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Substitution Lines of Wheat with Dominant Genes *Vrn*

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Abstract

KOŠNER J., PÁNKOVÁ K. (2001): Substitution lines of wheat with dominant genes *Vrn*. Czech J. Genet. Plant. Breed., 37: 41–49.

Lines with substituted homoeologous group 5 chromosomes with the loci *Vrn* that conditioned a change from winter to spring growth habit, were produced in the winter wheat cultivars Košutka, Vala and Zdar. The sources of the dominant alleles *Vrn-A1* (cv. Zlatka), *Vrn-B1* (cv. Česká Přesívka) and *Vrn-D1* (cv. Chinese Spring) as well as the expression of the individual loci *Vrn* and their vernalization responses were investigated. In the substitution lines the effects of individual chromosomes, and thus of the corresponding loci *Vrn* on time to heading, on response to different length of vernalization, and of the effects of genetic backgrounds on time to heading and on response to different day-length were studied. The results showed quite different effects of the chromosomes with dominant loci *Vrn* on time to heading and also their different dependence on vernalization. Lines with locus *Vrn-B1* were the latest, and lines with *Vrn-D1* were the earliest; the effect on earliness followed the order *Vrn-D1* → *Vrn-A1* → *Vrn-B1*. Estimates of genetic differences were: *Vrn-A1* vs. *Vrn-B1* = -3.53 days, *Vrn-A1* vs. *Vrn-D1* = 3.40 days and *Vrn-B1* vs. *Vrn-D1* = 10.85 days. Lines with *Vrn-B1* showed the least dependence on vernalization time (coefficient = -0.719); lines with both *Vrn-D1* (coefficient = -2.25) and *Vrn-A1* (coefficient = -3.14) were more dependent. The effects of individual genetic backgrounds on earliness was in the following order: Košutka → Zdar → Vala. The influence of genetic background on earliness parallels the differences found in photoperiod sensitivity. The differences between individual genetic backgrounds were: Košutka versus Vala = -15.95 days, Košutka versus Zdar = -9.83 days and Vala versus Zdar = 12.27 days.

Keywords: *Triticum aestivum* L.; wheat; growth habit; *Vrn* gene

Bread wheat, *Triticum aestivum* L., mostly occurs as spring or winter forms, but there are also alternative forms that can be sown either in fall or spring. Sensitivity of the plants to low temperatures and need of a cool period, i.e. vernalization requirement, determine this habit. Low need of a cool period – insensitivity to vernalization – determines spring habit, while requirement of a long period of cool temperatures conditions winter habit.

Genetic determination of growth habit is mostly based on the genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, formerly designated as *Vrn1*, *Vrn2* and *Vrn3* (MCINTOSH *et al.* 1998), which occur as two or more alleles (PUGSLEY 1971; SNAPE *et al.* 1976). The level of vernalization requirement is determined by a combination of the alleles present. Dominant alleles inhibit the vernalization requirement; their presence in a genotype is expressed by spring

growth habit. Recessive constitution of all three loci *vrn* determines winter growth habit (PUGSLEY 1972). The vernalization requirement of winter cultivars is strong but not identical; we can interpret the differences between cultivars as the results of diversity of recessive alleles *vrn* (PUGSLEY 1971), or as effects of modifying genes in the genetic background (GOTOH 1980; 1983). KOŠNER and PÁNKOVÁ (1997), in the study of winter wheat substitution lines with reciprocal substitutions of chromosomes carrying recessive alleles *vrn*, concluded that genotypes of winter wheat can be constituted of different alleles *vrn* which then determine the vernalization responses of individual cultivars. Alternative growth habit is usually conditioned by presence of one dominant locus *Vrn*, most frequently *Vrn-B1*, the weakest inhibitor of vernalization requirement, in combination with a

strong photoperiod sensitivity. The genes *Vrn* are located on chromosomes 5A, 5B, 5D (LAW *et al.* 1976; MAISTRENGO 1980). Besides *Vrn-A1*, *Vrn-B1* and *Vrn-D1* other loci are known, especially *Vrn5* on chromosome 7B (LAW 1966). An effect on time to heading was also detected in genes on homoeologous group 3 (MIURA & WORLAND 1994) and group 6 chromosomes (ISLAM-FARIDI *et al.* 1996).

The distribution of basic loci *Vrn* in spring wheat cultivars was determined by STELMAKH and AVSENNIN (1985). In 482 cultivars of the world assortment the following genotypes (with their numbers) were found: *Vrn-A1 vrn-B1 vrn-D1* = 125; *vrn-A1 Vrn-B1 vrn-D1* = 35; *vrn-A1 vrn-B1 Vrn-D1* = 18; *Vrn-A1 Vrn-B1 vrn-D1* = 251; *Vrn-A1 vrn-B1 Vrn-D1* = 29; *vrn-A1 Vrn-B1 Vrn-D1* = 22; *Vrn-A1 Vrn-B1 Vrn-D1* = 2. The frequencies of dominant alleles of these loci were: *Vrn-A1* = 84.4%; *Vrn-B1* = 64.3% and *Vrn-D1* = 14.7%.

After the vernalization requirement is satisfied, the life cycle of a wheat cultivar follows and includes order of growth and development stages. The course of these stages is probably markedly influenced by many agronomically important traits. Duration and course of each individual stage depends on environmental conditions and on the complex of genetic factors governing vernalization, photoperiod responses and earliness *per se*.

The effects of dominant genes *Vrn* on time to heading and on agronomic traits of wheat were studied by STELMAKH (1993). It was shown that the effect of genotypes *Vrn* in spring cultivars is related to mass of grains per plant; it was suggested that their effect is stronger than that of genetic background and environmental conditions.

A study of growth habit revealed the presence of alleles *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in 642 genotypes of spring wheat. A dominant allele, *Vrn5*, at another locus on chromosome 7B, was detected only in the accession Chinese Spring Hope, the source of which can be *T. dicoccum*, cv. Vernal (STELMAKH 1987).

The causes of irregular distribution of alleles *Vrn* on the globe was found by STELMAKH (1990, 1998) to lie in their different breeding qualities, resulting in their natural or artificial selection. This was most marked in the process of spreading of gene *Vrn3*, which was started at the beginning of the 20th century by its introgression by N. Strampelli from the Japanese wheat landrace Akakomugi into Italian cultivars. From these and by way of cv. Mentana the gene got into Mexican cultivars, which later became the background of many modern cultivars that are now distributed widely in countries near the equator.

Introgression of alien genes for growth habit was achieved by crossing with spring forms of related species or genera (STELMAKH & AVSENNIN 1996; STELMACH, 1998). Such introduced genes were located mostly on the homoeologous group 5 chromosomes and were allelic with the known genes *Vrn* in wheat (*T. aestivum*), *Vrn6^{sc}*, *Vrn7^{sc}* (from *Secale cereale*) and *Vrn8^{ts}* (from *T. sphaerococcum*). Since cultivated wheat originated only from part

of the genetic potential of diploid species, it is understandable that GONCHAROV (1998) showed the feasibility of transfer of new genes *Vrn* from species of the section *Sitopsis* of the genus *Aegilops*, and of a weak allele *Vrn-A1* from *T. dicoccum* into cultivars of wheat.

MATERIALS AND METHODS

Three genotypes of winter wheat differing in their photoperiod responses were chosen to produce genetically defined lines with changed growth habit.

Individual dominant loci *Vrn* were transferred into the winter cultivars Zdar, Vala and Košutka by substitution of homoeologous group 5 chromosomes, resulting in lines with spring growth habit.

The substituted chromosomes came from genotypes with earlier identified loci *Vrn* (LAW *et al.* 1976; KOŠNER 1984, 1987; KOŠNER & BROMOVÁ 1993). Individual loci were transferred by substitution of chromosome 5A of Zlatka (*Vrn-A1*), by substitution of 5B of Česká Přesívka (*Vrn-B1*) and of 5D of Chinese Spring (*Vrn-D1*). *Vrn-A1* of Chinese Spring was transferred as supplement into the background of Zdar; its weak action on inhibition of vernalization response lies between a dominant and recessive effect so that its substitution into winter background can result in heading without having satisfied the vernalization requirement (LAW *et al.* 1976), although it is still considered a recessive locus.

Lines created in this way were: Zdar (Zlatka 5A), Zdar (Česká Přesívka 5B), Zdar (Chinese Spring 5D), Vala (Zlatka 5A), Vala (Česká Přesívka 5B), Vala (Chinese Spring 5D), Košutka (Zlatka 5A), Košutka (Česká Přesívka 5B), Košutka (Chinese Spring 5D) and Zdar (Chinese Spring 5A) with atypical locus *Vrn-A1* of CS (designated from now on as *Vrn-A1[CS]*).

These substitution lines were produced by a series of backcrosses with monosomic lines for the given chromosomes of recipient cultivars which were obtained by eight generations of backcrossing with monosomic lines of the cultivar Zlatka, with inter-generations of self-pollination and permanent cytological tests for chromosome number. Some mistakes can occur during such chromosome manipulations, most typical of which is univalent shift, i.e. interchange of the chromosome substituted.

Tests to verify the source of the substituted chromosome, and thus checks of the presence of the substitution in individual sub-lines, were done on separate sisterly offsprings of the lines (examination of univalent shift or other errors) and at concurrent detection of differences in effects of loci *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in identical/different genetic backgrounds on time to heading. Materials in the most advanced generations of backcrossing were tested. The substitution lines are designated in the text as given in the abbreviations in the tables (Table 1).

