were compact, nonhomogeneous, turning brown. Lighter clusters not forming differentiated structures were observed in the basic mass. Callus induction was caused by each of other growth substances, though in a small degree.

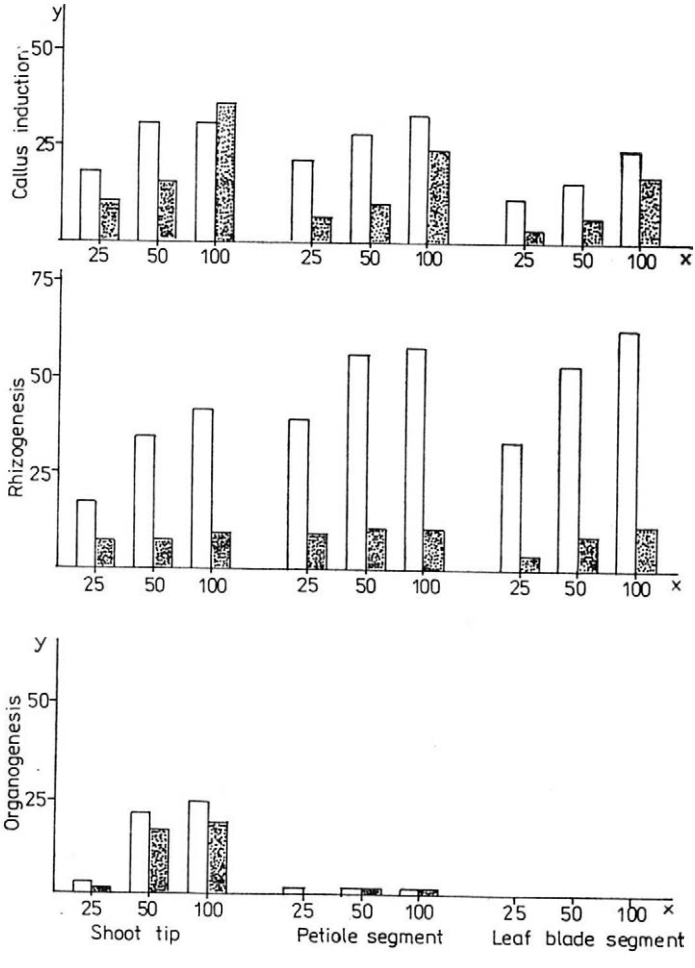
The best results in rhizogenesis were obtained on media with IAA. High percentage of root formation was observed in all five concentrations: 86—100 % in clone DA/3 and 50—79 % in L 4/1. Good results were observed also on media with NAA and IBA in higher concentrations. All the roots studied grew from the cut area of the explants.

Formation of shoots was relatively more profuse only on medium with BAP (7—29 % of explants). On other media it was sporadic, incidental, without any regularity. New shoots were formed on the rib of the leaf stalk, not on the cut area. Endogeneous growth substances have a positive influence on the shoot formation, too. It results from the fact that 93.7 % of all shoots regenerated *de novo* in our experiments were found on tissues of the shoot tip, having an active apical meristem. These shoots arose from residues of the leaf stalks of shoot tip explants.

By comparison of formation frequency of callus, roots and shoots in individual explants types, differences were found in morphogenetic reaction and callus induction upon the provided conditions of cultivation, as shown in Tab. II. While the differences were smaller in callus induction and rhizogenesis, the use of shoot tip showed to be unambigously most suitable for induction of organogenesis. In spite of

II. Percentage comparison of morphogenetic reaction and callus formation of individual types of explants after 100 days of cultivation (totally 216 explants of each clone were evaluated)

Type of reaction of explants	Clone	Used explants			Average
		growing tip	petiole segment	leaf blade segment	
Callus induction	DA/3	30.5	33.3	23.6	29.2
	L 4/1	36.1	23.6	16.7	25.5
Rhizogenesis	DA/3	45.8	56.9	62.5	55.1
	L 4/1	8.3	9.7	11.1	9.7
Organogenesis	DA/3	23.6	1.4	0	8.3
	L 4/1	18.0	1.4	0	6.5



x — days of cultivation
y — percentage of explants forming callus, roots or shoots

□ — clone DA/3
▨ — clone L 4/1

1. Comparison of frequency of callus induction, rhizogenesis and organogenesis in three explant types after 25, 50 and 100 days of cultivation

smaller differences in calogenetic and rhizogenetic abilities of individual explant types, callus was formed more often on the basis of shoot tips, while rhizogenesis was more intensive on segments of leaf blades.

Another interesting fact is that no great differences between the two clones used were found in callus induction and organogenesis, in contrast to rhizogenesis where large differences in the root formation were caused by genotype (Tab. 2). This result corresponds with our experience concerning the rooting of shoots of different sugar beet clones, maintained *in vitro* at our institute with differences practically from 0—100 %.

The dynamics of callus induction, rhizogenesis and organogenesis in both clones used and three explant types can be seen in Fig. 1.

Explants in the control treatment (14 from both DA/3 and L 4/1 clones), grown on medium without growth substances, lived through the whole period evaluated. No signs of callus and shoot formation were observed there. Root formation could be observed in 42.8 % of explants in clone DA/3, and no rhisogenesis in clone L 4/1.

DISCUSSION

Our experiments were carried out on explants isolated from two well growing, genetically different clones, cultivated *in vitro* for 5 years. No surface sterilization had to be done. Other authors worked either with surface sterilized explants, or with aseptically germinating plants.

In general, it is possible to say that callus and/or root formation in sugar beet can be induced without any difficulties. We found, and so did Greef (1978), that homogeneous and friable callus can be obtained only on media with 2,4-D. Callus formation can be induced on media with different composition. In our experiments the best callus formation was reached on medium with 2,4-D (0.1 and 0.3 mg/l). Ingram and Joachim (1971) used medium with 6.0 mg/l 2,4-D + + 0.1 mg/l NAA first, later with combination of 2,4-D 6.0 mg/l + KIN 1.5 mg/l. Greef (1978) used second combination with a low concentration of 0.1 mg/l 2,4-D + 0.1 mg/l KIN. On the other hand, Read (1977) observed good callus growth on medium with addition of 0.2 mg/l 2,4-D, but also on medium without growth substances. But Rogozinska and Goska (1983) used again high concentrations of growth substances; the combination of 5.0 mg/l NAA + 5.0 mg/l BAP caused callus formation in 88 % of cotyledons.

Root formation was observed on media with each growth substance used. IAA caused the highest percentage of root formation but on these media the roots were the first to die away. For rhizogenesis induction IBA and NAA in concentrations of 3.0—10.0 mg/l can be recommended. We have unpublished results with micropropagation of sugar beet haploids where a part of haploid clones is better rooted on media with NAA and another part on media with IBA. Rogozinska and Goska (1976) mention the growth substances 2.0 mg/l BAP + + 5.0 mg/l NAA which proved optimal for callus growth but also for the formation of roots from anthers. Greef (1978) described the best root formation on a medium with 4.0 mg/l IAA + 1.0 mg/l BAP, while Mohammad and Collin (1979) observed high root and shoot

formation on a medium with 1.0 mg/l KIN + 0.05 mg/l 2,4-D. The root induction in our experiments on a medium with 2,4-D (0.1, 0.3 mg/l) was observed after a longer period of cultivation. This phenomenon was probably caused by a gradual reduction of 2,4-D from the medium.

The *de novo* regeneration of shoots in sugar beet is very problematic. It is deeply influenced by the plant genotype, the medium used, and cultivation conditions. According to Il'enko (1983), nutrient media are a very important factor in regeneration of shoots. Another significant factor influencing shoot formation is the type of plant tissue. We obtained all regenerants from leaf stalks, regeneration from callus and leaf blade was not successful. Similarly, Slavova et al. (1982) mentions the greatest ability of direct organogenesis from leaf stalks. Miedema (1982) obtained regeneration of shoots from the basis of flower bud, Rogozinska and Goska (1983) from cotyledons.

Unlike callus induction and rhizogenesis which can be achieved with different growth substances, regeneration of shoots *de novo* in our experiments, as in experiments performed by other authors, was induced by BAP. According to Saunders and Mahoney (1981), shoots on leaves of young plants growing in a greenhouse were induced by spraying the plants with a BAP solution. 6-benzylaminopurine has an exceptional position among the seven growth substances tested, because it was the only substance that induced in a relatively high percentage calogenesis, rhizogenesis and *de novo* shoot formation at the same time.

Based upon our results and literary data, callus induction and rhizogenesis *in vitro* in sugar beet has been experimentally solved. Regeneration of shoots *de novo,* especially regeneration from callus, remains problematic. Attention should be focused on this field, because it is the first-rate prerequisite for the implementation of the latest biotechnological methods in sugar beet selection.

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FARAGO, J. — SEMAN, I. (Výskumný a šľachtiteľský ústav semenných okopanín a priemyselných plodín, Bučany): *Vplyv rôznych rastových látok na morfogenézu repy in vitro*. Genet. a Šlecht., *25*, 1989 (3) : 215-222.

Byly provedeny pokusy s vlivem sedmi růstových látek na explantáty cukrovky *in vitro*. Hodnotily se účinky těchto látek na kalogenézu, rizogenézu a organogenézu u dvou klonů cukrovky a jejich závislosti od typu explantátu. Kalogenéza byla nejsilnější na médiu Murashige-Skoog s přídavkem 2,4-D v koncentraci 0,1 — 0,3 mg/l, nejlepší výsledky v rizogenéze byly zaznamenány na médiích s IAA v koncentracích 0,1—10,0 mg/l a u NAA a IBA v koncentracích 3,0—10,0 mg/l. Tvorba výhonků byla velmi slabá. Jenom na médiích s BAP regenerovalo 7 — 29 % explantátů, účinné byly všechny použité koncentrace (0,1 — 10,0 mg/l).

cukrovka; kultivace *in vitro;* kalogenéza; rizogenéza; organogenéza

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Проводились исследования с влиянием семи ростовых веществ на эксплантаты сахарной свеклы *in vitro*. Оценивались действия этих веществ на калогенез, ризогенез и органогенез у двух штаммов сахарной свеклы и их зависимость от типа экспланта. Калогенез был самым сильным на среде Murashige-Skoog с добавлением 2,4-Д в концентрации 0,1—0,3 мг/л, самые лучшие результаты в ризогенезе были отмечены на средах с IAA в концентрациях 0,1—10,0 (мг/л) и у NAA и IBA в концентрациях 3,0—10,0 мг/л. Образование побегов было весьма слабым. Только на среде с BAP регенерировалось 7—29 % эксплантатов, действенны были все использованные концентрации (0,1—10,0 мг/л).

сахарная свекла; культивирование *in vitro*; калогенез; ризогенез; органогенез

FARAGO, J. — SEMAN, I. (Forschungs- und Züchtungsinstitut für Samenhackfrüchtebau und technische Pflanzen, Bučany): *Einfluß verschiedener Wuchsstoffe auf die Morphogenese der Zuckerrübe in vitro*. Genet. a Šlecht., *25*, 1989 (3) : 215-222

Es wurden Versuche über den Einfluß von sieben Wuchsstoffen auf Zuckerrübenexplantate *in vitro* durchgeführt Bewertet wurden die Auswirkungen dieser Stoffe auf die Kallo-, Rhizo- und die Organogenese zweier Klone der Zuckerrübe und ihre Abhängigkeit vom Explantattyp. Die Kallogenese war am stärksten auf dem Murashige-Skoog-Medium mit Beigabe von 2,4-D in Konzentration 0,1 — 0,3 mg/l, die besten Ergebnisse der Rhizogenese wurden auf Medien mit IAA in Konzentrationen von 0,1 — 10,0 mg/l und bei NAA und IBA in Konzentrationen von 3,0 — 10,0 mg/l verzeichnet. Die Triebbildung war sehr schwach. Nur auf Medien mit BAP regenerierten 7 — 29 % der Explantate, wirksam waren sämtliche angewandten Konzentrationen (0,1 — 10,0 mg/l).

Zuckerrübe; Kultivation *in vitro;* Kallogenese; Rhizogenese; Organogenese

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ACCURACY OF WHEAT WINTERHARDINESS EVALUATION BY A PROVOCATION METHOD IN NATURAL CONDITIONS

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PRÁŠIL, I. — ROGALEWICZ, V. (Research Institute of Plant Production, Praha-Ruzyně): *Accuracy of Wheat Winterhardiness Evaluation by a Provocation Method in Natural Conditions.* Genet. a Šlecht., *25*, 1989, (3) : 223-230

The proportions of separate factors, and their interactions in the total variability was investigated for winterhardiness testing by the provocation method, consisting in cultivation of plants in wooden boxes during the whole autumn — winter season. It was proved that the order of rows in boxes was insignificant. The significance of boxes was caused only with their position in the experimental area; pots themselves caused no differences. A significant effect of cultivars was proved, while the interaction cultivar × box was insignificant. The sowing date and the level above the soil surface showed a significant influence in interaction with cultivars. The results of the analyses were used in modification of the design of experiments. The analyses also showed that the influence of random „uncontrolled" factors is lower in quality numbers than in survivals.

winter wheat; winterhardiness evaluation; experimental errors; components of variance

Winterhardiness evaluation is an important test for genetic resources and breeding material in winter crops. Owing to very variable weather conditions in Czechoslovakia, evaluation in field is quite difficult. It is necessary to harmonize all experimental conditions — locality, sowing date, snow cover etc., so that the experimental members may differentiate. An experiment is worthless and results unwanted if most material either fully survive or is fully killed.

A sophisticated "provocation method" has been developed at the Research Institute of Plant Production, Prague-Ruzyně to evaluate winterhardiness of cultivars (Segeťa, 1957, 1970; Prášil et al., 1989a). Cultivars tested are sown and cultivated in wooden boxes during the whole autumn — winter period. The boxes are placed at various levels above the ground, which enables better differentiation under various weather conditions. Nevertheless, demands for the design and for a careful performance of an experiment increase when a large collection (several hundred accessions) is evaluted. The variability of experimental results increases with the number of experimental members. A description of modifications of this method and a discussion on them are available in Prášil et al. (1989a).

Experimental results are influenced by many factors of environment. Cultivars are sown on several dates and exposed at various levels above the ground, so that genotypes should differentiate at least in some

treatments. Only a limited number of genotypes can be sown into a single box, so the experiment is to be extended to a large number of boxes. Further errors may thus be introduced, caused by the row (influence of boundary rows), by the box, or by the position of the box within the experimental area (non-homogeneity of conditions over the whole area of experiment).

This paper is focused on influences of particular factors on experimental errors. A modification of the design of experiments is proposed, so as to offset the variability due to interfering factors as much as possible. The paper specifies and elaborates in more detail the results of a similar analysis of Segeťa (1970). The problems concerning the single experiment studied in this paper will be completed by a study comparing the results of various experiments (years, localities). A suggestion of a solution to this problem is the subject of a separate paper (Palovský et al., 1989).

MATERIALS AND METHODS

Let us start with a description of the design of winterhardiness trials at the Research Institute of Plant Production. Cultivars are sown into wooden boxes 40 x x 30 x 12 cm, with 6 rows. Three cultivars are evaluated in each box (1st and 4th row, 2nd and 5th row, 3rd and 6th row). In the autumn, 16 seeds are sown by hand into each row. Each genotype is represented by 4 rows (i. e. it is sown into two boxes). The same combination of three cultivars is placed in both boxes. Boxes (with the same combinations of genotypes) are placed both into the soil and 50 cm above the surface. (In the higher place plants are exposed to a stronger influence of lower temperature and other harmful factors of winter.) The same material is sown in 3 terms in 14- to 20day intervals, the middle term being the optimum sowing date. Each genotype is thus included in 6 variants (3 sowing terms and 2 levels). The variants are represented by rows of boxes which are placed one after the other.

The number n of well-developed plants is evaluated for each row at the beginning of winter. During the spring evaluation, the level of damage and the intensity of renewed growing of shoot as well as root parts are determined visually according to a six-point scale (1 — not injured to 6-killed). For each row, two numbers are calculated:

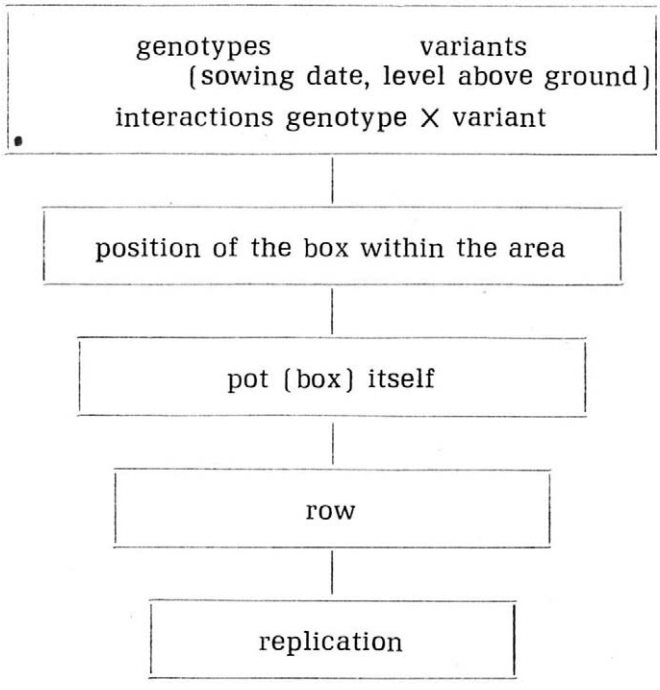
$$Z = (1 - b_6/n) \cdot 100$$

$$B = (b_1 + 0.8b_2 + 0.6b_3 + 0.4b_4 + 0.2b_5) \cdot 100/n$$

where b_i (i = 1, 2, 3, 4, 5, 6) is the number of plants in the particular row in the i-th quality group and n is the total number of plants in the row (during the autumn determination). The number Z is called a survival, the number 5 a quality number. Both are expressed in percentage.

To analyse the influences of the particular factors, four experiments were carried out during the winters 1986/87 and 1987/88. The standard desing was slightly modified due to different questions investigated. We used three cultivars of winter wheat (*Triticum aestivum* L.): Mironovskaya 808, Vala, Slavia. These cultivars differ considerably in winterhardiness. In trial A (1986/87) and C (1987/88), each cultivar was sown into four, resp. five boxes placed tightly one by one in each variant. The total extent was 432 rows in 72 boxes in 6 variants in trial A, and 180 rows in 30 boxes in 2 variants (only the middle sowing date) in trial C. In trials B (1986/87) and D (1987/88), the same cultivars were sown together, each, into two rows in the same box, in boxes situated regularly over the whole experimental area. We had 58 boxes (13 for the early sowing term, 12 for the middle one, and 4 for the late one, all at both height levels) in trial B, and 36 boxes (18 on the ground level and 18 on a bench, all only for the middle sowing term) in trial D.

Owing to problems wiht winterhardiness testing discussed above, not all variants provided data suitable for further analyses. Thus in the winter 1986/87, all cultivars were fully winterkilled in the variants on a bench 50 cm above the surface. In trial B only the results from the first sowing term could be used. In trial C and



1. Hierarchical classification of factors and their interactions

D (winter 1987/88), the values of survival for cv. Mironovskaya 808 were equal to 100 % in more than 75 per cent of cases. Nevertheless, we analysed them having in mind that the results might be distorted (the results for quality numbers are OK).

All results were transformed by the (normal) quantile transformation before executing the analyses. This transformation can be explained by intrinsic properties of winterhardiness experiments and by the central limit theorems from the probability theory (R o g a l e w i c z, P r á š i l, 1988) and seems to be more appropriate then speculative arcsin transformation.

I. Schema of the analysis of variance

Factor	Degrees of freedom (f)	Sum of squares (SS)	Mean square (MS = SS/f)	F-criterion
Factor A (cultivars)	f_A	SS_A	MS_A	MS_A/MS_{AB}
Factor B (variants)	f_B	SS_B	MS_B	MS_B/MS_{AB}
Interaction A × B	f_{AB}	SS_{AB}	MS_{AB}	MS_{AB}/MS_C
Factor C (boxes)	$f_C + f_{AC} + f_{BC} + f_{ABC}$	$SS_C + SS_{AC} + SS_{BC} + SS_{ABC}$	MS_C	MS_C/MS_D
Factor D (rows)	$f_D + f_{AD} + f_{BD} + f_{CD} + f_{ABD} + f_{ACD} + f_{BCD} + f_{ABCD}$	$SS_D + SS_{AD} + SS_{BD} + SS_{ABD} + SS_{CD} + SS_{ACD} + SS_{BCD} + SS_{ABCD}$	MS_D	MS_D/MS_R
Residual R	f_R	SS_R	MS_R	–

Effects of all the mentioned factors and of some of their interactions were tested by an analysis of variance — the model with random effects (W e b e r, 1980). The desing of experiments corresponds to the hierarchical classification, where cultivars, heighs above surface, sowing terms (and their interactions) are at the highest level, followed step by step by box positions, pot influences, rows and replications (Fig. 1). The rows with influences a priori taken for equal were considered for repetitions.) Formulae for the respective analyses are given in Tab. I. Particular analyses were derived as submodels of this basic scheme. All statistical estimations and tests were constructed at a significance level of 5 %.

Note. The design of trials B and D enabled to replace in the hierarchical scheme the factors „levels above surface" and „boxes", when the joint effect of box positions and of pots themselves was considered. This gave a possibility to investigate also the interactions „cultivar × box". We denote the analyses in the original hierarchy as B1, D1, and those in the modified one as B2, D2.

RESULTS

The results from the winter 1986/87 were not fully suitable for our analyses. The variants on the bench were fully winter killed. In the boxes on the ground there were only insignificant differences between variants from different sowing dates. Due to a relatively heigh autumn temperature, plants from different sowing terms reach a similar stage of development by the beginning of winter.

In some replications (both in the winters 1986/87 and 1987/88), the survivals reached 100 % and hence quality numbers provided better differentiation. In order to compare them, we calculated variance analyses both for quality numbers and for survivals; nevertheless, the

II. Components of variance (in %)

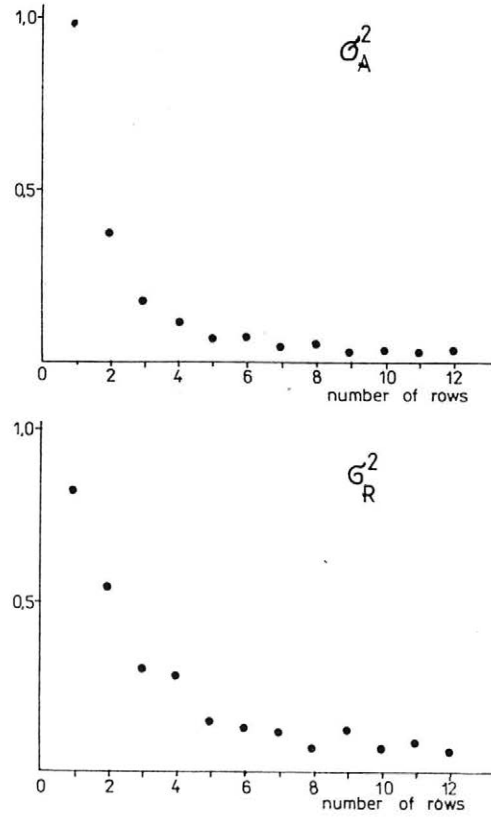
Factor	Quality number					Survival				
	trial A	trial B2	trial C	trial D1	trial D2	trial A	trial B2	trial C	trial D1	trial D2
repetition (residual)	45.96	27.35	24.47	29.75	27.75	74.53	78.83	39.94	46.33	47.59
row	0.05	—	0.15	—	—	−0.08	—	0.21	—	—
box pot itself	2.49	50.37	0.47	−0.11	9.82	0.53	4.12	−0.68	−0.08	−1.73
box position				3.76					−0.45	
sowing date	−6.37	—	—	—	—	6.17	—	—	—	—
level above ground	—	—	−2.84	−4.74	0.33	—	—	−0.35	−0.53	−0.05
Cultivar	30.65	21.76	64.19	57.78	60.10	10.09	30.37	61.15	54.30	56.11
sowing date × cultivar	27.23	—	—	—	—	8.76	—	—	—	—
box × cultivar	—	0.53	—	—	2.00	—	−13.31	—	—	−1.91
level ab. gr. × cultivar	—	—	13.55	13.56	—	—	—	−0.26	0.43	—
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

results for survivals are to be considered only for a rough estimations (great influence of errors occurs at values near 100 %).

The model of the analysis of variance used enables to estimate components of variance. These components, which represent the proportions of the variance corresponding to particular factors, give a global view on the significance of influences due to the respective factors (and their interactions if investigated). These components were expressed in percentage of the whole variance and are given in Tab. II.

Further, the hypothesis that a component of variance equals zero (= the respective factor is significant) can be subjected to F-test. In case of survivals, cultivars were the only significant factor (in trial A together with the interaction of cultivar X sowing date). For quality numbers, next to cultivars (and their interactions with sowing dates and with levels above surface), another significant factor appeared — the boxes. This factor in trials A, B2, D2. In trial D1 the factor box position was significant while the factor of pots themselves was not. The factor "boxes" was insignificant in trial C.

Finally, the influence of the number of plants was investigated (plants of each cultivar in each variant). In trial A, the components σ_R^2 (residual variance) and σ_A^2 (part of variance corresponding to cultivars) for survival evaluation were estimated by the one-way analysis of variance, survival being calculated from results of a single row, then of two, three, ..., up to twelve rows (with the corresponding number of replications). The results are given in Fig. 2.



2. Influence of number of rows on accuracy of the trial

σ^2_A — components of variance corresponding to cultivars;
σ^2_R — the residual error

DISCUSSION

The results of all trials show that there is a high dependence of winterhardiness evaluation (survivals as well as quality numbers) on cultivars. This is a positive result, because the method is aimed at a differentiation of cultivars.

On the other hand, the interactions of cultivars with sowing dates and with levels above the soil surface appeared to be significant, too. This corresponds to the experience that cultivars respond differently to various environmental conditions in each particular development stage. This affects primarily the results in various years or in various localities (the interaction of cultivar × year and cultivar × locality). Therefore it is very difficult to combine results from various trials and to interpret them uniformly. This problem is discussed in a separate paper (Prášil et al., 1989b). The significance of interactions of cultivars with sowing dates and with levels above the ground shows that every such variant should be examined as a separate trial, and the same method as in the case of various years should be applied.

The influence (an additive effect) of levels above the surface, considered alone, was proved to be insignificant and the results of trial A show that the same holds probably also for sowing dates.

It is very important how results are influenced by a given experimental design, i. e. by the distribution of cultivars over all boxes and rows. It was proved that there is no influence of rows. This enables to consider rows for pure replications. In the winter 1986/87, the effect of boxes was significant. This factor, however, comprised both the effect of pots themselves and the effect of positions of boxes in the experimental area. The results of experiments A and B2 showed that only the position of the box was likely significant and there was no effect of the pots. The design of the trials in the winter 1987/88 was somewhat modified so that both effects could be separated in analysis D1. The hypothesis was proved in this trial. The results concerning rows and boxes show that only one parameter of the arrangement of cultivars into rows and boxes affects the results: the position within the experimental area. Therefore it is desirable to distribute replications (of each cultivar) into more boxes over the whole area.

To design a trial using the provocation method, the number of plants of each cultivar is also to be determined. In the standard design, 16 seeds are placed in a row. We leave a reserve for the case that some seed will not germinate; hence we consider 15 plants in a row. Our task is now to determine the number of plants as a multiple of 15. If we start from the estimation of a parameter of an alternative distribution, the number of rows estimated will be quite high. We tried to analyse the random error and the component of variance corresponding to cultivars in trial A, when we took for replications every 2, 3, ... up to 12 rows (24 rows of each cultivar was sown into 4 boxes placed one next to the other). The results are given in Fig. 2. The residual variance as well as the component of variance coresponding to cultivars rapidly fell down with the number of rows growing from 1 to 5 or 6. Further, they did not change significantly, though showing a slight declination. Reflecting this fact, we can cinsider estimations of winter-

hardinnes (survivals and/or quality numbers) based on results from 6 rows (appr. 90 plants) for reasonable accuracy.

The trials also enabled to compare reliability of information given by survivals and by quality numbers. It was shown that when we replace survivals (yes-no experiment) by quality numbers (a more quantitative description), the percent expression of the component of variance corresponding to uncontrolled factors decreases considerably, while the share corresponding to the factor "cultivars" increases. This tendency was proved in all trials. Therefore it is better to use quality numbers and to use survivals as a complementary criterion only. However, we should remember that the role of "disturbing" factors is also higher in quality numbers than in survivals (see Tab. II).

Generally, it was proved that the provocation method is suitable for evaluation of cultivar (breeder's line, genetic resource) winterhardiness in the weather conditions of Czechoslovakia. On the basis of the results stated above, a minor modification of the method has been proposed. This includes:

1. to use quality numbers and to consider survivals for preliminary results only;
2. to sow each cultivar into six rows in each variant and to place into each box one row of a cultivar maximum;
3. to lower the non-homogeneity of the experimental area surface, which causes higher experimental errors in variant placed on the ground (this was done during the summer 1987 with a positive effect — see results of trials C and D);
4. to take each variant as an independent experiment and to compare them by the same methods as experiments from various years and localities.

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PRÁŠIL, I. — ROGALEWICZ, V. (Výzkumný ústav rostlinné výroby, Praha-Ruzyně): *Přesnost hodnocení zimovzdornosti pšenice provokační metodou v přirozených podmínkách.* Genet. a Šlecht., *25*, 1989 (3) : 223-230

Byl hodnocen vliv základních faktorů provokační metody a jejich interakcí na celkovou variabilitu výsledků. Při provokační metodě byly rostliny jednotlivých odrůd

pěstovány v dřevěných bedýnkách, které byly uloženy v různé výšce nad zemí během celého podzimně-zimního období. Byl prokázán nevýrazný vliv řádku v bedýnce. Významný vliv bedýnky byl způsoben pouze jejím umístěním v rámci pokusného areálu, nikoliv vlivem bedýnky jako kultivační nádoby. Významný vliv měla odrůda, zatímco interakce odrůda × bedýnka byla nevýznamná. Termín výsevu a úroveň uložení bedýnek nad zemí měly významný vliv v interakci s odrůdou. Výsledky analýz byly užity při návrhu pokusného uspořádání a úpravy pokusné plochy provokační metody. Z analýz rovněž vyplynul nižší vliv náhodných nekontrolovatelných faktorů při hodnocení rostlin pomocí bonitace než životnosti.

pšenice ozimá; hodnocení zimovzdornosti; chyba pokusu; komponenty rozptylu

ПРАШИЛ, И. — РОГАЛЕВИЧ, В. (Научно-исследовательский институт растениеводства, Прага-Рузыне): **Точность оценки морозоустойчивости пшеницы провоцирующим методом в природных условиях.** Genet. a Šlecht., *25*, 1989 (3) : 223-230.

Оценивалось влияние основных факторов провоцирующего метода и их взаимодействие на общую изменчивость результатов. При провоцирующем методе растения отдельных сортов выращивались в деревянных ящиках, которые находились на разной высоте над землей в период всего осенне-зимнего исследования. Было доказано недостоверное влияние рядка в ящике. Значительное влияние ящика было вызвано лишь местонахождением в рамках опытного ареала, а не самим ящиком как культивирующим сосудом. Большое влияние оказывал сорт, причем взаимодействие сорт × ящик было без значения. Срок посева и уровень нахождения ящиков над землей оказывали значительное влияние в взаимодействии с сортом. Результаты анализов использовались при проекте опытного проведения и оформления опытной площади провоцирующего метода. Анализы также показали более низкое влияние случайных неконтролируемых факторов при оценке растений с помощью бонитировки, а не жизнеспособности.

озимая пшеница; оценка морозоустойчивости; ошибка эксперимента; компоненты дисперсии

PRÁŠIL, I. — ROGALEWICZ, V. (Forschungsinstitut für Pflanzenproduktion, Praha-Ruzyně): *Genauigkeit der Bewertung der Kälteresistenz von Weizen mittels Provokationsmethode unter natürlichen Bedingungen.* Genet. a Šlecht., *25*, 1989, (3) : 223-230.