Table 1. Tested materials

Line	Designation	Transmitted gene	Generation	Subline	Check
Zdar (Zlatka 5A)	Zdar (Zl 5A)	<i>Vrn-A1</i>	B ₇	3/98	OK!
				4/98	OK!
				4/99	OK!
				3/99	–
Zdar (Chinese Spring 5A)	Zdar (CS 5A)	<i>Vrn-A1(CS)</i>	B ₄	1/98	OK!
				5/99	OK!
				5/98	OK!
				3/98	OK?
Zdar (Česká Přesívka 5B)	Zdar (ČP 5B)	<i>Vrn-B1</i>	B ₆	4/98	OK!
				4/99	OK!
				2/98	–
				2/99	–
Zdar (Chinese Spring 5D)	Zdar (CS 5D)	<i>Vrn-D1</i>	B ₃	1/97	OK!
				1/98	OK!
				1/99	OK!
Vala (Zlatka 5A)	Vala (Zl 5A)	<i>Vrn-A1</i>	B ₆	2/99	OK!
				6/99	OK!
				6/98	OK!
				1/98	OK!
				7/98	OK!
				2/98	OK?
				3/98	–
Vala (Česká Přesívka 5B)	Vala (ČP 5B)	<i>Vrn-B1</i>	B ₁	1/99	OK!
				6/99	OK!
				6/98	OK!
				4/98	OK!
				7/98	OK!
				1/98	OK?
				2/98	OK?
Vala (Chinese Spring 5D)	Vala (CS 5D)	<i>Vrn-D1</i>	B ₂	1/96	OK!
				15/97	OK!
				1/99	OK!
				2/99	OK!
Košetka (Zlatka 5A)	Košetka (ZL5A)	<i>Vrn-A1</i>	B ₃	2/98	–
				2/99	–
Košetka (Česká Přesívka 5B)	Košetka (ČP5B)	<i>Vrn-B1</i>	B ₄	3/98	OK!
				1/99	OK!
				6/99	OK!
Košetka (Chinese Spring 5D)	Košetka (CS5D)	<i>Vrn-D1</i>	B ₂	2/98	OK!
				2/99	OK!

OK! – all plants headed

OK? –all plants headed; some of them with a delay

– = mistake

Grains of substitution lines were germinated in weekly intervals and vernalized under temperatures from +1 to +3°C so that variants with 4, 3, 2, 1 and 0 weeks of vernalization were prepared for sowing on 20th April when photoperiod (day-length) in the latitude at the location of the experiment exceeded 14 h (long-day photoperiod). We can also suppose that after this date there is low probability of additional vernalization due to low temperatures. The grains of all lines and variants of vernalization were

sown in plots in the field, and grains of all lines vernalized for 4 weeks were also sown in a darkened plot with a 10 h light regime (8 a.m. to 18 p.m) to determine the influence of day length and eliminate different effects of the loci *Vrn*. Darkening was ended just after heading of the lines with background Košetka (on 15th July), to allow heading of photoperiod-sensitive materials with backgrounds of Vala and Zdar.

Time from germination to heading was determined. 20th April was considered sowing date, and the time of heading of a plant was the day when half of the spike of the first tiller was headed. Data obtained on approximately 1400 plants were statistically assessed and further processed.

Analysis of variance, regression analysis and statistical models of establishing estimates of differences were carried out according to MATHER and JINKS (1971).

RESULTS AND DISCUSSION

The experiments confirmed the effects of the dominant loci *Vrn*; lines with changed growth habit (from winter to spring type) were detected for all loci. The genotypes of cultivars Zlatka, Česká Přesívka and Chinese Spring are reliable sources of the loci *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, respectively (Table 1). The experiments also showed that a transferred chromosome or gene can be lost during the process. Some of the sub-lines did not head, indicating that they did not carry a dominant locus *Vrn*. These materials were not used for subsequent tests; they also underlined the necessity of frequent and precise tests to verify the transfer of a chromosome for obtaining chromosome substitutions.

Only materials in which no errors were detected in the transfer of chromosomes were used for the following assessment. Unfortunately, this excluded line Košutka (Zl 5A) from the experiment. Line Zdar (CS 5D), which significantly carries locus *Vrn-D1*, was affected by virosis. A proportion of its plants perished and the others headed with a delay; the line was excluded from most evaluations. Data for which it was included can be only considered informative. Since only materials were used further where the presence of loci *Vrn* was confirmed, the effect of the substituted chromosome on time to heading can be reckoned to be identical with the effect of the respective locus *Vrn*. However, in our interpretation of results we must take into account the possible presence of other genes that could influence the observed traits (earliness *per se* etc.).

All three dominant loci *Vrn* cause a change from winter to spring growth habit by inhibiting vernalization require-

ment. The differences between individual loci are subject of our study.

The effects of individual main factors on time to heading of the whole experiment were generally evaluated by analysis of variance (Table 2). It proved a significant influence of all studied factors, i.e. of the loci *Vrn*, genetic background and duration of vernalization on time to heading.

Divergence of the loci *vrn* is well known and was confirmed by many authors. The genetic background influenced markedly the time to heading in this experiment. We can suppose that differences between individual genetic backgrounds are influenced especially by different photoperiod sensitivity of the materials. For that reason their response to short-day conditions (10 h) was tested (Fig. 1). Košutka was least sensitive and thus the earliest, followed by Zdar, while Vala was most sensitive. The effect of duration of vernalization was also significant.

Considering that the lines Košutka (Zl 5A) and Zdar (CS 5D) could not be included in the evaluation of the whole experiment and the design of it became unbalanced, contrasts between main factors of the experiment as a whole could not be assessed, but only in the scope of each line. The means of numbers of days to heading conditioned by substituted chromosomes, and thus by

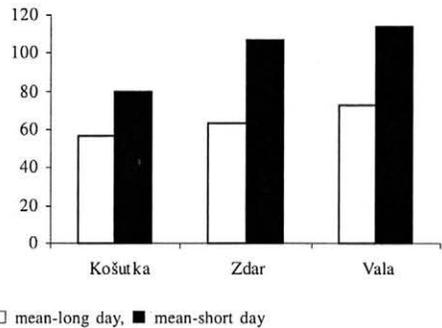


Fig. 1. Mean time to heading under long and short day of the three genetic backgrounds

Table 2. Analysis of variance

Source of variability	Sum of squares	Degrees of freedom	Mean square	Statistic <i>F</i>	Significance
Genes <i>Vrn</i>	16 169.15	2	8 084.57	163.80	***
Genetic background	73 425.61	2	36 712.81	743.81	***
Weeks of vernalization	15 724.07	4	3 931.02	79.64	***
Error	68 311.46	1 384	49.36		
Total	176 039.10	1 392	126.47		

*** *P* < 0.01

Table 3. Means of days to heading influenced by homologous group 5 chromosomes and thus by genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in different genetic backgrounds, and their significance (categorization into homologous groups)

Genes <i>Vrn</i> in individual backgrounds		<i>n</i>	Mean	Homologous group
Košetka	<i>Vrn-D1</i>	264	51.87	
	<i>Vrn-B1</i>	142	65.42	
Vala	<i>Vrn-D1</i>	356	68.97	
	<i>Vrn-A1</i>	149	75.19	
	<i>Vrn-B1</i>	199	77.50	
Zdar	<i>Vrn-A1</i>	121	63.10	
	<i>Vrn-B1</i>	154	63.55	

genes *Vrn* in individual genetic backgrounds, are shown in Table 3. Mutual differences between effects of the genes *Vrn* were statistically evaluated for each of the observed groups, and significance is expressed by classifying them into homologous groups. In plants with gene

Vrn-D1, and in both backgrounds which were available, the shortest time to heading and a significant difference from plants with *Vrn-B1* and *Vrn-A1* was found. A significant difference between the effects of genes *Vrn-A1* and *Vrn-B1* was detected when these were present in the background of Vala, while their effects in the background of Zdar were almost identical. Lines carrying *Vrn-B1* are the latest, which agrees with findings of other authors (STELMAKH & AVSEININ 1985).

To exclude other influences and obtain estimates of only those differences caused by the factors studied, we proceeded from models where mean values of averages were compared in pairs of genotypes which differed only in one of the factors studied and thus determined the differences observed. Significance was established by pair *t*-test. In this way an imbalance of the experiment was minimized and estimates better match to declared factors. However, only those data were used where corresponding pairs for a given difference existed. The basis for evaluations were statistical models according to MATHER and JINKS (1971). The estimates of the differences between effects of homoecologous group 5 chro-

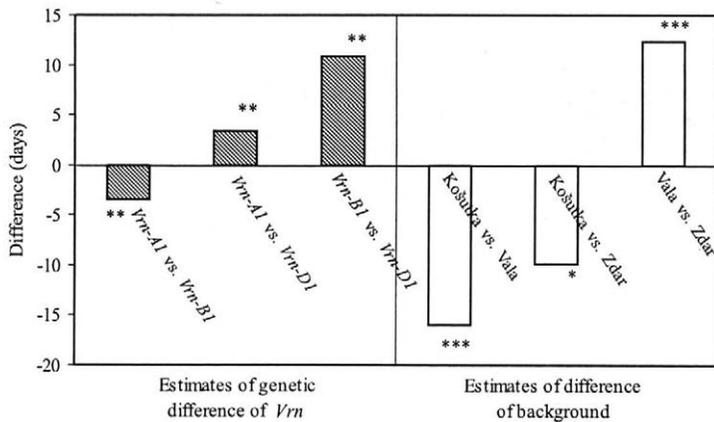


Fig. 2. Estimates of differences between the homoecologous group 5 chromosomes (genes *Vrn*), between the genetic backgrounds, and of the differences due to interactions genes *Vrn* × vernalization

Table 4. Estimates of differences between the homoecologous group 5 chromosomes and thus between the genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, individual genetic backgrounds, and differences as an effect of interactions genes *Vrn* × vernalization