Bewertet wurde der Einfluß der Grundfaktoren der Provokationsmethode und deren Wechselbeziehung auf die Gesamtvariabilität der Ergebnisse. Im Rahmen der Provokationsmethode waren Pflanzen einzelner Sorten in Holzkisten angepflanzt, die während der ganzen Herbst-Winter-Periode in verschiedenen Höhen über dem Erdboden deponiert waren. Es wurde ein unsignifikanter Einfluß der Reihe in der Kiste nachgewiesen. Der signifikante Einfluß der Kiste war ausschließlich auf ihre Aufstellung im Rahmen des Versuchsareals und nicht auf den Einfluß der Kiste als Kultivierungsgefäß zurückzuführen. Signifikant war der Einfluß der Sorte, während die Interaktion Sorte × Kiste unsignifikant war. Der Aussattermin und die Lagerungshöhe der Kisten über dem Erdboden hatten in Interaktion mit der Sorte signifikanten Einfluß. Ergebnisse der Analysen wurden beim Entwurf der Versuchsanordnung und der Gestaltung der Versuchsfläche der Provokationsmethode ausgenützt. Aus den Analysen ergab sich ebenfalls ein niedrigeren Einfluß der zufälligen nichtkontrollierbaren Faktoren bei der Bewertung der Pflanzen mittels Bonitierung gegenüber Bewertung mittels Vitalität.

Winterweizen; Kälteresistanztest; Versuchsfehler; Varianz-komponente

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POLLEN FERTILITY RESTORATION IN ENGLISH WINTER WHEAT CULTIVARS

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APLTAUEROVÁ, M. (Research Institute of Crop Production, Praha-Ruzyně): *Pollen Fertility Restoration in English Winter Wheat Cultivars.* Genet. a Šlecht., *25,* 1989 (3) : 231-238.

Fertility restoration was tested in 14 English winter wheat cultivars and 8 of them were found to have *Rf* genes for CMS *T. timopheevi.* The cultivars were divided into 3 groups according to the level of fertility restoration in CMS Ilyichevka and CMS Slavia. On an average for two testers and two years, the most effective restoration was recorded in the cultivars Durin (53.72 %, i. e. 71.55 % of that of the checks), Brigand (53.03 %, i. e. 70.63 % CH) and Norman (52.62 %, i. e. 70.08 % CH). The influence of genetic background of male sterile on fertility restoration level of hybrid was confirmed. The male sterile analogue of the Czech cultivar Slavia was restored by all restorers with difficulty, so it is not recommended as a female component of hybrid. Fertility restoration was influenced by weather factors in the experimental years. Fluctuating air temperature before and during flowering, and particularly low temperature just before the onset of flowering, had an adverse effect. The contribution of English cultivars with *Rf* genes to restorer breeding in Central European conditions is appreciated.

Cytoplasmic male sterility; *Rf* genes; restorer breeding

One of the prerequisites for heterosis breeding in wheat is a suitable system for hybrid seed production. The most suitable system, for the time being, is cytoplasmic male sterility and fertility restoration based on interaction of the plasmon of the tetraploid wheat *Triticum timopheevi* Zhuk. and the genome of the hexaploid *T. aestivum* L. Since 1974, hybrids obtained by means of this genetic male sterility have been grown in limited areas in North and South America and in Australia.

The comparatively slow rate of progress in hybrid breeding is also caused by the complexity of inheritance of fertility restoration in CMS *T. timopheevi* and by the lack of suitable donors of *Rf* genes which would ensure a full fertility restoration in F_1 generation. It was found that *Rf* genes in cultivars of *T. aestivum* are more effective in European conditions than the original *Rf* genes from *T. timopheevi* (Oehler, Ingold, 1966; Apltauerová et al., 1966). A high fertility restoration can be provided by a combination at least 2—3 *Rf* genes. A number of West European cultivars able to restore pollen fertility in CMS *T. timopheevi* were found by the screening of *Rf genes* in *T. aestivum* (Zeven, 1968; Apltauerová, 1968; Goujon, 1969; Apltauerová et al., 1979 aj.). From the point of view of use for breeding, English cultivars are particularly interesting. We want to draw attention to them in this contribution.

MATERIALS AND METHODS

Fourteen English cultivars of winter wheat were crossed with two sterile testers with *T. timopheevi* cytoplasm to identify *Rf* genes. A collection of the cultivars teseed is presented in a survey, including their genealogical origin. CMS Ilytchevka and CMS Slavia were used as testers. To find out the influence of environment on fertility restoration, experiments were carried out for 2 years.

A Survey of Cultivars Tested for Fertility Restoration in CMS *T. timopheevi*

Avalon (TJB 409/1088) — Maris Ploughman × Bilbo
Bounty (TJB 406/892) — Maris Ploughman × Durin
Brigand (TJB 370/491) — Maris Huntsman × Bilbo
Brimstone (CWW 1887/1) — (TJB 54/218) (M. Widgeon der. × Viking × Hobbit 30/2) × Hustler
Brock (CWW 1724/3/10) — Hobbit 30/2 × Talent
Durin — [(Vilmorin 29 × Vogel 8058) × Cappelle] × [(C. I. 12633 × Cappelle 4) × × (Heines 110 × Cappelle)] × Nord
Fenman (TJB 990/8) — [(Maris Ranger × Durin) × Maris Beacon] × Hobbit sib
Galahad (TJB 730/3637) — Joss Cambier × Durin) × Hobbit sib
Gawain (CWW 1681/1) — Durin der. × Brigand
Mardler — Maris Huntsman × (M. Ranger × Durin)
Norman (TJB 989/10) — [(Maris Ranger × Durin) × Maris Beacon] × Hobbit sib
Renard (CWW) 1724/3/4) — Hobbit 30/2 × Talent
Sarsen (CWW 2479/ 16) — Maris Marksman × Armada
Virtue (TJB 368/255) — Maris Huntsman × Durin

Seeds of the testing F_1 generation and the control cultivars Yubileynaya, Hana and Regina were sown in field conditions at a 15 × 15 cm spacing. In each variant, the first heading ear of each of 30 plants was isolated during heading. The number of spikelets, number of grains in the 1st and 2nd floret and total grain number in ear were recorded after harvest. Fertility restoration was estimated according to the formula

$$\% FR = \frac{A}{B.2} \cdot 100$$

where: A — grain number in the 1st and 2nd floret of spikelet per ear
B — spikelet number per ear

Cultivars which produced completely sterile progeny in both combinations in the 1st experimental year were not included in repeated evaluation.

After arcsin transformation, the data on fertility restoration were processed by analysis of variance, and the significance of the differences was tested by Tuckey's test.

RESULTS AND DISCUSSION

Primary donors of *Rf* genes used in restorer breeding represented extensive types with long lodged culm and a low yield potential. Systematic screening of West European cultivars has provided new fertility restoration sources with better agronomic characters, e. g. the English cultivars Maris Beacon and Maris Huntsman, for many years the highest yielding cultivar in our country, was particularly valuable and could be used directly as a restorer. Attention was therefore concentrated also on new semidwarf English cultivars. The aim was to find suitable restorers, or at least a new source of *Rf* genes for their breeding.

Testing of fertility restoration in CMS *T. timopheevi* revealed restoration ability in eight of the 14 English cultivars examined. Cultivars Avalon, Bounty, Brimstone, Brock, Mardler and Renard crossed with

I. Fertility restoration ability of English cultivars in CMS *T. timopheevi* Zhuk.

Cultivar	1986				1987			
	CMS Ilyichevka		CMS Slavia		CMS Ilyichevka		CMS Slavia	
	FR %	Rel. CH+ %	FR %	Rel. CH+ %	FR %	Rel. CH+ %	FR %	Rel. CH+ %
Brigand	56.43	74.64	27.29	36.10	79.04	106.02	49.36	66.21
Durin	62.67	82.90	32.25	42.66	80.86	108.46	39.08	52.42
Fenman	50.70	67.06	18.29	24.19	59.20	79.41	37.73	50.61
Galahad	52.68	69.68	22.58	29.87	60.61	81.30	28.91	38.78
Gawain	50.31	66.55	19.93	26.36	74.46	99.89	41.70	55.94
Norman	52.50	69.44	30.91	40.89	76.86	103.10	50.22	67.36
Sarsen	57.00	75.40	3.95	5.22	69.54	93.28	21.46	28.79
Virtue	54.51	72.10	24.07	31.84	71.93	96.49	33.02	44.29

CH+ — average seed set in the 1st and 2nd florets in Yubileynaya 50, Regina, Hana
1986 — 75.60 %, 1987 — 74.55 %

both testers, produced quite sterile progeny, i. e. they were carriers of recessive alleles of *Rf* genes. They can be used in hybrid breeding to obtain sterile analogues.

The presence of *Rf* genes was demonstrated in other 8 cultivars (Tab. I). Fertility restoration was very low in CMS Slavia progeny and did not reach a significant level. On the other hand, fertility restoration level was much higher in combination with CMS Ilyitchevka, i. e. it varied from 50.31 % to 62.67 %, and from 59.20 % to 80.86 % in 1986 and 1987, respectively. As even the common cultivars fail to reach a full seed set in the 1st and 2nd floret, the data were compared with the average seed set for three control cultivars. On the basis of this relation, fertility restoration was found to range from 74.64 % to 82.90 % in the three best cultivars tested on CMS Ilyitchevka in the first year. In

II. Cultivars with Rf genes ordered according to fertility restoration ability

Cultivar	FR %	Statist. signif.+	Rel. CH %	Ranking
Durin	53.72	a	71.55	1
Brigand	53.03	a	70.63	2
Norman	52.62	a	70.08	3
Gawain	46.60	b	62.07	4
Virtue	45.88	b	61.11	5
Fenman	41.48	b	55.25	6
Galahad	41.19	b	54.86	7
Sarsen	37.99	c	50.60	8

+ — differences in FR values denoted with the same letters are statistically insignificant

1987, when the level of fertility restoration was much higher, the best three combinations exceeded the average seed set of control cultivars by 3.10 %, 6.02 %, and 8.46 % in the 1st and 2nd florets.

Highly significant differences between the levels of all the factors observed, i. e. between cultivars, testers and years, were demonstrated by the statistical analysis ($F_{cult.} = 27.90^{++}$; $F_{test} = 1139.92^{++}$; $F_{year} = 310.71^{++}$).

The tested cultivars with *Rf* genes can be divided into three groups according to the level of restoration ability (Tab. II). The first group includes the most effective restores Durin, Brigand, and Norman, whose restoration ability reached 71.55 % — 70.08 % of check cultivars, on an average for the testers used and the experimental years. The cultivars Gawain, Virtue, Fenman and Galahad with a restoration ability of 62.07 % — 54.86 % of control cultivars were included in the second group with a significantly lower fertility restoration. The lowest restoration was found in the cultivar Sarsen — 50.60 % of that of the check cultivars.

All these cultivars originated from crosses with cultivars in which fertility restoration had been found before: Maris Huntsman, Maris Beacon, Maris Marksman, and Armada (see survey of the cultivars tested). It can be inferred from pedigree analysis of these cultivars that fertility restoration for CMS *T. timopheevi* origins from three original sources, including the English cultivar Hybrid 46, Belgian cultivar Prof. Marchal, and the American line C. I. 12633. The C. I. 12633 was used as a donor of the mildew resistance genes *Pm 2* and *Pm 6*. According to Hughes, Bodden (1977), there is a stronger linkage between these genes and *Rf* genes, which could influence positively the restorer breeding.

Statistical analysis also demonstrated highly significant differences between both testers. On an average for all cultivars and years, CMS Ilyitchevka was restored at 63.08 % whereas CMS Slavia only at 30.05 %. It was found some time ago that the genotype of the male sterile influences fertility restoration in the F_1 generation (Wilson, 1968; Apltauerová, Mikala, 1970; Milohnić, 1972). According to Wilson (1968) fertility genes in a CMS analogue can make either an additive or complementary contribution to phenotypic expression of restorer genes. It is therefore necessary to choose such steriles, which would favourably influence the fertility restoration level in the F_1 generation. From that point of view, CMS Slavia will not be a suitable female component. However, it may be used as a hard tester for a future evaluation of restoration ability (Apltauerová, Golubec, 1987).

It follows from the significant interactions between cultivars and testers ($F_{cult. \cdot test} = 13{,}34^{++}$) that the best restorer cultivars Durin, Brigand, and Norman are effective and stable with both testers, whilst less effective cultivars fluctuate (Sarsen, Gawain).

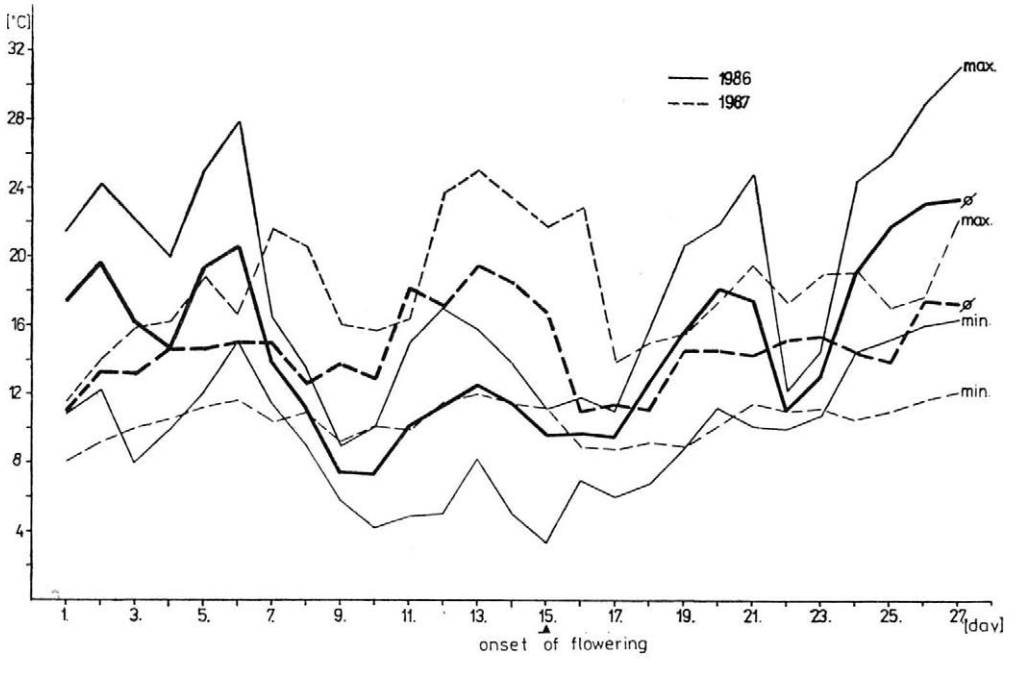
The level of fertility restoration in generation F_1 also depends on the environment. For example, fertility is explicitly reduced in greenhouse conditions (Rajki, Rajki, 1966). High temperature and low air humidity are most frequently regarded as the decisive factors leading to lower fertility restoration levels in field conditions (Žukovskij, Chvostova, 1971; Mihaljev, Borojević 1972).

However, low temperature also proves to be a "sterilizing" factor (Roberts, 1969).

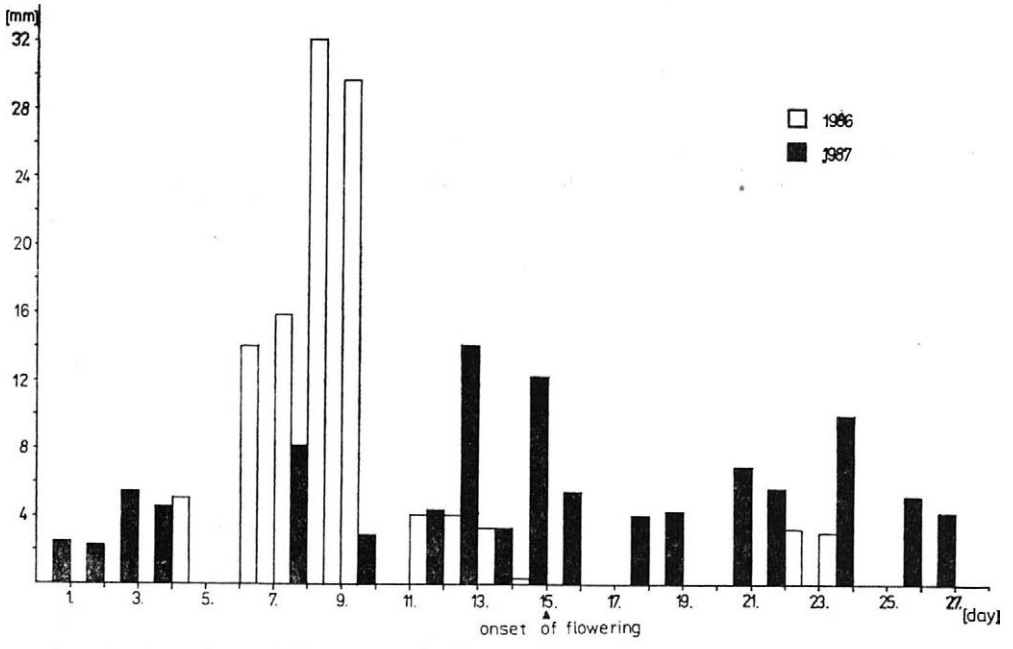
In our experiments we noticed high significant differences in fertility restoration between the two experimental years. On average, fertility restoration reached a level of 38.50 % in 1986, but it was much higher in 1987 — 54.62 %. In 1987, flowering was delayed by 10 days in comparison with the preceding year and was shifted to the latter half of June. Analysis of weather conditions in both years showed that in 1987 the period before flowering was warmer with a regular distribution of rainfall. In the same period in 1986, i.e. two weeks before flowering, the minimum temperature decreased to 3.4 °C. Six days with minimum temperature below 6 °C were recorded. A cold spell followed a very warm period, when maximum temperature reached even 27.9 °C.

During flowering in 1987, the temperature was lower but less fluctuating; the rainfall was distributed uniformly. In 1986, dry weather prevailed, with fluctuating temperatures (Figs. 1, 2). It could be concluded from different weather conditions in both experimental years, that the large changes in temperature, and particularly the low temperature just before flowering, are not favourable for fertility restoration. A high fertility restoration was reached when temperature just before and during flowering was adequately high and stable and rainfall was moderate and regularly distributed.

Fertility of the 1st and 2nd floret in the spike in the three controls Yubileynaya 50, Regina, Hana was about the same in both years, i. e. 75.60 % (average for check cultivars) in 1986 and 74.55 % in 1987. In this trait the order of cultivars was the same in both years.



1. Air temperature (average daily, maximum and minimum temperature two weeks before flowering and during flowering in 1986 and 1987



2. Distribution of rainfall two weeks before flowering and during flowering in 1986 and 1987

In conclusion it can be stated that the English winter wheat cultivars Durin, Brigand, and Norman can make a good contribution to an extension of the gene pool of restorer breeding for CMS *T. timopheevi*. Apart from a good restoration ability they exhibit a high productivity in Czechoslovak conditions (Bareš et al., 1985). They possess field resistance and have specific genes for resistance to mildew, yellow rust and leaf rust (Durin). It should be taken into account when the other partner for initial crossing is sought, that they have a lower quality and winter-hardiness.

The three cultivars referred to this paper could be used also directly as restorers in crosses with short stem male steriles. However, it is necessary first to test the character of their flowering because it is well known that some English cultivars (e. g. Hybrid 46) are cleistogamic. Nevertheless, effective pollination of sterile plants can be reached only in the case when there is an open — flowering pollinator which sheds in the air as much pollen as possible.

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APLTAUEROVÁ, M. (Výzkumný ústav rostlinné výroby, Praha-Ruzyně): *Obnova fertility pylu v anglických odrůdách pšenice ozimé*. Genet. a Šlecht., *25*, *1989* (3) : : 231-238.

Testováním obnovy fertility u 14 anglických odrůd pšenice ozimé bylo zjištěno, že osm odrůd má *Rf* gen (geny) pro CMS *Triticum timopheevi*. Podle výše obnovy fertility v CMS Iljičovka a CMS Slavia byly odrůdy rozděleny do tří skupin. Nejefektivnějšími obnoviteli v průměru dvou testerů a dvou let se projevily odrůdy Durin (53,72 %, tj. 71,55 % K), Brigand (53,03 %, tj. 70,63 K) a Norman (52,62 %, tj. 70,08 K). Byl potvrzen vliv genetického pozadí CMS analoga na úroveň obnovy fertility hybrida. Sterilní analog čs. odrůdy Slavia se obnovoval všemi odrůdami — obnoviteli velmi obtížně, proto se nedoporučuje jako mateřská forma hybrida. Obnova fertility byla ovlivněna povětrnostními faktory pokusných let. Nepříznivě působilo kolísání teploty vzduchu před kvetením a v průběhu kvetení a zejména nízké teploty krátce před začátkem kvetení. Uvádí se přínos anglických odrůd s *Rf* geny pro šlechtění obnovitelů ve středoevropských podmínkách.

cytoplazmatická samčí sterilita; *Rf* geny; šlechtění obnovitelů

АПЛТАУЕРОВА, М. (Научно-исследовательский институт растениеводства, Прага-Рузыне): **Восстановление фертильности пыльцы в английских сортах озимой пшеницы.** Genet. a Šlecht., *25*, 1989 (3) : 231-238.

Проводимые тесты восстановления фертильности у 14 английских сортов пшеницы озимой показали, что восемь сортов имеет *Rf* ген (гены) для CMS *Triticum timopheevi*. Согласно величине обновления фертильности в CMS Ильичовка и CMS Славия сорта разделили на три группы. Наиболее эффективными обновителями в среднем двух тестеров и двух лет проявились сорта Дурин (53,72 %, т. е. 71,55 % К), Бриганд (53,03 %, т. е. 70,63 К) и Норман (52,62 %, т. е. 70,08 К). Подтвердили влияние генетического фона CMS аналога на уровне восстановления фертильности гибрида. Стерильный аналог чехословацкого сорта Славия обновлялся всеми сортами-обновителями

с большим трудом, и потому не рекомендуется как материнская форма гибрида. На восстановление фертильности влияли погодные факторы опытных лет. Неблагоприятно влияло колебание температуры воздуха перед цветением и в период цветения и главным образом низкие температуры непосредственно перед началом цветения. Приводится вклад английских сортов с *Rf* генами для селекции восстановителей в среднеевропейских условиях.

цитоплазматическая мужская стерильность; *Rf* гены; селекция восстановителей

APLTAUEROVÁ, M. (Forschungsinstitut für Pflanzenproduktion, Praha-Ruzyně): *Restoration der Pollenfertilität in englischen Winterweizensorten.* Genet. a Šlecht., *25*, 1989 (3) : 231-238.

Anhand von Tests der Fertilitätsrestoration bei 14 englischen Sorten des Winterweizens wurde festgestellt, daß acht Sorten das *Rf* Gen (bzw. Gene) für CMS *Triticum timopheevi* führen. Dem Umfang der Fertilitätserneuerung in CMS Iljitschowka und CMS Slavia nach wurden die Sorten in drei Gruppen eingeteilt. Als effektivste Restorer im Mittelwert von zwei Testern und zwei Jahren erwiesen sich die Sorten Durin (53,72 %, d. h. 71,55 % K), Brigand (53,03 %, d. h. 70,63 K) und Norman (52,62 %, d. h. 70,08 K). Es wurde der Einfluß des genetischen Hintergrunds des CMS-Analoges auf das Niveau der Fertilitätserneurung des Hybriden bestätigt. Das sterile Analoge der tschechoslow. Sorte Slavia erneuerte sich durch sämtliche Sorten-Erneuerer nur schwierig und wird daher als mütterliche Form des Hybriden nicht empfohlen. Die Fertilitätserneuerung war auch durch die Witterungsfaktoren der Versuchsjahre beeinflußt. Ungünstig wirkten sich die Lufttemperaturschwankungen im Zeitraum vor und während der Blüte und insbesondere dann niedrige Temperaturen kurz vor Blütebeginn aus. Es wird der Beitrag der englischen Sorten mit *Rf* Genen für die Züchtung von Restorern unter mitteleuropäischen Bedingungen angeführt.

zytoplasmatische männliche Sterilität; *Rf* Gene; Restorer-Züchtung

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DISCRIMINATION OF WHEAT CULTIVARS AFTER THEIR RUST REACTIONS

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BARTOŠ, P. — STUCHLÍKOVÁ, E. — HANUŠOVÁ, R. (Research Institute of Crop Production, Praha-Ruzyně): *Discrimination of wheat cultivars after their rust reactions.* Genet. a Šlecht., *25*, 1989 (3) : 239-244.

The winter wheat cultivars registered in Czechoslovakia can be divided into several groups according to their seedling reactions to some leaf and stem rust isolates. Some cultivars can be determined according to their specific reactions to several leaf and stem rust isolates. Simple discrimination of the group of cultivars possessing *1B/1R* translocation, using one leaf rust isolate, can be used to distinguish cultivars with poor and good baking quality. The cultivar Branka, the only one with *1B/1R* translocation and medium baking quality, can be determined by one isolate among other cultivars possessing *1B/1R* translocation.

Triticum aestivum L.; leaf and rust isolates; *1B/1R* translocation; baking quality

Identification of wheat cultivars after harvest is often of practical and economic importance. Several methods are used for this purpose. Electrophoretic analysis of seed proteins belongs to the most efficient ones (Šašek et al., 1988). However, special laboratory equipment, skill and experimence are needed for this technique. Our study of rust resistance in the winter wheat cultivars registered in Czechoslovakia has shown characteristic differences in their rust reactions to certain isolates of rusts, conditioned by different resistance genes. The possibility to utilize these differences, observed in the seedlings greenhouse tests for identification of the registered winter wheat cultivars, is described and discussed in this paper.

MATERIALS AND METHODS

Resistance genes determined in genetic analyses or postulated according to responses to a number of rust isolates are presented in Tab. I (Bartoš, Valkoun, 1988). Postulation of yellow rust resistance genes was based on the results obtained by Dr. R. Johnson (PBI Cambridge) and R. W. Stubbs (IPO Wageningen). In our tests only leaf and stem rust reactions were considered for identification of the cultivars.

Tests were carried out in the greenhouse at temperatures ranging between 15 °C and 25 °C. Wheat seedlings at the first leaf stage were inoculated by rubbing the leaves with spores in talc. After inoculation the plants were sprayed with water by means of an atomizer and kept closed in covered glass cylinders at high air humidity for 24—48 hours. Rust reactions were evaluated after 8—12 days, using

I. Genes for rust resistance in winter wheats registered in Czechoslovakia

Cultivars	Baking quality	Genes		
		Sr	*Lr*	*Yr*
Agra	2	31, +	3,26	9,Ad
Branka	5	31	3,26,+	9,Ad
Danubia	4	31, (+ ?)	26,+	9
Hana	9	29	3	2
Iris	1	31,11, 6?	26	9
Košútka	7	+	+	+
Mara	7	29	3	Ad
Mironovská (808)	9	Tmp	3	Ad
Odra	7	–	3	2,+
Regina	8	–	–	1,2,H IV
Roxana	3	31	26,+	9+
Selekta	2	31	26	9,Ad
Slavia	5	29	–	2
Sparta	4	31	26,3	9,Ad
Vala	5	29	–	Ad
Viginta	7	5,+	3	2,3a+4a
Zdar	5	+	–	3a+4a,CV

Yr genes postulated according to the results by: R. Johnson (PBI Cambridge) and R. W. Stubbs (IPO Wageningen)
Ad. = Adult plant resistance
Baking quality: 1 – poor; 9 – very good

infection types according to S t a k m a n et al. (1962). The following isolates (races) of rusts were used: 243(77), 600(14SaBa), 628(61SaBa), 1938(58SaBa), 1943(11SaBa) for leaf rust and G 702(14), G 69(21), and G 425(11) for stem rust.

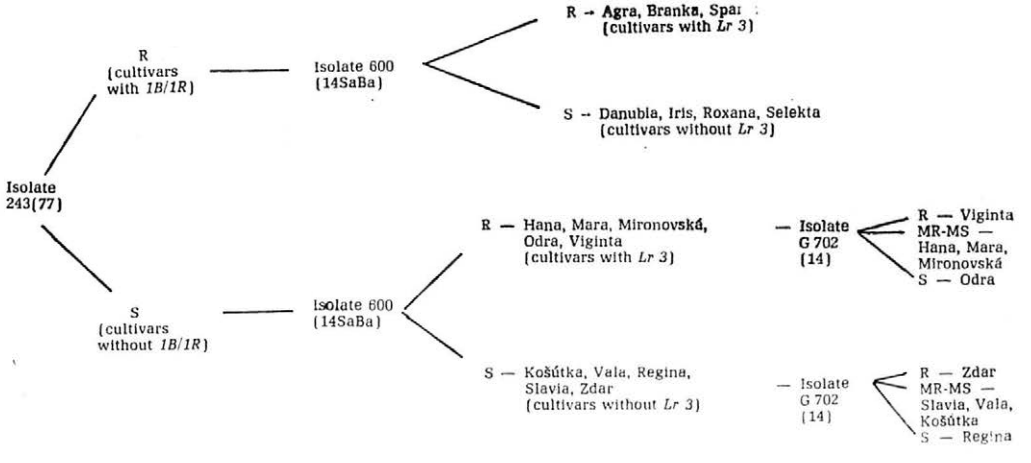
RESULTS

One leaf rust isolate, namely 243(77) is satisfactory for the differentiation of two important groups of cultivars, namely cultivars with translocation *1B/1R* and those without it. As shown in Tab. II, all cultivars possesing translocation *1B/1R* with rust resistance genes *Sr 31, Lr 26* and *Yr 9* are resistant to the isolate 243(77).

The group of cultivars with this translocation can be further divided into two sub-groups by the isolate 600(14SaBa). Cultivars Agra, Branka and Sparta are resistant to this isolate (possess *Lr 3*), whereas Danubia, Iris, Roxana and Selekta are susceptible. Out of the former sub-group of cultivars, Branka can be easily identified by its resistant reaction to the isolate 628(61SaBa). All other cultivars with *1B/1R* translocation are susceptible to isolate 628(61SaBa). Out of the latter sub-group of cultivars, Danubia can be identified by its resistant reaction to isolate 1938(58SaBa) and Roxana by its resistant reaction to isolate 1943-(11SaBa).

II. Reaction of the winter wheats registered in Czechoslovakia to 5 leaf rust and 3 stem rust isolates

Isolate	Cultivar	Leaf rust					Stem rust		
		243 (77)	600 (14 Saba)	628 (61 Saba)	1938 (58 Saba)	1943 (11 Saba)	G702 (14)	G69 (21)	G425 (11)
With *1B/1R* translocation	Agra	0	0	4	;1–3	0;	0	0	0
	Branka	0;	0;	0;	;1	0;	0;	0;	0
	Danubia	0;	3	3	;1	2–3	0;	0	0;
	Iris	0;	3	4	4	3	0;	0	0
	Roxana	0;	4	4	4	0;	0;	0;	0;
	Selekta	0;	4	4	4	3–	0;	0;	0;
	Sparta	0;	0;	4	4	0;	0;	0;	0;
Without *1B/1R* translocation	Hana	4	0;	4	4	0;	0;1–2	0; 1–2	0; 1–2
	Košútka	3	4	4	3	2–3	;1–2+	;1 –2+	;1–2+
	Mara	4	0;	4	4	0;	;1+	;1–2	;1–2+
	Mironovská	4	0;	4	4	0;	;1+	3	3
	Odra	3	0;	4	3	0;	3	3–	3
	Regina	4	4	4	4	3–	4	4	4
	Slavia	4	3	4	4	3	0;1–2+	;1 –2+	0;1–2+
	Vala	4	4	4	3	3–	1–2	;1 –2+	0;1–2+
	Viginta	4	0;	4	3	0;	0	0	0;1–2+
	Zdar	4	4	4	3	2–3	0;	3	3



1. Discrimination of winter wheat cultivars registered in Czechoslovakia based on leaf and stem rust reaction of their seedlings (Stem rust isolate designated by G and a number, leaf rust isolates designated by numbers; R — resistant, S — susceptible)

The cultivars not having translocation *1B/1R* can be further differentiated by isolate 600(14SaBa). Cultivars Hana, Mara, Odra, Viginta and Mironovská (Mironovskaya 808) are resistant (possess *Lr 3*), whereas Slavia, Vala, Regina, Košútka and Zdar are susceptible. Stem rust isolate G 702(14) enables further differentiation. Of the former sub-group of cultivars, Viginta is highly resistant, Hana and Mara medium resistant, and Odra susceptible. Of the latter sub-group of cultivars, Zdar is resistant, Slavia and Vala medium to highly resistant, Regina susceptible.