Estimate of difference	Estimates of genetic difference of the genes <i>Vrn</i>			Estimates of difference of background		
	<i>Vrn-A1</i> vs. <i>Vrn-B1</i>	<i>Vrn-A1</i> vs. <i>Vrn-D1</i>	<i>Vrn-B1</i> vs. <i>Vrn-D1</i>	Košetka vs. Vala	Košetka vs. Zdar	Vala vs. Zdar
Estimate of difference	-3.53**	3.41**	10.85***	-15.95***	-9.84*	12.27***
<i>t</i> -test	-2.43	2.55	6.79	-13.45	-1.95	8.38
<i>t</i> _{crit} (<i>P</i> _{0.10})	1.83	2.13	1.83	1.83	1.90	1.83
<i>t</i> _{crit} (<i>P</i> _{0.05})	2.26	2.78	2.26	2.26	2.37	2.26

* *P* = 0.05–0.10; ** *P* = 0.01–0.05; *** *P* < 0.01

Table 5. Regression analysis (dependence of time to heading on weeks of vernalization, on the action of individual genes *Vrn* and of these genes in different genetic backgrounds)

Dependent variable: days to heading							Confidence intervals			
			Coefficient	Standard error	<i>t</i> -stat.	Significance	limit		significant difference	
lower	upper									
Whole experiment		weeks of vernalization	-2.41	0.20	-11.86	***	-2.81	-2.01		
<i>Vrn</i>	<i>Vrn-A1</i>	weeks of vernalization	-3.15	0.37	-8.46	***	-3.88	-2.42	<i>Vrn-B1</i> , <i>Vrn-A1</i> (CS)	
		<i>Vrn-B1</i>	weeks of vernalization	-0.72	0.31	-2.29	**	-1.334	-0.10	<i>Vrn-A1</i> , <i>Vrn-D1</i> , <i>Vrn-A1</i> (CS)
			weeks of vernalization	-2.26	0.32	-7.00	***	-2.89	-1.62	<i>Vrn-B1</i> , <i>Vrn-A1</i> (CS)
			weeks of vernalization	-6.88	0.64	-10.78	***	-8.14	-5.62	<i>Vrn-A1</i> , <i>Vrn-B1</i> , <i>Vrn-D1</i>
Košutka	<i>Vrn-B1</i>	constant	67.92	0.91	74.52	***	66.12	69.72	<i>Vrn-D1</i>	
		weeks of vernalization	-1.73	0.45	-3.89	***	-2.61	-0.85	-	
	<i>Vrn-D1</i>	constant	56.47	0.47	121.12	***	55.55	57.38	<i>Vrn-B1</i>	
		weeks of vernalization	-2.4041	0.19	-12.35	***	-2.79	-2.02	-	
Vala	<i>Vrn-A1</i>	constant	78.98	0.73	107.49	***	77.53	80.43	-	
		weeks of vernalization	-3.08	0.44	-6.98	***	-3.943	-2.21	<i>Vrn-B1</i>	
	<i>Vrn-B1</i>	constant	77.14	0.77	99.92	***	75.61	78.66	-	
		weeks of vernalization	-1.04	0.54	-1.94	*	-2.11	0.02	<i>Vrn-A1</i> , <i>Vrn-D1</i>	
	<i>Vrn-D1</i>	constant	76.73	0.84	91.02	***	75.07	78.38	-	
		weeks of vernalization	-3.46	0.32	-10.81	***	-4.09	-2.83	<i>Vrn-B1</i>	
Zdar	<i>Vrn-A1</i>	constant	66.82	0.73	91.82	***	65.38	68.26	<i>Vrn-A1</i> (CS)	
		weeks of vernalization	-2.17	0.34	-6.43	***	-2.84	-1.50	<i>Vrn-A1</i> (CS)	
	<i>Vrn-B1</i>	constant	65.09	0.54	121.02	***	64.03	66.15	<i>Vrn-A1</i> (CS)	
		weeks of vernalization	-1.16	0.29	-4.03	***	-1.73	-0.59	<i>Vrn-A1</i> (CS)	
	<i>Vrn-A1</i> (CS)	constant	94.41	1.08	87.64	***	92.28	96.55	<i>Vrn-A1</i> , <i>Vrn-B1</i>	
		weeks of vernalization	-6.88	0.64	-10.78	***	-8.14	-5.62	<i>Vrn-A1</i> , <i>Vrn-B1</i>	

* $P = 0.05-0.10$ ** $P = 0.01-0.05$ *** $P < 0.01$

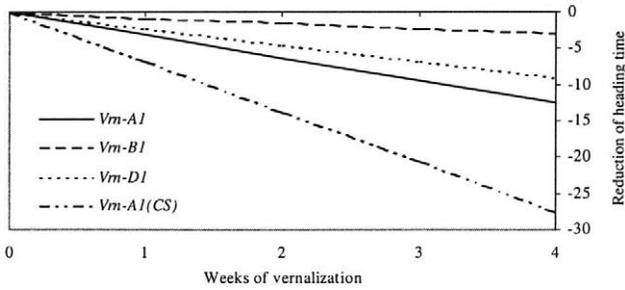


Fig. 3. Dependence of heading time of lines with loci *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-A1(CS)* on duration of vernalization; reduction of time to heading as effect of vernalization

mosomes, and thus of genes *Vrn*, and for the genetic backgrounds are shown in Table 4. The differences between lines carrying homoeologous group 5 chromosomes with genes *Vrn* were significant in all cases; *Vrn-A1* vs. *Vrn-B1* = -3.53 days, *Vrn-A1* vs. *Vrn-D1* = 3.40 days, and *Vrn-B1* vs. *Vrn-D1* = 10.85 days. The effect on earliness followed the order *Vrn-D1* → *Vrn-A1* → *Vrn-B1*. We can conclude that the genes *Vrn* influence earliness significantly. STELMAKH & AVSENIN (1985) established additive effects of loci *Vrn* on earliness: *Vrn-A1* (-12.8 days), *Vrn-D1* (-12.2 days), *Vrn-B1* (-10.2 days). The least effect was found in *Vrn-B1*; effects of *Vrn-A1* and *Vrn-D1* were similar.

Estimates of differences between genetic backgrounds were also significant in all cases; Košutka vs. Vala = -15.95 days, Košutka vs. Zdar = -9.83 days, and Vala vs. Zdar = 12.27 days. An effect on earliness was detected in

the order of Košutka → Zdar → Vala. This significant influence by the genetic background parallels the different photoperiod sensitivity and is attributed to it (Fig. 1). Estimates of differences are depicted in Fig. 2.

Different length of vernalization showed itself to be an overall significant factor (Table 2). The experiment as a whole also shows a significant dependence of time to heading on the degree of vernalization (Table 5). To determine the dependence on vernalization time of lines carrying individual loci *Vrn* is important for a better understanding of their response to vernalization. The results are shown in the upper part of Table 5, including a line with locus *Vrn-A1(CS)* which, as expected, borders spring and winter growth habit and responds to vernalization very markedly with a coefficient of -6.87. Then follow the lines carrying loci *Vrn-A1*, *Vrn-D1*, *Vrn-B1*, with decreasing response to vernalization. Surprisingly, the

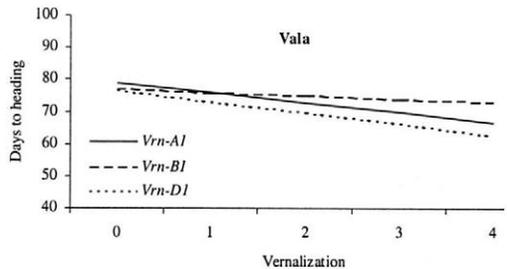
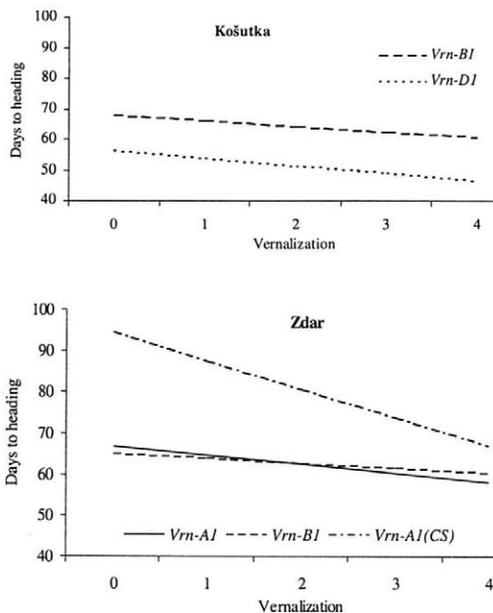


Fig. 4. Dependence of heading time of lines with loci *Vrn-B1* and *Vrn-D1* in genetic background of Košutka, lines with loci *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in the background of Vala, and lines with loci *Vrn-A1*, *Vrn-A1(CS)* and *Vrn-B1* in the background of Zdar

weakest of them was the line with *Vrn-B1*, a gene known to delay time to heading, and the cause of this delay was thought to be a certain level of vernalization requirement. The lowest response to vernalization was expected in locus *Vrn-A1* which is reckoned to be epistatic over the others and without vernalization requirement. A certain extent of caution is thus necessary considering that the experiment was carried out with not yet completed materials.

Establishing confidence intervals allowed the detection of significant differences between regression coefficients of the lines which express responses to vernalization time. As expected, the semi-winter line with *Vrn-A1(CS)* reacted most markedly and differs from the others. But the line with the lowest coefficient, carrying *Vrn-B1*, also differs from the other lines. The difference between the lines carrying *Vrn-A1* and *Vrn-D1* was not significant (Fig. 3).

Table 5 shows results of regression analyses of lines with the loci *Vrn* in individual genetic backgrounds, including the semi-winter line Zdar (CS 5A). The course of dependence of the lines on vernalization time (Fig. 4) shows a similarity between the lines carrying the respective loci *Vrn* in the genetic backgrounds; the response of lines with locus *Vrn-B1* was the weakest, lower than that of lines with either *Vrn-A1* or *Vrn-D1*, which responded similarly. The differences are noticeable in coefficients of constant which characterize time to heading of unvernallized plants. This is probably a result of interaction between the genetic background (mainly its photoperiod sensitivity) and the respective locus *Vrn*.

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Abstract

KOŠNER J., PÁNKOVÁ K. (2001): **Substituční linie pšenice s dominantními geny *Vrn***. Czech J. Genet. Plant. Breed., 37: 41–49.

Ve třech rozdílných genetických pozadích pšenice ozimé (Košetka, Vala a Zdar) jsou vytvářeny substituční linie, u kterých je substitucí chromozomů páté homoeologické skupiny nesoucích lokusy *Vrn* měněn růstový typ z ozimého na jarní. U vytvářených materiálů byla ověřována správnost použitého zdroje a byl studován projev jednotlivých lokusů *Vrn* a jejich reakce na jarovizaci. Použité zdroje dominantních lokusů – *Vrn-A1* z genotypu odrůdy Zlatka, *Vrn-B1* z genotypu odrůdy Česká Přesívka a *Vrn-D1* z genotypu odrůdy Chinese Spring – lze považovat za ověřené. U vzniklých substitučních linií byl sledován vliv jednotlivých chromozomů, potažmo příslušných lokusů *Vrn*, na dobu do metání, reakce na různou dobu jarovizace a vliv genetického pozadí na dobu do metání a jeho reakce na délku dne. Výsledky prokázaly odlišné působení sledovaných chromozomů s dominantními lokusy *Vrn* na dobu do metání i jejich rozdílnou závislost na jarovizaci. Nejpozdnější byly linie s lokusem *Vrn-B1*, nejranější s lokusem *Vrn-D1*. Vliv na ranost se projevoval v následujícím pořadí: *Vrn-D1* → *Vrn-A1* → *Vrn-B1*. Odhady genetických diferencí byly: *Vrn-A1* versus *Vrn-B1* = –3,53 dní, *Vrn-A1* versus *Vrn-D1* = 3,40 dní a *Vrn-B1* versus *Vrn-D1* = 10,85 dní. Nejmenší závislost na jarovizaci vykazovaly linie s lokusem *Vrn-B1* (koeficient = –0,719), s lokusy *Vrn-D1* (koeficient = –2,25) a s lokusy *Vrn-A1* (koeficient = –3,14) vykazovaly závislost shodně vyšší. Vliv genetického pozadí na ranost se projevil následovně: Košetka → Zdar → Vala. Působení genetického pozadí na ranost je přisuzováno zjištěné různé fotoperiodické citlivosti, kterou kopíruje. Odhady diferencí mezi genetickými pozadími byly: Košetka versus Vala = –15,95 dní, Košetka versus Zdar = –9,83 dní a Vala versus Zdar = 12,27 dní.

Klíčová slova: *Triticum aestivum* L.; pšenice; růstový typ; geny *Vrn*

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Reactions of Registered Winter Barley Varieties to *Wheat dwarf virus* Infection

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Abstract: Small-plot field trials were conducted to study the reactions of 13 registered winter barley varieties to infection with the *wheat dwarf virus* (WDV). The varieties were artificially infected with a barley strain of WDV at the tillering stage in the autumn. All studied varieties reacted with strong symptoms of plant stunt, heading disorders, leaf yellowing, reduced aboveground biomass production and grain yield depression. There was no resistant variety among the tested cultivars. The varieties Agrilo, Marinka, Kromir, Kromoz, Okal and Sigra with grain yield depression of more than 90% were classified as very susceptible. The other varieties, Babylone, Borwina, Kamil, Lunet, Luxor, Marna and Monaco, were classified as susceptible with grain yield depressed by 79.2% to 88.7%.

Keywords: winter barley; barley strain of wheat dwarf virus; reaction to infection

Wheat dwarf virus (WDV) belongs to the group of geminiviruses (LINDSTEN *et al.* 1980). It is transmitted by nymphs and adults of the leafhopper *Psammotettix alienus* Dahlb. (VACKE 1962). It occurs mostly at lowland regions of the Czech Republic, where it causes in some years heavy losses of grain yield in winter wheat and winter barley. Two strains of WDV were described in this country in the early nineties: a wheat strain and a barley strain (LINDSTEN & VACKE 1991). A complex of agrotechnical and/or chemical measures helps to protect winter crops against the WDV. The efficiency of the measures could be increased by growing resistant varieties. The identification of resistant varieties within the collection of registered winter wheat varieties was described in a preceding paper (VACKE & CIBULKA 2000). The present communication deals with the reaction of a group of registered winter barley varieties to infection with WDV.

MATERIAL AND METHOD

Thirteen registered varieties of winter barley were included in a trial conducted in 1999–2000. The varieties were grown on 1 m² plots, at a plant spacing of 8 × 8 cm. The trial was divided in two parts – one infected and one non-infected (the control). The varieties in both the infected and non-infected part were grown in three random-

ized replications. A barley strain of WDV, kept at the RICP Prague-Ruzyně, was used for the infection of plants on the trial plots. The virus was transmitted by adults of the leafhopper *Psammotettix alienus*, collected on volunteer plants from cereal fields. The varieties in the experiment were inoculated at the tillering stage in the autumn. The plots were isolated with dense nylon meshwork to prevent the escape of vectors. Acquisition feeding of leafhoppers on the source of infection (winter barley variety Borwina) lasted for a week, inoculation feeding on young barley plants for 2 weeks. Subsequently the leafhoppers were killed by spraying with Phosdrin. The trial was kept free of insects during the subsequent vegetation period by means of insecticides.

The reaction of winter barley varieties to WDV infection was assessed throughout the vegetation period and also after harvest. Following traits were investigated: plant color, plant height, share of heading plants, aboveground biomass weight and grain yield. A total of 60 plants were assessed in each variety.

Relative virus content in the sap of infected plants was determined by enzyme-linked immunosorbent assay (ELISA) according to CLARK and ADAMS (1977). Fresh leaf samples were frozen to –20°C immediately after sampling and stored at this temperature till analysis. Leaf material was homogenized in a press (Meku Press, E Pollahne, Germany) while extraction buffer was added at the same time.

RESULTS AND DISCUSSION

The proportion of infected plants of the particular varieties ranged from 97% to 100%. This high proportion was achieved after two weeks of inoculation feeding by the vector, with 2 leafhoppers per plant on average.

No symptoms of WDV infection appeared on the tested varieties in the autumn. This was due to the late infection (inoculation feeding 30.10.–14.11.) and the onset of low temperatures instantly after inoculation. Virus incubation in plants was slow, in the course of several warmer periods in winter. The first marked symptoms of virus infection appeared on barley in mid-March of the next year. They consisted in growth retardation and light green color of youngest leaves. In some cases these leaves were deformed with bulges, curved blades and serration. Later on, the leaves started yellowing from the tips and margins, and necrotic spots appeared at the same time. Finally, leaf tips and margins dried off. Unlike winter wheat plants that withered away within a relatively short time in the spring, barley plants continued retarded growth until maturation. The plants were dwarfed throughout the whole vegetation period, and their development was retarded by 1–2 growth stages of the Feekes scale (Fig. 1).

The harvested plants were analyzed. Plant height was much reduced, ranging from 40.1 to 75.3% reduction in the particular varieties (Table 1). The lowest reduction was observed in Lunet (40.1%) and Kamil (47.4%), the

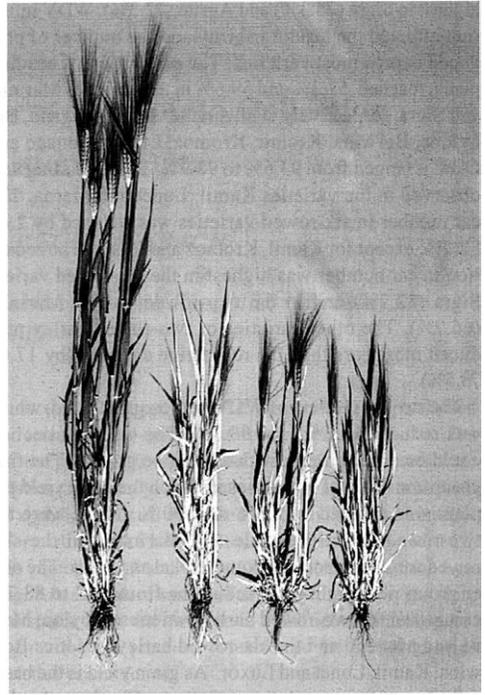


Fig. 1. Symptoms of WDV infection in the susceptible winter barley variety Kamil; uninfected plant on the left

Table 1. Values of studied traits characterising winter barley varieties response to the infection with WDV