A diagram of the identification of the winter wheat cultivars registered in Czechoslovakia by means of 2 leaf rust- and 1 stem rust isolate is given in Fig. 1.

DISCUSSION

The genetic basis of leaf and stem rust resistance of the Czechoslovak cultivars registered at present enables to discriminate many cultivars by their reactions to the selected isolates of leaf and stem rust.

Simple discrimination of the group of wheats possessing *1B/1R* translocation by means of isolate 243(77) can be of particular importance. Translocation *1B/1R* indicates almost in all cases a low baking quality, ranging in the registered cultivars from 1 to 4, except Branka, classified 5. Branka can be easily distinguished from other cultivars with *1B/1R* translocation by its resistant reaction to isolate 628(61SaBa).

Reaction to two races can be examined simultaneously on single plants, the top of the leaf being infected with one and the base with another isolate. Thus the group of the cultivars with *1B/1R* translocation, and out of them Branka, can be determined in a single test. This simple technique also enables to reveal mixtures of cultivars with and without *1B/1R* translocation.

In most cases classification of infection types is very easy. Classification of the reactions may be uncertain in the cultivar Košutka where the range is from intermediate to susceptible and both types of reactions often occur. Also the cultivars with *Sr 29,* (Hana, Mara, Vala, Slavia), and the cultivars Mironovská (808) having *Sr Tmp* can also display variable reactions to the stem rust isolates, and can be misclassified. It is therefore advisable to test samples of the registered cultivars simultaneously as controls with the samples analyzed.

However, considering only differentation into two groups, low quality wheats (1—4) and medium to high quality wheats (5—9), critical rust reactions are stable and easily distinguishable. The same is true of most of the cultivars of high baking quality (7,9), namely Hana, Mironovská, Mara, Odra, Viginta. Discrimination of the cultivars Zdar, Slavia, Vala, Košutka and Regina needs some experience and has to be confirmed sometimes by tests with more various rust isolates.

Reactions to 5 leaf and 3 stem rust isolates (Tab. II) show the possibilities to confirm cultivar identification by tests with other suitable isolates. The suggested isolates can be also replaced by other suitable rust isolates that discriminate the genes summarized in Tab. I.

The collection of leaf and stem rust isolates is kept at the Research Institute of Crop Production, Prague-Ruzyně and samples (except isolates 1943 and 1938 not available at present) will be supplied on request. Samples can be stored for several months in refrigerator at 5—8 °C or for many years (over 10) in liquid nitrogen.

Seed samples treated with systemic fungicides effective against rust cannot be analyzed by the method described above.

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BARTOŠ, P. — STUCHLÍKOVÁ, E. — HANUŠOVÁ, R. (Výzkumný ústav rostlinné výroby, Praha - Ruzyně): *Rozlišení odrůd pšenice podle jejich reakce ke rzím*. Genet. a Šlecht., *25*, 1989 (3) : 239-244.

Odrůdy pšenice ozimé povolené v současné době v Československu je možné rozdělit do několika skupin podle reakce ve fázi 1-3 listů k vybraným izolátům rzi pšeničné a rzi travní. K odlišení odrůd s translokací *1B/1R* s nízkou potravinářskou kvalitou postačí test jedním izolátem rzi pšeničné. Odrůda Branka jako jediná z povolených odrůd, majících translokaci *1B/1R* a střední kvalitu, může být odlišena od všech ostatních odrůd s uvedenou translokací testem jiným izolátem rzi pšeničné.

Triticum aestivum L.; rez pšeničná; rez travní; *1B/1R* translokace; potravinářská kvalita

БАРТОШ, П. — СТУХЛИКОВА, Е. — ГАНУШОВА, Р. (Научно-исследовательский институт растениеводства, Прага-Рузыне): **Различия сортов пшеницы согласно их реакции к ржавчинам**. Genet. a Šlecht., *25*, 1989 (3) : 239-244.

Сорта озимой пшеницы апробированные в настоящее время в Чехословакии можно разделить на несколько групп согласно реакции в фазе 1—3 листов к выбранным изолятам бурой и стеблевой ржавчины. Для различия сортов с транслокацией *1B/1R* с низким пищепромышленным качеством достаточен тест одним изолятом бурой ржавчины. Сорт Бранка, как единственный из апробированных сортов с транслокацией *1B/1R* и средним качеством, может различаться от всех остальных сортов с указанной транслокацией тестом иным изолятом бурой ржавчины.

Triticum aestivum L.; бурая ржавчина; *1B/1R* транслокации; пищепромышленное качество

BARTOŠ, P. — STUCHLÍKOVÁ, E. HANUŠOVÁ, R. (Forschungsinstitut für Pflanzenproduktion, Praha-Ruzyně): *Differenzierung von Weizensorten anhand ihrer Reaktion gegenüber Rostkrankheiten*. Genet. a Šlecht., *25*, 1989 (3) : 239-244.

Die z. Z. in der ČSSR zugelassenen Weizensorten können ihrer Reaktion in der Phase von 1—3 Blättern gegenüber ausgewählten Isolaten des Braun- und Schwarzrostes nach, in mehrere Gruppen eingeteilt werden. Zum Auseinanderhalten von Sor-

ten mit der Translokation *1B/1R* mit niedrigem lebensmitteltechnologischem Wert genügt der Test mittels eines Braunrostisolats. Die Sorte Branka, als einzige unter den zugelassenen Sorten, die mit der Translokation *1B/1R* und mittelmäßiger Qualität, kann von allen übrigen Sorten mit der angeführten Translokation durch einen Test mit einem anderen Braunrostisolat auseinandergehalten werden.

Triticum aestivum L.; Braunrost; Schwarzrost; Translokation *1B/1R;* lebensmitteltechnologische Qualität

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VARIABILITY OF NITRATE CONCENTRATION IN FORAGE OF LUCERNE AND RED CLOVER

O. Chloupek

CHLOUPEK, O. (OSEVA-Plant Breeding Station, Želešice): *Variability of Nitrate Concentration in Forage of Lucerne and Red Clover.* Genet. a Šlecht., *25*, 1989 (3) : 245-250.

Concentration of nitrates in the herbage of lucerne and red clover was investigated in several localities, varieties and years. It was influenced first of all by the age of plants (younger plants had mostly higher concentration), by the site of growing and by mutual interaction. Percentage of non-genetic variability of the total variability was predominant (95—99 %). The influence of the varieties tested was significant in lucerne and in tetraploid red clover even when their percentage of the total variability was low (up to 2 %). Tetraploid varieties of the clover were characterized by higher concentration than the diploid ones. Breeding of new varieties at plant breeding stations where all varieties tested had a higher nitrate concentration (probably caused by higher N-fertilization), resulted in a higher nitrate concentration of the new varieties bred there. We recommend therefore lower N-fertilization at the plant breeding stations where the new varieties are produced.

nitrates; forage; lucerne; red clover; genetic variability; breeding

Lucerne is grown in Czechoslovakia on about 330 thousand hectars and red clover on about 350 thousand ha. About 100 kg nitrogen in mineral fertilizers is used now on average per hectare, and therefore the concentration of nitrates in all plants, including lucerne and red clover, increases too.

The objective of this paper is to find whether plant breeding could lower the nitrate concentration in forage of lucerne and red clover.

MATERIALS AND METHODS

Nitrate concentration in the herbage of lucerne (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) was evaluated in experiments testing new varieties in Czechoslovakia in recent years. Lucerne harvested in 1985 was grown at five sites in three- or four year old stands and harvested three times each year. Fifteen new varieties and a control variety were evaluated. In the next year (1986) lucerne was grown at six sites in the sowing year and was harvested twice at three sites and once in other three sites. Sixteen new varieties and the control variety were tested.

The extent of experiments with clover is given in the Tab. I. Because of different numbers of cuts in experiments with clover in individual harvest years, average concentration from 1—3 cuts in individual years was used for evaluation.

Concentration of nitrate nitrogen (NO_3^-—N) was determined, using ion-selective liquid electrode.

I. Survey of experiments with red clover

Exp. No.	Plants	Number of varieties		Number of sites	Year of	
		new	controls		sowing	harvest
1	2*n*	8	3	3	1984	1985, 1986
2	2*n*	12	3	5	1985	1985, 1986
3	4*n*	7	2	2	1984	1985, 1986
4	4*n*	3	2	5	1985	1985, 1986

RESULTS

Analysis of variance (Tab. II) shows significant mean squares (MS) of all evaluated factors (age of plants, site of growing, cuts, varieties) and also of all (with one exception) interactions of the second and third order of these factors. The greatest MS was found for cuts as its percentage of the total MS amounted 34.1 %. Nitrate concentration was significantly different between all three cuts and reached in the first cut 0.187 %, in the second one 0.064 % and in the third one 0.043 % NO_3^--N. It was therefore nearly three times higher in the first cut than in the second and more than four times higher in comparison with the third cut.

The second significant factor was the age of plants the MS of which amounted to 27.3 % of the total MS. While the concentration was 0.138 % in three-year-old plants, it was significantly lower in four-year-old plants (0.058 %). The third and fourth factors participating in the total MS were sites (11.3 %) and the interaction of second order of plants age × cuts (also 11.3 % of the total MS). The highest concentration was found at the site Čejč (0.172 %), then followed Bučany (0.139 %), Borovce (0.094 %), Želešice (0.048 %) and the last Libochovice (0.037 %). All differences between the sites were highly significant.

II. Variance analysis for concentration of nitrate nitrogen (. 10^3) in dry matter of lucerne (%)

Source of variability	*dF*	*MS*	Source of variability	*dF*	*MS*
a) age of plants	1	772 683^{++}	b × d	60	565^{+}
b) sites of growing	4	318 792^{++}	c × d	30	1 131^{++}
c) cuts	2	964 827^{++}	a × b × c	8	109 424^{++}
d) varieties	15	1 195^{++}	a × b × d	60	445
a × b	4	101 946^{++}	a × c × d	30	688^{+}
a × c	2	319 887^{++}	b × c × d	120	546^{+}
a × d	15	737^{+}	error	120	366
b × c	8	237 060^{++}			

$^{+}$ $P < 0.05$ $^{++}$ $P < 0.01$

Interaction of the second order of plants age with cuts influenced the total MS at a level of 11.3 % as can be seen from the following comparison (% NO_3^--N):

	3 year-old plants	4 years-old plants
1st cut	0.278	0.096
2nd cut	0.086	0.042
3rd cut	0.050	0.036

The most important factor influencing concentration in the experiment can be seen in the above comparison — it was the age of plants. The younger the plants the higher was the percentage of nitrates.

The total percentage of non-genetic factors, including interactions, amounted to more than 99.9 % of the total MS observed in NO_3^--N concentration. MS for the tested varieties was also significant, but their proportion of the total MS amounted to less than 0.0005 %. All interactions of the varieties tested with non-genetic factors were lower than 0.0005 %, too. Nine varieties (eight developed at Želešice and one at Libochovice) had significantly lower concentration (0.085 — 0.097 %) than other seven varieties (0.100 — 0.108 %) which were bred at Borovce (3 of them), Bučany (3) and Libochovice (1). The significant interaction of varieties with sites is very interesting in this comparison. As stated above, forage from Borovce and Bučany contained more nitrates than that from Želešice or Libochovice. The varieties developed at these stations on soils probably rich in nitrogen contained more nitrates. Substantially lower concentration of nitrates was found in forage of all the varieties tested at Želešice and Libochovice, and therefore new varieties developed there (especially in Želešice) contained significantly lower concentrations of nitrates. This interaction of varieties with sites gives evidence of great importance of ecology, especially of growing technology for plant breading.

Similar results were also found in the second experiment with other lucerne varieties in the sowing year (1986). Average concentration in the whole experiment was 0.128 % NO_3^--N with significant differences between sites, cuts and interactions but significant differences were not found between varieties. The MS for varieties was less than 0.09 % of the total MS in experiments cut twice and less than 0.34 % in experiments cut only once.

In clover the average concentration in the sowing year was 0.110 % NO_3^--N, in the second year 0.130 % and in the third one 0.082 % and was therefore lower than in lucerne. Variability of the concentration was controlled first all by non-genetic factors, primarily by the age of plants (% NO_3^--N):

	1st year	2nd year	3rd year
2n	0.110	0.130	0.082
4n	0.110	0.136	0.097

Tetraploid varieties contained more nitrates than the diploid ones. The concentration was also influenced by sites and by the interaction of age of plants × sites (Tab. III). Varieties were significantly different

III. Variance analysis for concentration of nitrate nitrogen (. 10^3) in dry matter of red clover (%)

	dF	Sowing 1984		Sowing 1985	
		2*n*	4*n*	2*n*	4*n*
a) age of plants	1	21 025^{++}	40 334^{++}	14 386^{++}	54
b) sites of growing	1—4	1 946^{++}	4 074^{++}	3 164^{++}	2864^{++}
c) varieties	4—14	162	83	61	151^{+}
a × b	1—4	2 175^{++}	4 467^{++}	6 366^{++}	2636^{++}
a × c	4—14	160	77	159	158^{+}
b × c	8—56	120	45	179	49
error	8—56	134	43	121	39

$^{+}$ $P < 0.05$ $^{++}$ $P < 0.01$

only in one experiment with tetraploid varieties. In the given case a significant interaction of varieties with age of plants was found. The total proportion of non-genetic influences in the four experiments was 93.3 — 99.5 and percentage of MS for varieties 0.2 — 2.5 % of the total MS.

The influence of environment on the breeding process can be also seen in experiments with clover. In the first experiment with tetraploid varieties the concentration amounted to 0.154 % in new varieties, 0.128 % in controls, in the second experiment new varieties contained 0.112 %, controls 0.105 %. It can be assumed that the breeding of new varieties was carried out in conditions with a higher rate of nitrogen fertilization in comparison with the development of control varieties bred before 1970. During that period the fertilization rates were only about half the level used today.

DISCUSSION

Our results confirm the earlier ones (Smith, Sund, 1965) on higher concentration of nitrates in forage of lucerne in comparison with other leguminous fodder plants. We also confirm the findings about higher nitrate concentration in tetraploid varieties in comparison with the diploid ones (Míka, Našinec, 1974). Our results correspond with experiments in which predominant influence on non-genetic factors on nitrate concentration was found (e. g. in potatoes it was fertilization, the age of plants, etc., while the influence of varieties was small). The studies are complicated in forage plants since older plants can be harvested in the earlier stage of development and on the contrary — — younger plants in a later stage of development.

Concentration of NO_3^--N in forage higher than 1.4 g/kg (i. e. 0.140 %) of dry matter can not be profitable (Kemp et al., 1978). In our experiments this was found in lucerne at the site Čejč but it was not found clover, even though in forage of tetraploid clover from Hladké Životice it was 0.138 %. However, harmful concentration is 0.250 % (Míka, 1985).

Significant variation in nitrate reductase activity (NRA) was found within, but not among 6 *Medicago sativa* populations. A quantitative genetic study showed that general combining ability effects for NRA accounted for 86 % of mean squares among crosses. High and low NRA populations were developed by selection (Nelson, 1986).

Other possibility to lower the nitrate concentration in forage of lucerne is breeding for higher symbiotic nitrogen fixation which lowered it significantly (Chloupek et al., 1989).