Varieties	Plant height		Percent of heading plants	Number of ears per plant		Weight of above-ground biomass		Grain yield		ELISA absorbance 450 nm (I)	Degree of susceptibility
	cm (I)	A		(I)	A	g/plant (I)	A	g/plant (I)	A		
Six-rows											
Borwina	25.7	54.4	92.9	1.5	24.4	1.7	71.8	0.4	88.7	1.380	S
Kamil	26.7	47.4	100.0	2.0	-1.7	1.8	67.2	0.7	79.2	1.379	S
Kromir	23.7	58.7	93.0	3.2	10.2	1.8	83.2	0.6	90.8	1.356	VS
Kromoz	20.0	64.7	94.0	2.8	-1.8	1.3	83.0	0.3	92.8	1.072	VS
Lunet	33.3	40.1	100.0	2.0	2.4	2.4	63.8	0.7	81.6	1.241	S
Luxor	29.0	53.2	98.9	2.3	-4.6	1.9	74.7	0.7	83.7	1.272	S
Okal	23.3	52.7	93.6	1.8	30.1	1.2	85.7	0.3	94.0	1.262	VS
Sigra	14.3	75.3	59.6	0.7	82.7	0.7	94.6	0.1	99.3	1.096	VS
Two-rows											
Agrilo	15.3	72.3	96.6	5.2	-73.5	1.1	80.6	0.3	91.0	1.082	VS
Babylone	19.7	66.1	95.8	4.1	-17.1	1.8	76.1	0.5	86.9	1.380	S
Marinka	24.3	54.9	57.3	1.1	66.7	2.3	65.4	0.1	96.4	1.415	VS
Marna	23.3	53.9	100.0	5.2	-30.0	2.1	71.9	0.8	80.8	1.403	S
Monako	28.3	48.5	98.1	5.4	-23.7	2.3	71.1	0.6	86.3	1.308	S

A = 100 - I/C.100; I - infected variant; C - uninfected variant; VS - very susceptible; S - susceptible

highest in Sibra (75.3%) and Agrilo (72.3%). WDV infection affected the heading ability and the number of produced ears in most varieties. The proportion of heading plants reached 57.3% and 59.6% in the varieties Marinka and Sibra, respectively, while in the varieties Agrilo, Babylone, Borwina, Kromir, Kromoz, Luxor, Monaco and Okal it ranged from 93.6% to 98.9%. 100% heading was observed in the varieties Kamil, Lunet and Marna. The ear number in six-rowed varieties was reduced by 2.4–82.7%, except for Kamil, Kromoz and Luxor. The reduction in ear number was highest in the six-rowed variety Sibra (82.7%) and in the two-rowed variety Marinka (66.7%). The other varieties of two-rowed barley produced more ears than the respective controls (by 17.0–73.5%).

The strongest effect of WDV was on grain yield, which was reduced by 79.2 to 99.3%. The tested collection could be accordingly divided into two groups. The first group consisted of 6 varieties in which the grain yield per plant was reduced by more than 90 %. These were the two-rowed varieties Agrilo and Marinka and the six-rowed varieties Kromir, Kromoz, Okal and Sibra. The other group with grain yield reduction from 79.2 to 88.7% comprised the two-rowed barley varieties Babylon, Marna and Monaco and the six-rowed barley varieties Borwina, Kamil, Lunet and Luxor. As grain yield is the basic criterion of how much the varieties suffer from the disease, the first group of cultivars can be classified as highly susceptible, the other as susceptible. The weight of the aboveground biomass of infected plants was reduced from 63.8% (Lunet) to 94.6% (Sibra).

Abstrakt

VACKE J., CIBULKA R. (2001): **Reakce registrovaných odrůd ječmene ozimého na infekci virem zakrslosti pšenice.** Czech J. Genet. Plant Breed., 37: 50–52.

V maloparcelkových polních pokusech byla studována reakce na infekci ječným kmenem viru zakrslosti pšenice (WDV) u 13 registrovaných odrůd ječmene ozimého. Tyto odrůdy byly experimentálně infikovány na podzim ve fázi odnožování. Ječný kmen WDV vyvolal na sledovaných odrůdách silnou symptomatickou reakci, která se projevila zakrslostí rostlin, poruchami v metání, žlutým zabarvením listů, redukcí hmotnosti nadzemní biomasy a výnosu zrna. V testovaném sortimentu nebyla zjištěna žádná odolná odrůda. Jako silně náchylné, s poklesem výnosu zrna nad 90 %, byly klasifikovány odrůdy Agrilo, Marinka, Kromir, Kromoz, Okal a Sibra. Zbývající odrůdy (Babylone, Borwina, Kamil, Lunet, Luxor, Marna a Monaco) byly ohodnoceny stupněm náchylný. Redukce výnosu zrna u této skupiny odrůd se pohybovala od 79,2 do 88,7 %.

Klíčová slova: ječmen ozimý; ječný kmen viru zakrslosti pšenice; reakce na infekci

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ELISA technique was used to determine the relative virus content in the sap from leaves of infected plants. Based on this, we tried to determine the degree of susceptibility. However, the ELISA results did not reveal any significant differences in the relative content of virus protein between the cultivars. The average value of absorbance was 1.213 and 1.337 in the group of very susceptible and susceptible cultivars, respectively.

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Estimation of Genetic Parameters of a Potato Collection from Incomplete Historical Data

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Abstract: A highly non-orthogonal data set from a potato collection with 226 genotypes of diverse origin was evaluated from 1981 to 1984 for important agronomic traits. It was statistically analysed by methods suited for incomplete data. The most likely genetic ranking was obtained by the Least Squares (LS) analysis of individual traits. Heritability estimates were obtained independently from variance components and from correlations between years. The estimates obtained with both methods were very similar, highly correlated ($r = 0.92$) and highly significant for all evaluated traits, indicating a considerable potential for genetic improvement. Heritability was highest for earliness (0.89 and 0.88), tuber yield (0.55 and 0.61) and virus infection (0.46 and 0.45), lowest for percentage of crude protein (0.19 and 0.22). The combination of the best trait values was chosen as the target ideotype for multitrait ranking. After standardisation of all traits the genotypes were ranked according to similarity to the ideotype, measured as linear distance from the ideotype in a multidimensional space. Since most of the evaluated genotypes still exist, the analysis might still be of practical value for potato breeding, since among the top ranking genotypes promising parents or potential varieties can be found.

Keywords: potato breeding; genetic resources; collection of genotypes; heritability; variance components; multivariate selection; similarity with ideotype; least squares

Although large potato collections exist in the world, see for example HUAMAN and SCHMIEDICHE (1999), usually only passport and basic agronomic data of individual entries are known, while population parameters are missing. A set of historical data of potential value for potato breeding, not yet statistically evaluated, stimulated us to extract useful genetic information from the data. The data referred to a collection of 226 potato genotypes of world-wide origin, a greater part of which still exists. This collection was between 1981 and 1984 evaluated for a number of agronomic traits in a screening program, aimed to select potential parents for breeding purposes. The screening trials consisted of non-replicated plots. Every year some dissatisfying entries had been eliminated and a part of them replaced by new ones. The data set was therefore highly non-orthogonal and decreased in size during the screening. Although the data were not suited for a conventional statistical analysis, valuable information was evidently contained in the data. We tried therefore to obtain by exploratory statistical analysis at least rough estimates of genetic parameters of the investigated collection and derive some practical suggestions for potato breeding.

MATERIALS AND METHODS

Biological material

The evaluated samples were clones of 226 genotypes of various origin, already pre-screened from larger collections according to published data or previous observation. The collection consisted of samples from the Gene Bank of the Potato Research Institute (PRI) at Havlíčkův Brod in Bohemia and from the Potato Research Institute at Velká Lomnica, Slovakia. It included advanced breeding lines from several resistance breeding projects, including inter-specific hybrids with *Solanum acaule*, *S. stoloniferum* and *S. demissum* (RAJČANOVÁ *et al.* 1980; VIDNER & NOVOTNÁKOVÁ 1980). Only 29 genotypes were evaluated in all four years. A survey of all clones and of years, in which they were evaluated, is given in Table 1. The samples had been grown on non-replicated 5 m² plots consisting of 2 rows, each with 20 plants. Data relating to following traits were available for the statistical analysis:

Primary traits:

1. Earliness (in scores from 1 – very late to 9 – very early)

2. Yield of tubers (t/ha)
3. Dry matter (DM) content (% in tubers)
4. Crude protein content (% in tubers)
5. Soluble protein content. (% in tubers)
6. Proportion of virus diseased plants (%)
7. Proportion of fungus diseased plants (%)

Derived traits:

8. DM yield (t/ha)
9. Crude protein yield (t/ha)
8. Soluble protein yield (t/ha)
9. Crude protein in DM (%)
10. Soluble protein in DM (%)

Except earliness, all traits were quantitative. Since the population was large and the earliness scores could be regarded as rounded transformed values, we treated the scores also as quantitative values. This might result in a slight bias of the resulting earliness statistics, however without relevance for practical breeding, since this does not affect the ranking of the evaluated genotypes.

Statistical procedures

The principle aim was to estimate the performance of the evaluated genotypes. Since the data were highly non-orthogonal, a Least Squares Analysis (LSA) was used, which is since the paper of FINNEY (1980) the usual tool to estimate variety effects from incomplete data. The collection did not represent a random sample from a larger population. We regarded the 226 genotypes therefore as the base population, whose parameters had to be estimated. Due to non-orthogonality the values of individual genotypes were estimated with different precision. The error variances of the primary data were unknown, but were contained in the residual variance obtained by the statistical analysis.

To estimate the genotype effects, year effects and residual effects we used for the LSA the linear model:

$$Y_{ij} = u + a_i + b_j + r_{ij}$$

where: Y_{ij} – trait value of genotype i at year j
 u – general mean
 a_i – effect of genotype i
 b_j – effect of year j ,
 r_{ij} – residual effect

Since the data did not permit to distinguish between genotype \times year interactions and random errors, the residual effects included both. The system of linear equations for the genotypes was solved iteratively, using a standard procedure of the FTAB – plant breeding spreadsheet. Broad sense heritability of the base population for the individual traits was independently estimated by two methods:

1. Since the LSA procedure of FTAB provides estimates of variance components, estimates of broad sense heritability were calculated as

$$\frac{V_g}{V_g + V_r} \quad (1)$$

where: V_g – variance of genotypes
 V_r – residual variance for the given trait

The procedure estimates the genetic variance by subtracting the error variance of genotype means (V_r/n), properly weighted, from the variance of LS-adjusted genotype means.

2. Heritability was also estimated by calculating the correlation between the years for a given trait across all genotypes. This is justified by the following reasons: The correlation coefficient r_{xy} between the years x and y is defined as

$$r_{xy} = \frac{\text{Cov}_{xy}}{\sqrt{V_x V_y}} \quad (2)$$

where: Cov_{xy} – covariance of genotypes between the years x and y
 $V_x V_y$ – total variance within the years x and y

Since Cov_{xy} is entirely due to genotype effects, it reflects the variance of genotypes. Since the denominator represents the geometric mean of the phenotypic variances within both years, i.e. the sum of genetic and error variances, the formulas (1) and (2) can be regarded as equivalent and the correlation coefficient between years can be regarded as a heritability estimate. Since evaluation was performed in four years, the average of all 6 possible correlations was used, in order to obtain more precise estimates. To calculate the correlations and their means the FTAB program was used.

For the selection of genotypes, the multiple trait selection procedure of FTAB was used. This involves the calculation of linear distances between genotypes and an ideotype in a multidimensional space, where each trait in standardized units represents one dimension. The closeness to the ideotype, measured as linear distance, was taken as measure of the genotype value. The intensity of selection in the traits was specified by assigning subjective weights, based on breeders experience, to the individual traits. One out of three methods of standardization (i.e. divide the primary values by the standard deviation from the mean, by the trait mean or by the variation range) had to be chosen. We used the variation range as the standardization unit, since this constructs the ideotype as the combination of the best trait values in the collection. In contrast to Mahalanobis' generalised distance, correlations between traits are ignored, since for the breeder the actual trait values of the selected genotypes matter, regardless of how the source population was composed. Existing correlations within the source population have just the effect, that the frequency of desired genotypes in the source population is smaller or higher than expected.

Table 1. Evaluated potato clones and their evaluation in individual years

Genotype	Origin	81	82	83	84	Genotype	Origin	81	82	83	84	Genotype	Origin	81	82	83	84	Genotype	Origin	81	82	83	84
Abnaki	US	+	+	+		KE 17/276	CZ	+	+			Sosna	SK	+	+	+	+	VE 64/9	CZ	+	+	+	
Adretta	D	+	+			KE 47/21	CZ	+	+	+	+	SR 10	SK		+			VE 83/62	CZ	+	+	+	
Aguti	D		+	+	+	Ker. rohl.	CZ	+				SR 42	SK	+	+	+		VE 104/39	CZ	+	+		
Astilla	D	+				Kontr. C-2	D	+				SR 44	SK	+				VE 109/16	CZ	+			
Binia	D	+	+			Kontr. D-2	D	+				SR 58	SK	+	+	+	+	VE 227/179	CZ	+			
Bison	D	+				Lin. 975/57	D	+				SR 70	SK	+	+	+	+	Vera	CZ	+			
B 74-51/106	D		+			Linz. Fruhe	D	+				SR 77	SK	+	+	+	+	Vindika	NL	+			
Condea	D	+				Lipa	SK	+	+	+		SR 78	SK	+	+	+	+	VL 9/78	SK				+
Dirus	D		+	+		Lucia	D		+	+		SR 80	SK	+				VL 30/73	SK	+	+		+
Div.hyb.	D	+				Lu 5	D	+				SR 81	SK	+				VL 40	SK				+
Duet	PL		+			Lu 7	D	+				SR 85	SK	+	+	+	+	VL 50/77	SK				+
Edith	D		+			Lu 8	D	+	+			SR 96	SK	+				VL 60/70	SK	+	+	+	+
Elan	D		+	+	+	Lu 105	D	+				SR 97	SK	+				VL 69/73	SK	+			
Epic.× Kat.	SU	+				M 6	H	+	+			SR 101	SK	+	+	+	+	VL 80/70	SK	+	+		
Erna	D		+			M 7	H	+				SR 102	SK	+	+			VL 84/70	SK	+			
Eta	SK	+	+			M 8	H	+	+			SR 105	SK	+	+	+	+	VL 88/77	SK				+
Fanal	D	+	+	+	+	M 9	H	+	+			SR 107	SK	+	+			VL 163/68	SK	+			
Fausta	D		+			M 10	H	+	+	+		SR 127	SK	+	+	+	+	VL 239/68	SK	+			
Galina	D		+			Manuella	D		+			SR 130	SK	+				VL 265/72	SK	+			
Geisha	D		+	+		Marion	D	+				SR 132	SK	+	+	+	+	VL 269/72	SK	+			
Gitte	D	+	+			Miranda	D	+	+			SRLP 1	SK	+	+	+		VL 292/71	SK	+	+		
Granola	D	+	+	+		Monalisa	D		+	+		SRLP 4	SK	+	+	+		Wachtel	D		+	+	+
Gresus	B	+				Moni	D		+	+	+	SRLP 5	SK	+	+			Werta	D	+			
Gusto	D		+	+	+	MPI 10	D	+	+	+		SRLP 9	SK	+	+			Wirgo	NL		+		
Hertha	D		+	+	+	MPI 12	D	+				SRLP 10	SK	+	+	+	+	X 66 58/58	CZ	+	+		+
Hindenburg	D	+				MPI 61	D	+				SRLP 15	SK	+	+			Y 56/6	CZ	+	+		
HR 17	CZ		+			MPI 63	D	+	+	+		SRLP 19	SK	+				Y 56/145	CZ	+	+		
H.v.1	SK		+			MPI 110	D	+				SRLP 21	SK	+				Y 65/3	CZ	+	+		
H.v.2	SK		+			MPI 151	D	+	+	+	+	SRLP 29	SK	+				Zi 1	SK	+			
H.v.4	SK		+			N 2	D/SK	+				SRLP 30	SK	+	+	+	+	Zi 2	SK	+	+		
H.v.6	SK		+			N 9	D/SK	+				SRLP 32	SK	+				Zi 3	SK	+	+		

RESULTS AND DISCUSSION

The estimates of heritability with both methods are summarised in Table 2, together with the trait means and the genetic standard deviations. Since the distributions of percentage of virus and fungus diseased plants are very skewed, as indicated by variation coefficients (i.e. the genetic standard deviation relative to the trait mean) close to 1, the percentage of diseased plants was logarithmically transformed. This improved the distribution, as is reflected by the smaller coefficients of variation, but did not have an apparent effect upon the heritability estimates, indicating the robustness of the estimation methods.

There were no significant differences between heritability estimates obtained by the two methods. The estimates were highly correlated ($r = 0.92^{**}$) and highly significant in all evaluated traits. Therefore a considerable potential of genetic improvement can be assumed for each trait. The space for possible genetic improvement can be roughly deduced from the genetic standard deviations of the traits, since approx. 95% of the genetic variation is expected in the interval of two such deviations above and below the mean.

The heritability estimates might be biased due to the changing composition of the evaluated population during the four years. Effective selection shifts the population mean and the selected sub-population is expected to be more homogeneous. In contrast, new genotypes replacing out-selected ones might increase the variation. Therefore we tried to monitor, what actually happened

due to the changing composition of the collection. Since at least two years were necessary for the estimation of genetic variance, we estimated the genetic variance separately for the first two and the last two years, taking the covariance between each two years as the estimate. For the same periods we calculated also the trait means and adjusted them by subtracting the year effects, according to the LSA. To calculate the covariances, means and year effects, standard functions of the FTAB program were used. Table 3 summarises, what actually happened during the four years.

The data indicate, that in the last two years the collection contained considerable less virus infected plants. Since year effects were largely removed by the Least Squares procedure, the adjusted means might reflect real shifts towards more virus resistant genotypes. The shift might even be underestimated, since the genotypes are clones, in which viruses may accumulate during the years. In contrast, the collection was in the last two years considerably more susceptible to fungi. The shifts in other traits were generally small and not statistically significant. The estimates of genetic variance generally increased between the compared periods. This might be due to new genotypes entering the collection, but also to the screening for virus resistance at the expense of other traits. The estimates of genetic variance of the disease traits are, however, biased by the skewness of the distribution (see Table 2), as can be seen from the comparison of not-transformed and of logarithmically transformed disease data.

Table 2. Heritability h^2 of traits estimated by two methods, trait means and genetic standard deviations of the whole collection ($n = 226$)

Trait	h^2 by pooled correlation between years	h^2 from variance components	Trait mean	Genetic standard deviations from variance components
Earliness score (9 = earliest)	0.89	0.88	5.12	1.42
Tuber yield (t/ha)	0.55	0.61	29.87	7.66
DM in tubers (%)	0.44	0.61	19.11	1.66
Crude protein (%)	0.19	0.22	2.52	0.14
Soluble protein (%)	0.36	0.41	1.40	0.14
Virus infected plants (%)	0.46	0.45	7.24	6.43
log virus infected ^{*)}	0.51	0.42	1.72	0.62
Fungi infected plants (%)	0.20	0.24	3.53	2.61
log fungi infected ^{*)}	0.29	0.22	1.39	0.36
Dry matter yield	0.56	0.64	5.72	1.67
Crude protein yield	0.52	0.46	0.75	0.17
Soluble protein yield	0.56	0.59	0.41	0.17
Crude protein in DM (%)	0.25	0.39	13.33	1.28
Soluble protein in DM (%)	0.21	0.32	7.28	0.79

^{*)} transformed as $y = \log_{10}(x + 1)$

All heritability estimates are highly significant ($P < 0.01$, $DF = 179$)

Table 3. Effect of composition of the population on population mean and on genetic variance

Trait	Adjusted mean		Change (%)	Genetic variance	
	81–82	83–84		81–82	83–84
Earliness*)	5.18	4.97	-4.1*	1.70	1.88
Tuber yield (t/ha)	29.70	30.68	3.3	43.7	79.6
DM in tubers (%)	19.29	19.55	1.3	1.61	2.75
Crude protein (%)	2.55	2.62	2.7	0.016	0.025
Soluble protein (%)	1.42	1.46	2.8	0.017	0.012
Virus infected plants (%)	7.70	4.22	-45.2**	17.4	30.0
log virus infected plants	1.70	1.44	-15.3**	0.300	0.400
Fungi infected (%)	3.68	4.37	18.8**	6.11	3.96
log fungi infected	1.35	1.50	11.1**	0.149	0.191

*) earliness in scores, 1 = latest, 9 = earliest

Correlations between traits offer another way of monitoring population changes. In an artificial collection of fixed clones without segregation, correlations between traits reflect the composition of the collection and are not necessarily related to a genetic or physiologic relationship between the primary traits. Comparison of trait correlations of the first two years with those of the last two years, therefore, reflect changes in the composition of the population across the two periods.