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CHLOUPEK, O. (OSEVA-Šlechtitelská stanice, Želešice): *Variabilita koncentrace nitrátů v píci vojtěšky a jetele lučního.* Genet. a Šlecht., *25*, 1989 (3) : 245-250

Koncentrace nitrátů v píci vojtěšky a jetele lučního byla sledována na více lokalitách, odrůdách a letech. Byla především ovlivněna stářím rostlin (mladší rostliny měly většinou vyšší koncentraci), místem pěstování a vzájemnou interakcí těchto vlivů. Podíl negenetické proměnlivosti na celkové proměnlivosti byl převažující (95 — 99 %). Vliv sledovaných odrůd byl významný u vojtěšky a u tetraploidního jetele lučního, i když jejich podíl na celkové proměnlivosti byl malý (do 2 %). Tetraploidní odrůdy jetele měly vyšší koncentraci než diploidní odrůdy. Šlechtění nových odrůd na šlechtitelských stanicích, kde všechny sledované odrůdy měly vyšší koncentraci nitrátů (pravděpodobně v důsledku vyššího dusíkatého hnojení v osevním postupu), se projevilo ve vyšší koncentraci nitrátů ve zde vyšlechtěných odrůdách. Doporučujeme proto nižší dusíkaté hnojení na šlechtitelských stanicích, kde se šlechtí vojtěška a jetele.

nitráty; píce; vojtěška; jetel luční; genetická variabilita; šlechtění

ХЛОУПЕК, О. (ОСЕВА — Селекционная станция, Желешице): **Изменчивость концентрации нитратов в фураже люцерны и красного клевера.** Genet. a Šlecht., *25*, 1989 (3) : 245-250.

Эту концентрацию определяли на многих местах и сортах, в разные годы. Концентрация дана, главное, возрастом растений (у молодых она выше), местом выращивания и взаимодействием этих влияний. Доля негенетической изменчивости в общей изменчивости преобладает (95—99 %). Влияние изучаемых сортов значимое и у люцерны, и у тетраплоидного клевера, хотя их участие в общей изменчивости слабое (до 2 %). В тетраплоидных сортах клевера концентрация больше, чем в диплоидных. Селекция

на станциях, где все прослеживаемые сорта содержали много нитратов (вероятно, из-за повышенных азотных норм в севообороте), проявилась в росте концентрации нитратов в выведенных здесь сортах. Поэтому рекомендуем понижать нормы азотного удобрения на тех станциях, где селекционируются клевер и люцерна.

нитраты; фураж; люцерна; красный клевер; генетическая изменчивость; селекция

CHLOUPEK, O. (OSEVA-Züchtungsstation, Želešice): *Variabilität der Konzentration von Nitraten im Luzerne- und Rotkleefutter.* Genet. a Šlecht., *25*, 1989 (3) : 245-250.

Die Konzentration der Nitrate im Luzerne- und Rotkleefutter wurde auf mehreren Lokalitäten, in mehreren Sorten und Jahren untersucht. Sie wurde vor allem vom Alter der Pflanzen (jüngere Pflanzen wiesen überwiegend eine höhere Konzentration auf), vom Anbauort als auch von der Wechselwirkung dieser Einflüsse beeinflusst. Der Anteil der nichtgenetischen Veränderlichkeit an der Gesamtveränderlichkeit war überwiegend (95 — 99 %). Der Einfluss der untersuchten Sorten war bei der Luzerne und dem tetraploiden Rotklee bedeutend, obwohl ihr Anteil an der Gesamtveränderlichkeit nur gering war (bis 2 %). Die tetraploiden Kleesorten wiesen eine höhere Konzentration als die diploiden Sorten auf. Die Züchtung neuer Sorten auf Züchtungsstationen, wo alle untersuchten Sorten eine höhere Konzentration von Nitraten (wahrscheinlich infolge einer höheren N-Düngung in der Fruchtfolge) aufwiesen, fand in einer höheren Konzentration von Nitraten in den hier herausgezüchteten Sorten ihren Niederschlag. Wir empfehlen deshalb eine niedrigere N-Düngung auf den Züchtungsstationen, wo die Luzerne und der Wiesenklee gezüchtet werden.

Nitrate; Futter; Luzerne; Rotklee; genetische Variabilität; Züchtung

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OPTIMUM CONDITIONS FOR *IN VITRO* CULTIVATION OF LUCERNE SEEDS

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RAUSCHEROVÁ, L. — HOFBAUER, J. (Institute of Systematical and Ecological Biology, Czechoslovak Academy of Sciences, Brno; Research and Breeding Institute for Fodder Plants, Troubsko): *Optimum Conditions for in vitro Cultivation of Lucerne Seeds.* Genet. a Šlecht., *25*, 1989 (3) : 251-256

The possibilities of *in vitro* cultivation of lucerne seeds were tested using material originating both from field conditions and from growth chamber. After pollination, excised flowers with stems of the minimum length of 5 cm were cultivated in 25ml flasks containing nutrient solutions. Cultivation was carried out under controlled conditions using growing chambers (16 klux, temperature 26/16 °C and 16 h day). Analyses of nutrient media for the cultivation of lucerne seeds showed that solutions containing only mineral substances were quite insufficient; on the other hand, solutions containing 2 % of sugars (glucose, fructose and saccharose) were very suitable. Mixtures of lower sugars showed no markedly higher effect. Two-per-cent solution of honey which was used to replace the mixture of sugars was also insufficient. The decomposition was not prevented by the supplement of 6-hydroxiquinoline citrate (0.6 %) to the medium. On the contrary, the dipping of stems in a solution of $AgNO_3$ (1000 ppm) for 30 s to 1 min proved suitable for further cultivation. It is possible to conclude that this *in vitro* method can be used in breeding practice.

Medicago sativa L.; *in vitro* cultivation; solutions; flowers

The use of the method of *in vitro* cultivation of lucerne seeds is very promising above all for breeding practice expecially when producing the seed material. In this way it is possible to obtain seed material on a relatively small area even under unfavourable conditions or outside the growing season. Laboratory conditions (or controlled conditions of growing chambers) eliminate at the same time unfavourable factors during the period of pod formation and seed ripening.

The *in vitro* method has been used, and also patented, to cultivate ears of cereals (Smoček, Psotová, 1981). It was tested also in other fodder crops, above all in genotypes of grasses (Fojtík, et al., 1987) and clovers (Battle, 1949).

For lucerne this method has not been elaborated yet, and it should be said that more frequently used is the method of tissue culture for the cultivation of unripe embryos. However, the laboriousness of this method as well as the necessity to use sterile conditions limit a little its wider use (Wang et al., 1984). The same also applies to the method described by Li et al. (1986). For that reason we have tried to cultivate isolated flowers of lucerne under laboratory conditions.

MATERIALS AND METHODS

Experiments were carried out under controlled conditions in a growing chamber at the temperature 26 °C/16 °C; the length of the day was 16 h and the intensity of illumination 16 klux. Samples of the material were taken either in field experiments or from plants, grown as water cultures in the growing chamber (Rauscherová, Hofbauer 1983).

In the period of flowering, racemes with a stem at least 5 cm long were separated from plants and transferred into the nutrient solution in flasks with the volume of 25 ml. Each treatment involved 20 racemes and was repeated three times. Flowers were pollinated by hand; if the lower flowers were already opened, they were removed.

The following characteristics were evaluated the number of young pods (on the 6th day), numer of ripening pods (on the 14th day), number of persisting flowers which failed to form pods, and the number of shed flowers. Using these data, percentages of shedding and fertility (i. e. of produced and ripened pods) were calculated, in all cases, for the total number of flowers per raceme.

RESULTS AND DISCUSSION

The composition of nutrient solutions is one of the cardinal problems of the use of *in vitro* methods. These media are based either predominantly on mineral substances or on relatively high concentrations of sugar (Góral, Spiss, 1982). The aim of this study was to find the most suitable composition of nutrient solution for the cultivation of lucerne seeds.

We tested media based on macroelements (i.e. Hoagland's and Richter's nutrient solution) in normal and doubled concentrations and on individual microelements and their mixtures (according to Hoagland) in two-fold to five-fold concentrations. Concentrations of sugars were tested within the range from 1 to 6 % either individually or in a mixture; a 2-per-cent solution of honey was also used.

We tested also some recommended media (Demarly, Chezneaux et al., 1964; Góral, Spiss, 1982; Wang et al., 1984) and our modifications, which were based on different concentrations of sugars mixed with Hoagland's nutrient solution of macroelements and some microelements (mainly boron and zinc) in different concentrations up to 1 mg/l. The potato extract (starch) and 2-per-cent honey solution with the admixture of 0.6 % 6-hydroxiquinoline citrate were tested as well. To reduce the infection of stem bases a solution of $AgNO_3$ was used in concentrations of 100 to 1,000 mg/l. Stem bases were dipped in this solution for 30 s to 10 min or it was added as one drop into the medium (in 100 ppm concentrations). Some minerals showed no effect; this is true, above all, of the different concentrations (1 to 5 mg/l) of Ca and K, which we expected to be associated with the shedding of generative organs.

Owing to the great extent of experiments only some results are presented. In Tabs. I and II, individual nutrient solutions are compared, which were used for the cultivation of seeds using the *in vitro* method. Percentages of pods produced from the total number of flowers within the 6th to 14th day after the pollination of clones, cultivated in garden, are given in Tab. I, and those of the cultivar Palava cultivated in water culture under controlled conditions in a growing chamber are given in Tab. II.

I. Average percentages of pods produced (field conditions)

Solution	Concentration	Material-clone No. 7	
		6th day % of pods	14th day % of pods
Water	—	3.1	2.1
Richter	1 ×	3.9	2.3
Demarly	1 ×	4.0	2.0
Glucose	1 %	29.1	21.5
Glucose	2 %	44.0	28.0
Glucose	4 %	26.4	29.7
Saccharose	1 %	18.1	11.8
Saccharose	3 %	31.6	20.2
Fructose	1 %	33.7	18.2
Honey	2 %	31.6	—
Microelements	1 ppm		
B + Mo + Mn + Zn	0.1 ppm Mo	8.7	6.8

II. Average percentages of pods produced (growth chamber)

Solution	Concentration	cv. Palava	
		6th day % of pods	14th day % of pods
Water	—	2.8	4.5
Hoagland	1 ×	3.7	3.9
Glucose	1 %	52.8	45.8
Glucose	2 %	52.1	46.3
Glucose	4 %	26.4	29.7
Saccharose	2 %	31.6	38.3
Fructose	1 %	54.1	42.0
Glu + starch	2 % + 2 %	14.3	—
Glu + B	2 % + 1 ppm	10.2	49.7
Glu + Zn	2 % + 1 ppm	27.4	27.9
Glu + Hoagl.	2.5 % + 2.5 %	23.9	27.0
Glu + Hoa + B	2 % + 1 % + 1 ppm	9.0	12.5
Glu + Hoa + Zn	2 % + 1 % + 1 ppm	12.7	11.5
Honey	2 %	21.5	—
Honey + $AgNO_3$.	2 % + 100 ppm	19.1	—
Honey + $AgNO_3$	2 % + 15 m	38.6	—
Honey + HQC + + $AgNO_3$	2 % + 100 ppm	3.7	—
Honey + B	2 % + 1 ppm	1.0	—
Starch	2 %	—	—

Data presented in Tabs. I, II demonstrate that solutions containing only mineral substances are quite insufficient for the cultivation of racemes. On the other hand, 2-per-cent solutions of sugars were suitable; they were satisfactory even under conditions of reduced light intensity (Hofbauer et al., 1985). High concentrations (6 % of sugars: Góral, Spiss, 1981) were also unsuitable.

Similarly, treatment with a mixture of sugars in different ratios showed no marked effect on the number of pods produced. Although a 2-per-cent solution of honey as a replacer for the mixture of sugars was sufficient for the nutrition, it is necessary to say that a decomposition of this solution took place during the cultivation associated with necroses of stem bases and with infections; the addition of $AgNO_3$ or 6-hydroxiquinoline citrate to these solutions did not prevent a blockade of conductive bundles as was the case when these compounds were used to prolong the viability of cut flowers. Chemicals, which are used to prolong the durability of cut flowers, delay the closure of vascular bundles in stems or control the transpiration, and for that reason they are not suitable for *in vitro* cultivation of lucerne flowers. Their application resulted not only in a prolongation of the time of flowering (by 3 days) but also in a decrease in the number of pods produced. The expected effect, i. e. preventing of shedding, was manifested in flowers which remained in racemes after getting dry; they did not develop into pods. Dipping of the bases in $AgNO_3$ solution for 30 s to 1 min (concentrations of 1000 ppm and 100 ppm, respectively) seemed to be more suitable. The medium recommended for white clover (Góral, Spiss, 1982) was not suitable in our experiments even after modification. Similarly, a starch solution (Wang et al., 1984) gave also unsatisfactory results; it was quite insufficient for nutrition, and decomposed rapidly during the experiment.

Sugar solutions remain a very suitable source of energy for metabolic processes in plants. They may be also combined with growth regulators which can show a positive effect not only on the course of pollination and pod formation but also on the subsequent development of seeds. Effect of the application of these substances will be studied in our further experiments.

The method of *in vitro* cultivation of lucerne may be used above all in the breeding practice to obtain seed material. A great advantage is the possibility of rapid cultivation of seeds under laboratory conditions when it is not necessary to cultivate whole plants. This method seems to be suitable also for studies of causes of the shedding of generative organs although the situation in isolated racemes is considerably different from that of whole plant.

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Použití metody *in vitro* u vojtěšky umožňuje rychlé dopěstování a získání semen v laboratorních podmínkách. Předložená práce shrnuje výsledky počtu nasazených a dozrávajících lusků (v přepočtu na celkový počet květů v hroznu). Množství nasazených lusků slouží pro srovnání vhodnosti živných roztoků (médií) při dopěstování semen vojtěšky. Dopěstování semen bylo ověřeno na materiálu z polních podmínek i řízených podmínek fototronu. Odříznutá květenství s květní stopkou o délce nejméně 5 cm byla po opylení dále kultivována v živných roztocích v klimatizovaných komorách při 16 klux, teplotě 26/16 °C a 16hodinové délce dne v 25ml baňkách. Zhodnocení vhodnosti médií pro dopěstování semen vojtěšky prokázalo, že roztoky složené pouze z minerálních látek jsou nevhodné a pro dopěstování květů zcela nedostatečné, vhodné jsou naopak 2% roztoky cukrů. Směsi jednotlivých cukrů neměly výrazně vyšší efekt. K zastavení mikrobiální činnosti bylo vhodné krátkodobé (30 sekund až 1 minuta) máčení stopek v roztoku $AgNO_3$ (1000 ppm). Využití metody *in vitro* lze očekávat ve šlechtitelské praxi.

Medicago sativa L.; kultivace *in vitro;* roztoky; květy

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Использование метода *in vitro* у люцерны дает возможность быстрого доращивания и получения семян в лабораторных условиях. Авторы предложенной работы подытоживают результаты числа завязанных и созревающих бобов (в пересчете на общее число цветков в кисти). Количество завязанных бобов служит сравнению пригодности питательных растворов (сред) при доращивании семян люцерны. Доращивание семян проверялось на материале, происходящем из полевых условий и фититрона с кондиционированием воздуха. Срезанные соцветия с цветочным черешком длиной меньше всего 5 см после опыления культивировали в питательных растворах в кондиционированной камере при 16 клк, температуре 26/16 °C и при 16 часовой длине в 25 мл-колбах. Оценка пригодности сред длины доращивания семян люцерны подтвердила, что растворы, состоящие только из минеральных веществ, являются непригорными и для доращивания цветов вполне недостаточными, пригодными, наоборот, являются растворы сахаров в 2%-ной концентрации. Смеси отдельных сахаров не оказали заметно высшего эффекта. Для прекращения микробиальной деятельности оказалось пригодным краткосрочное (30 сек — 1 мин) замачивание черешков в растворе $AgNO_3$ (1000 ppm). Использование метода *in vitro* возможно ожидать в селекционной практике.

Medicago sativa L.; культивация *in vitro*; растворы; цветы

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Die Anwendung der *in vitro*-Methode ermöglicht bei der Luzerne ein rasches Aufziehen und die Gewinnung von Samen unter Laborbedingungen. Die vorliegende Arbeit fasst Ergebnisse der Zahl der angesetzten und reifenden Hülsen (in Umrichnung auf die Gesamtzahl der Blüten in der Traube) zusammen. Die Zahl der angesetzten Hülsen dient als Masstab beim Vergleich der Geeignetheit einzelner Nährlösungen (Medien) beim Laborbau der Luzernesamen. Der Laborbau des Samen wurde an Material sowohl aus Feld- als auch aus gesteuerten Phytotronbedingungen überprüft. Abgeschnittene Blätenstände mit Blütenstiel von mindestens 5 cm Länge wurden nach der Bestäubung in Nährlösungen in klimatisierten Kammern bei 16 klux, einer Temperatur von 26/16 ° C und 16stündiger Tageslänge in 25-ml-Kolben weiter kultiviert. Eine Beurteilung der Geeignetheit einzelner Medien für die Kultivierung der Luzernesamen ergab, daß ausschließlich aus mineralischen Substanzen zusammengesetzte Lösungen ungeeignet und für das Aufziehen von Blüten völlig unzureichend sind, während sich dagegen 2 %ige Zuckerlösungen als gut geeignet erwiesen. Mischungen einzelner Zucker wiesen keinen markant höheren Effekt auf. Zur Einstellung der mikrobiellen Tätigkeit erwies sich ein kurzes (30 Sekunden bis 1 Minute) Eintauchen der Stiele in eine $AgNO_3$-Lösung (1000 ppm) als angebracht. Ein Einsatz der *in vitro*-Methode ist in der züchterischen Praxis zu erwarten.