The correlations indicate, that the population did not much change across the compared screening periods. In the last two years the population contained a markedly higher proportion of early genotypes with less virus infections and also of higher yielding genotypes with lower protein content. In both periods the genotypes with higher tuber yield were less virus infected than the lower yielding ones. Different infection pressure in the four years may also have influenced the results, although the

statistical procedures eliminate at least mathematically the year effects.

Unfortunately, it is not well documented, how the data on the observed traits have been used in the subsequent years in practical breeding, since programs, persons and organisations have changed in the meantime. A part of the genotypes still exist in the potato gene bank of the PRI at Havlíčkův Brod (DOMKÁŘOVÁ 1999) and a part can be traced back in Slovak official trials. However, it is possible to show, what could have been done, based on the statistically analysis.

We applied therefore the mentioned multiple trait selection procedure of FTAB to the whole collection, using all available data and choosing the variation range of each trait as the unit of standardisation. An ideotype was constructed automatically by the combination of the best LS-adjusted trait values within the collection. Economic weights, based on breeders experience, were given to the

Table 4. Correlation between traits in the first and the second half of the screening period

Trait	Years	Tuber yield	DM (%)	Crude protein (%)	Sol. protein (%)	Infected (%)	
						virus	fungus
Earliness (9 = earliest)	81–82	0.15*	-0.15*	0.12*	-0.06	-0.01	-0.10
	83–84	0.14	-0.21	0.20	0.04	-0.41**	0.05
Tuber yield	81–82		0.10	-0.19**	-0.04	-0.35**	-0.24**
	83–84		0.05	-0.43**	-0.23	-0.43**	-0.26
DM content	81–82			0.16**	0.39**	-0.02	-0.02
	83–84			0.17	0.47**	-0.04	-0.11
Crude protein (%)	81–82				0.45**	-0.06	-0.06
	83–84				0.72**	0.02	0.19
Soluble protein (%)	81–82					-0.05	-0.03
	83–84					0.01	0.18
Virus infected	81–82						-0.06
	83–84						0.10

The figures are pooled correlations for the 81–82 or the 83–84 period, respectively

Table 5. Multivariate ranking of the collection based on ideotype similarity

	Rank	Earliness (9 = earliest)	Tuber yield (t/ha)	DM (%)	Crude protein (%)	Solub. protein (%)	Virus diseased plants (%)	Fungus diseased plants (%)	Multivariate value*)	Known utilisation
Weight		4	10	5	2.5	2.5	-15	-15		
IDEOTYPE		9.0	54.4	26.4	3.6	1.9	0.0	0.0	9.00	
The top 30 entries										
H.v.15	1	8.0	46.6	20.0	2.6	1.5	0.0	2.6	7.86	
Rotkehlchen	2	6.0	43.5	21.9	2.7	1.5	4.6	2.6	7.77	
VL 9/78	3	6.0	46.4	20.5	2.3	1.5	0.9	2.7	7.74	1997 ALVA
VL 239/68	4	8.9	41.7	21.0	2.8	1.3	6.1	0.0	7.71	1981 ETA
Fausta	5	6.0	39.3	24.3	2.6	1.6	2.3	4.8	7.63	
VL 265/72	6	5.9	44.4	18.3	2.8	1.7	6.1	0.0	7.59	off. trials 81
Erna	7	6.0	50.9	21.4	2.0	1.2	9.2	2.6	7.57	
Sosna	8	6.0	38.6	21.3	2.7	1.5	3.5	2.1	7.57	
M 7	9	7.9	36.9	21.1	2.5	1.6	3.8	0.0	7.56	
Gusto	10	4.7	45.6	19.7	2.5	1.2	1.2	2.1	7.55	
Galina	11	6.0	37.6	24.0	2.1	1.5	0.0	2.6	7.54	
H.v.29	12	7.0	39.9	19.9	2.4	1.4	0.0	2.6	7.53	
Y 56/145	13	5.5	42.4	18.6	2.6	1.5	1.9	1.3	7.53	
VL84b/70 ^{x)}	14	6.0	45.0	20.2	2.4	1.4	5.2	5.2	7.53	1983 REMA
H.v.27	15	4.0	38.4	23.0	2.7	1.6	0.0	2.6	7.52	
Miranda	16	7.5	38.9	19.8	2.7	1.4	1.9	3.5	7.51	
MPI 10	17	7.0	38.4	19.3	2.7	1.5	4.7	1.8	7.47	
65/3	18	5.9	37.7	20.3	2.5	1.6	3.8	2.2	7.45	
VL 50/77	19	4.0	45.9	20.5	2.4	1.3	3.2	4.9	7.45	off. trials 84
Rental	20	5.0	46.4	19.0	2.2	1.2	4.6	2.6	7.44	
Edith	21	4.0	36.8	23.2	2.5	1.7	0.0	2.6	7.44	
Wachtel	22	6.0	40.8	18.4	2.7	1.4	5.0	2.1	7.43	
VL 88/77	23	6.0	36.4	22.7	2.5	1.4	3.2	4.9	7.39	off. trials 84
Kar.308	24	3.9	44.4	21.8	2.3	1.1	8.4	2.2	7.39	
Fanal	25	4.0	39.7	20.7	2.5	1.7	6.4	2.7	7.39	
H.v. C.kl 7	26	3.0	39.7	24.5	2.5	1.5	3.2	4.9	7.38	
H.v.19	27	3.0	40.6	21.0	2.4	1.5	0.0	2.6	7.37	
N 20	28	7.9	51.7	18.4	2.4	1.4	15.3	0.0	7.37	
VL 84/70 ^{x)}	29	5.9	39.9	19.1	2.5	1.6	6.1	4.4	7.36	1983 REMA
VL 40	30	4.0	43.6	26.4	2.5	1.4	12.4	4.9	7.36	off. trials 83
Population mean		5.2	29.8	19.2	2.5	1.4	9.5	4.8	6.6	
The bottom 10 entries										
SRLP 29	217	6.9	20.0	19.1	2.4	1.5	8.4	26.4	4.86	
N 41	218	5.9	17.8	13.9	2.5	1.5	45.2	0.0	4.66	
H.v.57	219	8.0	18.9	17.9	2.5	1.2	48.3	2.6	4.65	
N 18	220	3.9	17.5	18.4	2.2	1.6	49.8	0.0	4.49	
Kontr.C-2	221	2.9	20.5	16.2	2.6	0.9	3.8	28.6	4.46	
N 42	222	3.9	12.9	20.7	2.4	1.3	52.1	0.0	4.24	
78.56/142	223	4.9	26.1	17.4	2.5	1.7	8.4	35.2	4.06	
SR 97	224	3.9	13.5	21.7	3.6	1.8	61.3	8.8	3.68	
SSB 1301	225	6.9	14.7	18.3	2.1	1.3	3.8	39.6	3.33	
SR 96	226	3.9	11.2	23.0	2.7	1.6	68.2	0.0	3.29	

*) linear distance to the ideotype transformed to a 9 to 1 scale, with 9 = closest (= zero distance)

x) replicates of the same genotype

traits. The entries were finally ranked according to their closeness to the ideotype. For the convenience of breeders, familiar with scores, the distance from the ideotype was converted to a 9 to 1 scale (9 = closest). The results are summarised in Table 5. The signs of the weights in the table specify the direction of selection. For simplicity, only the top 30 and the last 10 entries are shown in Table 5. The last column indicates, which entered official trials or were released as varieties.

From the 226 entries 15 were tested in official trials in Slovakia and four of them entered the national variety list. Three of these four released varieties were among the top 30 entries in Table 5. This clearly indicates the benefits, breeders can expect from this kind of statistical evaluation, even though the statistical assumptions were far from being satisfied. Greater benefits could possibly be expected from the selection of parents for crossing, but, unfortunately, no data of this kind were available to us.

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Abstrakt

DEBRE F., SCHWARZBACH E. (2001): **Odhad genetických parametrov kolekcie brambor z neúplných historických dát.** Czech J. Genet. Plant Breed., 37: 53–60.