Medicago sativa L.; Kultivation *in vitro;* Lösungen; Blüten

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GENOTYPE DIFFERENCES IN ECONOMICALLY IMPORTANT TRAITS OF TWO- AND SIX-ROW WINTER BARLEY

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Two years experiments performed in Kroměříž in 1987—1988 showed that yield potential of the six- and two-row cultivars and new-bred lines of winter barley are at about the same level. Erfa represented the most productive six-row cultivar, Flamenco was the best one among the two-row cultivars. No considerable difference was found between the six- and two-row cultivars even in other economically important traits, such as winterhardiness or resistance to lodging and diseases. The two-row barleys were superior in the thousand grain weight (by 10 g) and in proportion of grain above 2.5 mm mesh sieve (by 20 %). They do not meet, however, the present demands of malting industry in Czechoslovakia, owing to their protein content above 11 %, and extract content below 80 %. The line KM 948, however, proved superior to the most productive cultivar Flamenco, and — with respect to the parameters of malting quality — to Kaskade cultivar. Further aspects of utilizing the 2-row winter barley are discussed.

winter barley; yield; yield components; quality

Winter barley attracted the attention of Czechoslovak agricultural practice in 1976—1988 for economic, agronomic and organizational reasons. Six-row winter cultivars are the only winter barleys to be grown on a large scale at present. Two-row winter barley of both fodder and malting types can be expected to spread wider in the future. These tendencies are marked in the countries of West Europe (Schildbach, 1986a).

The aim of the presented contributions is to compare the results concerning the yield potential of six- and two-row winter barleys and the malting parametrs of two-row spring and winter barley, obtained in experiments performed in 1987—1988 in Kroměříž.

MATERIALS AND METHODS

The genotype differences were evaluated in performance tests which were established in Kroměříž with 4 replications in 1987 and 1988. Seeds were sown within the agronomic term on September 25, 1986 and September 28, 1987. The fertilizer was applied before sowing at rates of 54 kg N, 72 kg P_2O_5, 100 kg K_2O.

The following cultivars were tested in both years: Alraune, Kaskade, Flamenco (two-row), Erfa and Borwina (six-row). The new-bred lines of two-row barley were

also tested — KM 60, KM 61 in 1987, KM 948 and KM 1055 in 1988. KM 60, KM 61 were excluded from testing in 1988 for poor results in interstational trials.

The origin of the new breeding lines:

KM 60 — Erfa × Br 8
KM 61 — Kaskade × Co 80 201
KM 948 — Co 80 201 × Pan
KM 1055 — Co 80 201 × Dorad

The sowing rate was the same for all genotypes, i. e. 4 mil. germinable seeds. All genotypes were treated with Baytan Universal at a rate of 150 g per 100 kg of seed.

The protein and extract contents of two-row cultivars and new-bred lines were determined by standard methods at the Cereal Research and Breeding Institute, Kroměříž. Malting parameters were compared, using the Rubín and Zenit cultivars, which were grown in these years as standards in the spring barley performance tests after sugar beet as forecrop. The course of weather is given in Fig. 1, in comparison with long-term means.

RESULTS

As follows from Tab. I, the yield potentials of the two- and six-row winter barleys are about the same. Erfa was the most productive cultivar on an average of the two years. As concerns the two-row cultivars, the yields of cv. Flamenco were close to those of cv. Erfa. No statistically significant yield differences were recorded between the cultivars. The number of fertile tillers and the thousand grain weight showed the most pronounced differences within the yield components. While Erfa had 608 ears on an average of two years cv. Flamenci produced 812 ears. The difference in the thousand grain weight between six- and two-row barleys was 9.2 g in 1987 and 11.0 in 1988; this was

I. Comparison of yield components in six- and two-row winter barleys in performance tests, Kroměříž 1987—1988

Cultivar	1987					1988				
	Yield		Number of fert. stems	1000 grain weight	Grain above 2.5 mm sieve	Yield		Number of fert. stems	1000 grain weight	Grain above 2.5 mm sieve
	t/ha	%				t/ha	%			
Erfa	8.23		592	43.2	81.2	8.16		624	38.9	72.8
Borwina	7.72		576	42.2	79.2	7.87		668	41.4	82.8
$\bar{x}$	7.97	100.0	584	42.6	80.2	8.01	100.0	646	40.1	77.8
Alraune	7.88	98.8	880	52.6	91.1	8.19	102.1	736	49.1	92.5
Kaskade	7.36	92.2	712	46.3	91.3	7.18	89.5	752	47.7	92.8
Flamenco	7.88	99.8	872	53.8	91.6	8.23	102.6	744	53.5	94.0
KM 60	7.35	98.9	912	57.1	95.4					
KM 61	7.25	90.9	848	48.1	84.6					
KM 948						8.22	102.5	712	55.3	96.8
KM 1055						7.77	96.9	752	50.0	94.0
$\bar{x}$	7.54	94.6	845	51.8	90.4	7.91	98.8	740	51.1	94.0

II. Comparison of resistance to lodging and diseases in six- and two-row winter barleys

Cultivar	1987						1988					
	Stem height	Winter-hardiness	Resistance to				Stem height	Winter-hardiness	Resistance to			
			lodging	powdery mildew	rust	*Pyrenophora teres*			lodging	powdery mildew	rust	*Pyrenophora teres*
Erfa	104	8	8	8	7	9	103	9	3	5	7	8
Borwina	102	9	8	7	9	9	99	9	2	6	7	7
Alraune	100	9	8	7	9	9	99	9	9	5	7	8
Kaskade	101	8	7	6	9	9	100	9	7	5	8	8
Flamenco	110	9	6	7	9	9	106	9	9	4	8	8
KM 60	112	9	8	7	9	9						
KM 61	109	9	8	8	9	9						
KM 948							104	9	9	5	8	8
KM 1055							97	9	9	6	8	8

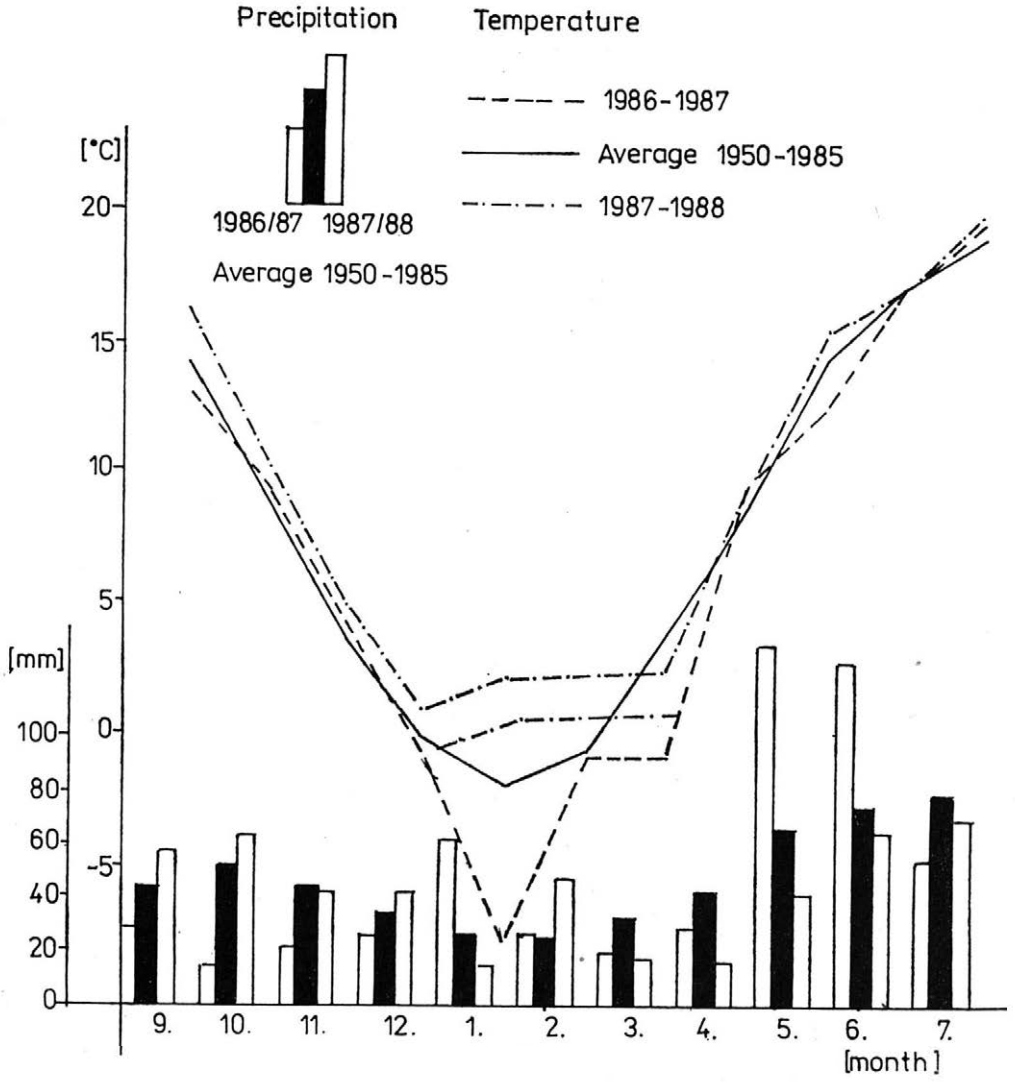
also associated with an increased proportion of grain above 2.5 mm in size, which was by 10—16 % better in the two-row cultivars.

Tab. II shows that results obtained in yield and yield parameters were conditioned by stabilizing yield factors. In both years there were no differences between six- and two-row genotypes in winterhardiness and resistance to lodging and diseases.

As follows from Fig. 1, winter temperature of both years were above the long-term mean, which enabled good overwintering especially for the two-row genotypes, which normally have a lower interhardiness. Lodging resistance was better in the two-row barleys than in the six-row

III. Basic parameters of malting quality in two-row winter barleys

Cultivar	1987				1988			
	Protein content	Starch content	Extract content	Relat. extract 45 °C	Protein content	Starch content	Extract content	Relat. extract 45 °C
Alraune	11.1	61.1	78.0	31.1	12.2	61.8	78.8	49.4
Kaskade	12.1	62.4	80.4	37.1	13.2	62.2	78.2	42.7
KM 60	12.7	61.0	78.2	38.3				
KM 61	11.2	60.7	78.1	35.2				
KM 948					12.0	60.3	79.6	42.6
KM 1055					12.5	60.7	78.9	41.9
Rubín	10.5	63.5	81.2	37.4	11.0	64.0	82.3	51.9
Zenit	11.3	62.4	77.4	36.2	11.7	62.2	81.0	42.5



1. Course of temperature and precipitation in Kroměříž 1986—1988 in comparison with long term average 1950—1985

ones in 1988. This was due to increased number of fertile tillers above the value of 600, which is optimal for the cultivars of "ear type", represented by Erfa and Borwina.

Tab. III gives the basic parameters of malting barleys. The results show that no genotype of the two-row winter barley can compare with the top-quality spring malting barley cultivar Rubín. The winter barleys tended to increased protein content and reduced content of starch which was manifested in reduced extract. Winter barley modification was worse: it showed a reduced proteolytic activity, as proved by the values of RE 45 °C. Some winter barleys, especially the Kaskade cultivar, had the same or even better level not only of this trait but also of starch and extract contents, in comparison to the medium-quality malting cultivar Zenit.

DISCUSSION

The area of winter barley in Czechoslovakia increased from 16,000 ha to 200,000 ha during the period 1976—1988. Although the mean yield of the six-row winter barley was higher by 0.5 t/ha in comparison with spring barley in this period, there are problems of the quality of production. Therefore, the possibility of growing two-row barleys is assessed in Czechoslovakia.

The results of growing two-row winter barleys are considerably variable in the European continent. For example, Wustrack (1988) assumes that the two-row winter barley will be grown in GDR only for malting purposes because the contemporary cultivars and new varieties have a yield potential representing 90—92 % of that of the six-row barleys. According to Demontis (1982), two-row winter barley has a higher yield potential in South Italy, six-row barley in the North, in fertile lowlands along the Po. The results of EBC (Anonym, 1987) show that in 1986 there was no yield difference between two- and six-row winter barleys in FRG, six-row barley was by 2 t/ha more productive in France, and two-row barley was better — on the contrary — by 0.2 t/ha in Hungary. Only two-row winter barley is grown in Great Britain. Wicke (1988) asserts that 25 % of malt came from the winter barley in EBC countries. The main disadvantages of the two-row winter barleys, concerning malting quality, are the reduced extract content (by 2—3 %) and increased protein content (by 1—2 %), in comparison to the quality spring malting barleys (Schildbach, 1986b). This was confirmed by our experiment, too.

Aufhammer (1984) and Hlavinková (1986), however, found in FRG and Czechoslovakia, respectively, that there were no significant differences between the Kaskade and Korál cultivars in selected areas. From the standpoint of brewing, the wintér barleys have undesirably increased viscosity of wort, which consequently has a negative effect on the time of straining, beer stability, colour, and durability of foam (Schildbach, 1986).

Jestin (1986), Narziss et al. (1987) proved that new lines, tested in France and FRG, were quite close to peak parameters of spring barley.

Lelong (1978), Vaculová (1988) found that there were no significant differences in feeding quality between winter and spring two-row barleys; however feeding quality can be expected to be higher, owing to the tendency to increased protein content.

Although the climatic and soil conditions of Central Europe are considerably different from the main growing regions of two-row winter barley, this crop deserves increased efforts of genetic-breeding researchers and breeding in Czechoslovakia.

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ŠPUNAR, J. — VACULOVÁ, K. — ZAVADIL, M. (OSEVA - Výzkumný a šlechtitelský ústav obilnářský, Kroměříž): *Genotypové rozdíly v hospodářsky důležitých znacích 2-řadého a 6-řadého ozimého ječmene.* Genet. a Šlecht., *25*, 1989 (3) : 257-262

Ve dvouletých pokusech provedených v Kroměříži v letech 1987 — 1988 bylo zjištěno, že výnosový potenciál 6-řadých a 2-řadých odrůd a novošlechtění ječmene ozimého se vyrovnává. Z 6-řadých byla nejvýnosnější odrůda Erfa, z 2-řadých odrůda Flamenco. Mezi 6-řadými a 2-řadými nebyl zjištěn významný rozdíl ani v dalších hospodářsky důležitých znacích jako zimovzdornost, odolnost k poléhání a chorobám. Dvouřadé ječmeny dosáhly o 10 g vyšší hmotnosti zrna a o 20 % vyššího podílu zrna na sítě 2,5 mm. Avšak obsahem bílkovin nad 11 % a obsahem extraktu pod 80 % nesplňují současné požadavky sladovnického průmyslu v ČSSR. Linie KM 948 však překonala nejvýhodnější odrůdu Flamenco a v parametrech sladovnické kvality odrůdu Kaskade. Jsou diskutovány další aspekty využití 2-řadých ječmenů ozimých.

ječmen ozimý; výnos; výnosové prvky; kvalita

ШПУНАР, Я. — ВАЦУЛОВА, К. — ЗАВАДИЛ, М. (ОСЕВА — Научно-исследовательский и селекционный институт зерновых культур, Кромержиж): **Генотипические различия по хозяйственно-ценным признакам двухрядного и шестирядного озимого ячменя.** Genet. a Šlecht., *25*, 1989 (3) : 257-262.

В двухлетних опытах, проводимых в г. Кромержиж в течение 1987—1988 гг. установлено, что потенциальная урожайность 6-рядных и 2-рядных сортов и перспективных линий озимого ячменя выравнивается. Среди 6-рядных сортов наиболее урожайным является сорт Erfa, среди 2-рядных Flamenco. Между 6-рядными и 2-рядными сортами не было определено резких различий ни по дальнейшим хозяйственно-ценным признакам как зимостойкость, устойчивость к полеганию и болезням. Двухрядные ячмени достигли повышенной массы зерна на 10 г и повышенной доли переднего зерна (2,5 мм) на 20 %. Однако содержание белка, превышающее 11 % и выход экстракта ниже 80 % не соответствуют современным требованиям пивоваренной промышленности в ЧССР. Линия КМ 948 превысила наиболее урожайный сорт Flamenco и по показателям пивоваренного ячменя сорт Kaskade. Обсуждаются дальнейшие вопросы использования 2-рядных озимых ячменей.

озимый ячмень; урожай; элементы структуры урожая; качество

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SUPPLEMENT

GENERAL METHOD OF MAINTENANCE BREEDING OF SYNTHETIC VARIETIES IN FODDER PLANTS

J. Rod, O. Chloupek

A synthetic variety is a population originating in crossing of components possessing combining ability. This population is propagated and maintained under conditions of random pollination in spatial isolation till a state of equilibrium is achieved (Rod, Vondráček, 1982).

Breeding a new synthetic variety consits in finding possible components, in testing their breeding value and combining ability, in crossing selected components in order to obtain a desirable population and in several generative reproductions reaching a state of equilibrium. This procedure takes adventage of the laws of population genetic and respects the fact the components are represented by heterozygous open-pollinated genotypes with the possibility of their vegetative propagation as clones.

The method of maintenance breeding must respect these genetic, technical and methodological assumption. It is possible to approach this task by means of several procedures, differing in the respective levels at which they take into account the genetic substance of the material, the technical and material requirements, and time factor involved.

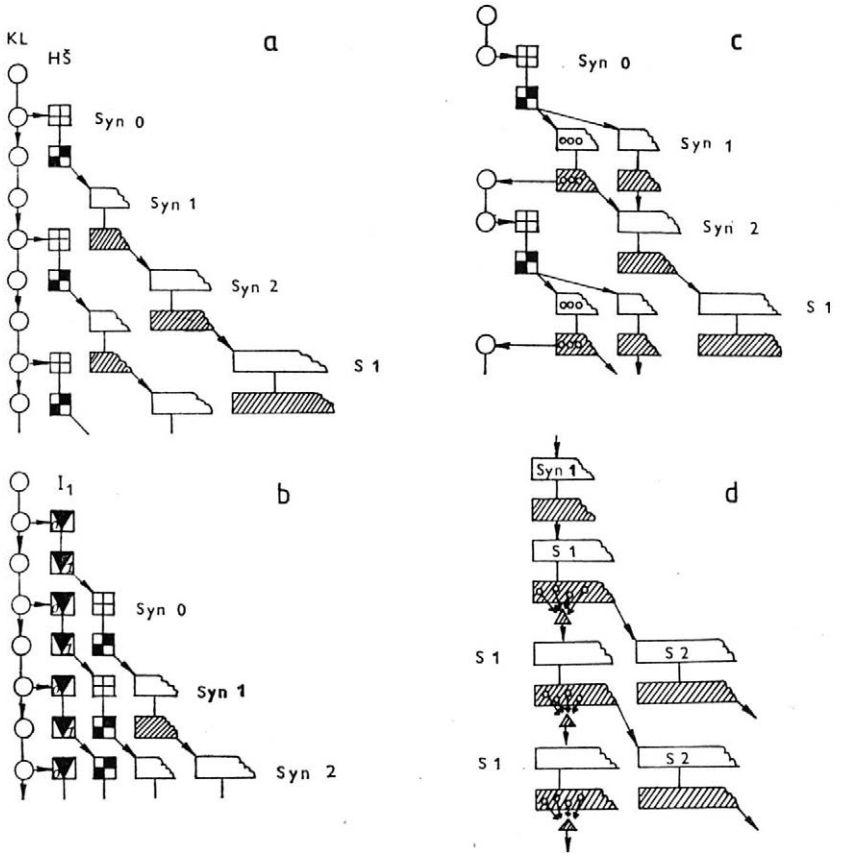
Maintenance breeding has to respect the principle that the basis of a real synthetic variety should be immutable, as well as the composition of components to be synthetized, and that it should guarantee a permanent and stable variety performance during its duration. Under these circumstances the question arises whether an immutable character of components, hence the stability of the future variety, can be attained, in other words how and for how long it can meet these demands. This task can be solved in several ways, differing mainly in their reqirement for labour and time (Rod, 1977; Chloupek, Rod, 1982).

In essence the solutions can be divided in two methodically different groups. The first one corresponds to current methodical practice and, with certain modifications, copies the procedure of breeding new varieties (group A). The second one is based on once and for all seed production, formation of a reserve, and its subsequent exploitation throughout the variety's existence (group B).

The first group (A) of solutions is based on the assumption of an immutable character of components and on the possibility of their preservation in a vegetative way. In view of the fact that this assumption is seldom met compromise solutions have been sought (Fig. 1).

A—a: Classic procedure, requiring an unchangeable and permanent character of components. A repeated vegetative reproduction of components is required which would guarantee an unchangeable character of the synthetis. This would build on the stability of the genetic base, i. e. predominantly on clones. How realistic this postulate is, depends mainly on the species, but also on varietal characters (fitness for clonal propagation, level of shooting and the like). Techniques of tissue cultures are supposed to be useful for this purpose in the future.

The appropriate procedure can be described as follows: Maintenance of clone nursery, i. e. components selected according to suitable comb-



1. Group A: a — Classic procedure, assuming on unchanging character of vegetatively propagated components; b — „Line" procedure, utilizing I_1 generation for building up of synthesis; c — Procedure based on a successive combination of two or more component into a synthetic population; d — Labour-saving procedure without preservation of componets, utilizing the resulting population and its maintenance by means of mass selection

ining ability. Layout, or repeated laying out of hybridization nurseries, the purpose of which is a mutual crossing of components. Every component has to be represented in the hybridization nursery several times, and in such an order as to guarantee maximal mutual pollination. The decisive factor will be the flower biology of the respective species. There are not such strict demands for alternation in insect pollinated plants but mutual crossing can be improved by the use of pollinators in isolation. With wind pollinated plants proximity must be respected, as well as synchronization of flowering time and direction of prevailing winds.

The seed obtained from all parts of components (clones) is mixed, observing the principle of equal amounts, and represents generation Syn 0. Further generations, produced from the mixture of this seed, are Syn 1, followed by Syn 2, possibly Syn 3. The seed gained in this generation is used to establish the breeder's seed plot — S1 (superelite).

The number of generative reproductions (Syn 1 to Syn 3) is dictated by the need to gain a genetically balanced population. This

number is given mainly by the genetic character of the species, being shorter in diploids, substantially longer in tetraploids. A certain part will also be played by the degree of component heterozygozity entering the synthesis. In synthetic populations, consisting of several components, balance and maximal heterosis effect are attained in later generations than in populations consisting of fewer components. The yields in generations Syn 2 to Syn 4 are even (Chloupek, Babinec, 1989).

These measures, concerning the number of components, their variation in a hybridization nursery and the number of synthetic generations, must in the given case be agreed on by the team of breeders with respect to the peculiarities of species and improved types in their framework.

A—b: "Line procedure". The hybridization nursery can be laid out, instead of clones, by means of "lines" of selected clones, i. e. from generative progenies of clones after self-pollination (mostly in generation I_1). After mutual crosing in a hybridization nursery, which is laid out in a similar manner as in A—a, seed of generation I_1 — Syn 1, I_1 — Syn 2, 3 possibly I_1 — Syn 4, is obtained, representing in tetraploid species a balanced open pollinated population at the level of propagation degree S 1.

This procedure has the advantage that the I_1 generation can be stockpiled as an adequate compensation for the procedure A—a (Kehr, 1965; Bula et al., 1974).

A—c: Compromising procedure, enabling substitution of lost components. It is based on the knowledge that in some species, especially in lucerne and clovers, the components cannot be maintained on a biologically suitable level. In the case of a lost component, repeated reproduction of selected combinations in a hybridization nursery is not possible and the synthesis loses its original character. For this reason a compromise solution was worked out, which can be methodically described as follows:

According to the principles described, a hybridization nursery (Syn 0) is laid out. The seed of individual components in this nursery is used to lay out generation Syn 1 as in the procedure described as A—a, but with the basic difference that the seed of individual components is not mixed, or only half of it is mixed. From the non-mixed seed, generation Syn 1 is laid out, respecting the identity of progenies and under conditions of individual planting. Under these conditions an individual (genotype) can be selected in each progeny, phenotypically corresponding to the original component and replacing it. The disadvantage of this procedure is that genetic identity with the original component is not guaranteed. On the other hand, a certain improvement of the genotype can be expected, as it arose under conditions of pollination by other selected components, in this case under conditions of recurrent crossing and selection. The mixed seed is used to lay out generation Syn 1 in the familiar way; for generation Syn 2 seed of both types can be used. In the case of a presumed improvement of the synthetic variety the change could be expressed by adding a Roman numeral to the variety name. If there is a demonstrable deterioration, maintenance breeding of the variety is not continued.

A—d: Economical procedure, working with a resulting population without maintenance of original components. The synthetic variety

comes into existence by manifold generative reproduction of material from the hybridization nursery — generation Syn 0. The purpose to these reproductions is to attain a genetically balanced population, which, while keeping up plasticity, will exhibit stability of performance and traits important for the breeder.

It is possible to assume that unless this population is exposed to extreme environmental conditions, its performance and character will be maintained in further reproductions.

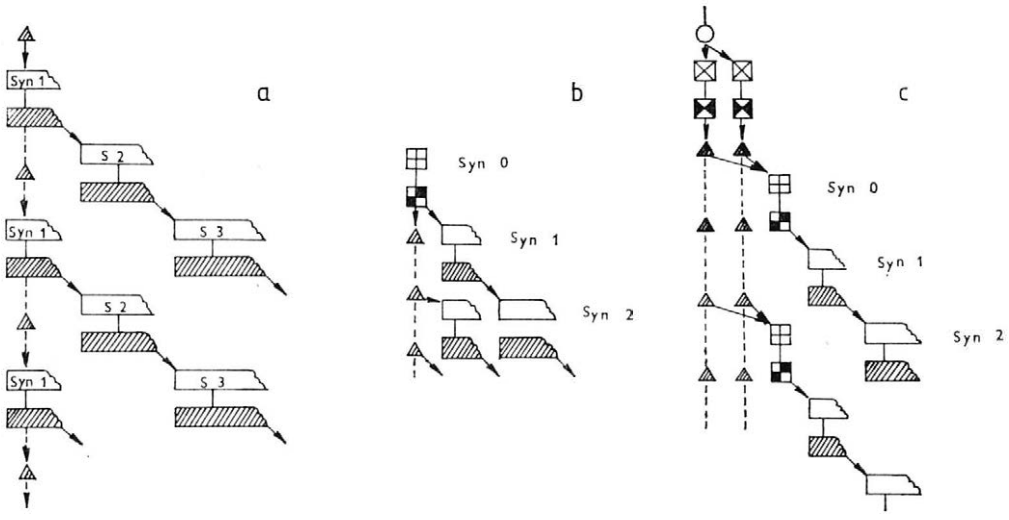
In the framework of maintenance breeding it is possible on the basis of this consideration to go over to mass selection from the resulting population — mostly Syn 3. This can be carried out as positive or negative selection. In the first case the procedure is more effective, though with risk that a gradual change of population character can appear. In the second case the risk is much lower and it is a matter of removing the unhealthy individuals. This should be applied exceptionally only where exists an opportunity to maintain an outstanding variety without technical conditions to use other procedures.

B—a. Procedure based on once-and-for-all building of a synthetic population (Fig. 2). If the original genetic composition of components cannot be maintained for technical and biological reasons and the character of the final synthetic population should be strictly preserved, once-and-for-all production of some of the synthetic generations remains at present the only way. The seed of the relevant generation (Syn 0, 1, 2, or 3) is then long-term stored under conditions of constant humidity and temperature and further multiplied at given intervals. The life of a variety is therefore limited by the supply of this initial seed. With respect to the several-year exploitation cycle of fodder plants and according to repeated cycles of propagation, a life of 15 to 20 years can be reckoned with. In addition, the seed supply and consequently the variety continuance can be extended by repeated production of generation Syn 1 throughout component's persistence. A maximal propagation coefficient can be achieved by taking advantage of technical measures (cultivation and planting of seedlings, cultivation in phytothrons, glass-houses, etc.), which further prolong the life of a variety.

The advantage of this procedure can be summarized as follows: Conservation of an unchangeable variety character, saving of time and material costs of classis maintenance breeding, relieving working capacities for breeding new varieties, i. e. building new, more efficient synthetic populations.

B—b: Procedure based on a single gradual building of a synthetic population. The above procedure can be divided into several stages, which often seems more advantageous for biological and technical reasons. The original components (genotypes-clones) should be stored. This task will be tackled after a satisfactory mastering of techniques of tissue culture. Further it is necessary to stress that the components cannot be represented by the seed, obtained from an arbitrary open — pollination. Accordingly, it is possible to store seeds of individual components separately, but after mutual crossing in a hybridization nursery, hence after hybridization of all components. This principle has to be observed in all kinds of propagation in order to save the character of a synthetic variety.

B—c: Another (more frequent) method consists in succesive combin-



2. Group B: a— Procedure based on a onefold building of a synthetic population and on a successive utilizing of seed gained in this way up to spending; b — Procedure based on once and for all building up Syn 1 seed and its successive utilization; c — Procedure based on a successive combination of two and more components into a synthetic population

ation of two or more components. Seed obtained and stored in this way is gradually used for further hybridization where the hybrids mentioned will represent the components. Thus we start with dialleles of components, the performance of which in the consecutive generation (F_1) allows estimation of their combining ability. These basic dialleles can be carried out in a maximal number of combinations of components taken into account. The seed of the most efficient dialleles is used for crossing either at once, or after storing in a hybridization nursery, so that generation Syn 1 of 4 components results. There can be several such Syn 1 generations (always from 4 other components), so that by means of their mutual hybridization a Syn 2 is obtained from 8, 12 or more components. This procedure is equivalent to procedure A—a (K e h r, 1965; B u l a et al., 1974). It is also an advantage of this procedure that components may be used as single, double or more complex hybrids, which makes it possible to produce and maintain synthetic varieties at a high level also on the basis of information about specific combining ability.

Final Remarks

Individual procedures of maintenance breeding of synthetic varieties were drawn at an entirely general level in a way permitting compliance with lucerne, clovers and grasses. Selection cycles will have to be adjusted according to the growth and development requirements of particular species, if need be of types within their framework. This relates chiefly to graphic supplements conceived for the most simple case, i. e. for cycle with a lay-out year and one year with seed harvest. In species with several years' use it will be necessary to adjust the procedure accordingly and have it approved by a relevant council of breeders.

In the course of reproduction of a synthetic population in generations Syn 0 to Syn 4 evaluations of performance, health condition, etc. are no longer made. If an infestation of clones by viruses occurs, restoration to health by means of tissue cultures is applied, and if fails a substitute procedure is used, as described above.

In drawing the graphs we used the "Proposal for uniform symbols for construction of breeding schedules in fodder plants" (R o d, 1980), approved by the breeders' council for fodder plants.

A fundamental question remains: the choice of one of the above-mentioned procedures and its appropriate modifications. Until now endeavours to apply procedures based on the maintenance of original components (A—a, b, c) has prevailed. For biological and economic reasons, another procedure was exceptionally admitted, based on mass selection in the resulting population (A—d), but only when all other possibilities were exhausted. This happens when the variety loses its character and its advantage as a synthetic population.

For technical and economic reasons it will be appropriate to pass over to procedures based on the principle of once-and-for-all seed production in the framework of one of the selection steps and propagation of the variety until this supply is exhausted (B—a, b, c). In this sense the attitude of responsible authorities has to be revised and the relevant directives adjusted. It is necessary to stress that from a realistic point of view, only procedures ad B can guarantee an innutable and stable character of a synthetic variety during the whole period of its existence (R o d, 1985; R o d, C h l o u p e k, 1988).

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