Soubor historických veľmi neortogonálnych dát z kolekcie 226 genotypů brambor, ktorá bola v letech 1981 až 1984 vyhodnocovaná podľa dôležitých užitkových znakov, bol štatisticky spracovaný metódami vhodnými pre neúplné dáta. Najpravdepodobnejší genetické ťaženie odrůd v jednotlivých vlastnostiach bolo zisťované metódou najmenších čtvrců. Odhady heritability boli získané z komponent variance a rovněž, nezávisle, z medzi-ročníkových korelácií v rámci jednotlivých vlastností. Odhady heritability oboch metódami boli veľmi podobné, vysoce korelované ($r = 0,92$) a vysoce průkazné vo všetch sledovaných znakov, čo poukazuje na značný priestor pre šlechtiteľský pokrok. Odhad heritability bol najvyšší pre rannosť (0,88 a 0,89), výnos hlíz (0,55 a 0,61) a podíl virózných rastlín (0,46 a 0,45) a najnižší pre percento hrubých bielkovín (0,19 a 0,22). Kombinácií najlepších hodnôt v jednotlivých znakov, zistených metódou najmenších čtvrců v celej kolekcii, bol definovaný cieľový ideotyp pre multivariátnu triedu. Po štandardizácii všetch hodnotených znakov boli všetky genotypy seřazené podľa podobnosti s ideotypem, mēřená jako lineární vzdálenost od ideotypu ve vícerozměrném prostoru. Protože většín část vyhodnocovaných genotypů stále ještě existuje, muže provedená štatistická analýza být prospěšná pro šlechtění brambor, protože mezi nejvyšě řazenými genotypy lze nalézt slibné výchozí materiály pro šlechtiteľský pokrok vo všetch sledovaných znakov.

Klíčovú slova: šlechtění brambor; genové zdroje; kolekcie genotypů; heritability; komponenty variance; multivariátna selekcie; podobnosť s ideotypem; metóda najmenších čtvrců

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Use of Simple Sequence Repeats Analysis to Reveal DNA Polymorphism in Potato

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Abstract: Twenty-two primer pairs flanking simple sequence repeats (SSRs) were examined for their potential use in DNA fingerprinting of thirty potato cultivars, mainly of Czech origin. Amplification with twenty-two primer pairs yielded 76 products, 56 of which were polymorphic. The amplification products based on polymerase chain reaction, generated from each primer pair, consisted of 1 to 6 bands within individual cultivars. A similarity matrix derived from fourteen SSRs distinguished clearly all cultivars. The utilisation of the SSRs analysis is discussed.

Keywords: potato; DNA polymorphism; SSRs; microsatellites; cultivar identification

The identification of varieties of field crops is important at every stage of the agricultural production chain. All those involved in crop production – plant breeders, seed producers, merchants, farmers and other users – benefit from variety identification and discrimination. There is also increasing interest in the descriptive definition of varieties for the purpose of intellectual property protection and verification or quality control (COOKE 1999).

Traditionally, potato cultivars have been identified by morphological characteristics such as flower colour, growth habit, leaf type, disease reaction and sprout and tuber type. However, limitation to this type of identification exists since many of these characteristics are subjective and often influenced by the environment. Furthermore, these morphological traits cannot be scored simultaneously nor cultivar identification can be performed quickly and efficiently (SCHNEIDER & DOUCHES 1997). There are thus good reasons to investigate other means of variety identification to minimise these problems and a number of different approaches have been suggested that are proving useful in many crops, including potatoes:

- various methods of gel electrophoresis to analyse isozymes and proteins
- DNA profiling, including probe-based (RFLP), amplification-based (RAPD, SSRs analysis) and 'second generation' methods such as AFLP (COOKE & REVEES 1998).

One of these approaches consists in the analysis of simple sequence repeats (SSRs). SSRs are also referred to as short tandem repeats or microsatellites. SSRs or microsatellites are highly polymorphic tandemly repeated DNA sequences with a basic repeating unit of 2–8 that are flanked by conserved DNA sequences (RAMEL 1997). The polymorphism found in microsatellites is due to variations in the copy number of this basic repeat unit. The most frequently observed repeat in plant species is (AT)_n, which has been observed to be randomly distributed across the genome, tri- and tetra- nucleotide repeats are also abundant within plant species (WANG *et al.* 1994). This class of DNA markers is attractive because of their abundance and relatively simple technical requirements. Simple sequence repeats have demonstrated high levels of polymorphism in many plant species including barley (SAGHAI MAROOF *et al.* 1994), tomato (PHILLIPS *et al.* 1994), rice (MCCOUCH *et al.* 1997), maize (SENIOR *et al.* 1993) and brassica (POULSEN *et al.* 1993). Microsatellites were used to study polymorphism among potato cultivars (SCHNEIDER & DOUCHES 1997; PROVAN *et al.* 1996b), to study somatic hybrids of potato (PROVAN *et al.* 1996a) and in many others applications.

Our study has been aimed at developing useful molecular markers for characterisation of potato varieties by means of SSRs analysis.

MATERIALS AND METHODS

Plant material and DNA isolation. DNA polymorphism has been assessed in 30 cultivars registered in the Czech Republic:

- a) very early variety: Karmela, Koruna, Krasa, Krystala, Satina, Vera, Rosara and Impala
 b) early variety: Karin, Kobra, Kreta, Veronika, Vilma, Secura and Dali
 c) medium – early variety: Keřkovské rohličky, Korela, Krista, Tara, Velox, Vladan, Granola and Folva
 e) medium – late variety: Amylex, Javor, Ornella, Pacov, Zlata, Saturna, and Asterix.

DNA was extracted from fresh leaf material collected in greenhouse and in field, from *in vitro* plants and from tubers using Dneasy Plant Mini kit (Qiagen). The DNA samples were quantified by fluorometry (Perkin Elmer) and were diluted to 200 ng/μl for PCR.

SSRs analysis. Simple sequence repeat polymorphism analysis was performed using the 19 primer sets listed in MILBOURNE *et al.* (1998) with the addition of two primer sets (PP and PIG) mentioned in SCHNEIDER and DOUCHES (1997) and one primer set (PIP) introduced by PROVAN *et al.* (1996a). The description, the sequence of the repeats and the localisation in genom are shown in Table 1.

DNA from each potato sample was amplified in a 25 ml reaction consisting of 200 ng template DNA, 200 μM each

of dATP, dTTP, dCTP and dGTP (Sigma), 10 pmol each flanking primer (IDT – Integrated DNA Technologies, Inc.), 1 × reaction buffer – magnesium free (50 mM KCl, 10 mM Tris-HCl, pH 9.0 at 25°C, 0.1% Triton X-100) and 0.5 U Taq DNA polymerase (Promega). The MgCl₂ concentration for this reaction was optimised for each primer set (Table 2). Amplification was performed using the thermal cycler PTC-100, and the protocol was: initial denaturation 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at T_{ann} and 1,5 min at 72°C, last step was extension 5 min at 72°C (PROVAN *et al.* 1996b). T_{ann} temperature for annealing was also optimised by primer set as shown in Table 2. The complete reaction products were held at 4°C until electrophoretic evaluation.

The PCR products were separated on a 3% agarose gel stained with ethidium bromide (0.25 μg/ml). The gels were run at 100 V for 4 hours at room temperature and the products were viewed by ultraviolet light, photographed and saved.

Statistical analysis. The SSRs were visually scored. Band presence is indicated by 1 and absence by 0. The similarities of SSRs profiles were estimated with the Jaccard's coefficient (JACCARD 1908). The resulting data were subjected to a cluster analysis using the UPGMA (unweighted pair group average). Data analysis was performed using the software SPSS Base+.

Table 1. Oligonucleotide sequences and associated information for the used primer pairs

Name	Primer sequence 5'–3'	Repeat	Product size (bp)	Location	LG
STM 0040	F GCAATAATGGCCAACACTTC R TGGGAAATGTTAGTCAAAAATAGC	(AT)20AG...(AT)2(GT)11	140 85–100		III, XII
STM 0051	F TACATACATACACACACGCG R CTGCAACTTATAGCCTCCA	(AC)7...(AC)7(AT)4	115		X
STM 1008	F GTACACAGCAAAAATAGCAAG R TAGACACTCTCACATCCACT	(AT)10	140	CP55	II, IV, IX
STM 1009	F ATTAGCATACGACTCAAC R TTATTTTCATTTTCAGC	(AT)30	198	CP54	VII, XI
STM 1020 (II KIG)	F TTCGTTGCTTACCTACTA R CCCAAGATTACCACATTC	(T)12(A)9...(TA)7...(ATA)6	216	HK inhibitor gene intron	III, V
STM 1045	F GAAGTTTTATCAGAATCC R ATCACCTCATCAGCAATC	(TGG)5	181		II, XII
STM 1050	F GTACATATATACAATTATCTAACCG R TTCTCTATGTTAGGCTAGAGTG	(AT)16	130	5'–UTR	II, IV, VI, VII
STM 1056	F AGGTAAGTTTTATTTTCAATTGC R GGGTATGGGAATAGGTAGTTT	(AAAAT)4	229	INTRON2	VIII
STM 1102	F GGAAGAATTTGTAGGTTCAA R AAAGTGAAACTTCTAGCATG	(TA)8	167	5'–UTR	I, IX
PP (SSR 7)	F CAACCAACAAGGTAAATGGTACC R TGGTCTGGTGCATTAGAAAAA	(AATT)5	386	<i>patatin</i> pseudogene	

Table 1 to be continued

Name	Primer sequence 5'–3'	Repeat	Product size (bp)	Location	LG
PIG (SSR 2)	F CTGCAACTGTAGTACCCC R AAATCCTTTGTGACCTCCC	(TC)12...(TA)18	180	<i>proteinase inhibitor gene</i>	
PIP	F TGTACTGGGAGCCTCAAAG R AATTTAACCTCGTGACATGGG	(TA)23	500	<i>proteinase inhib. pseudogene</i>	
STM 0004	F CGAGGGCGTAAACTCATGATA R AGGTATTGTGGACACAGTCTTCA	(AC)9(AT)4(AC)5	149		VII
STM 1024	F ATACAGGACCTTAATTTCCCAA R TCAAAACCCAATTCAATCAAATC	(TTG)6	143	5'–UTR	VIII
STM 1041	F GTTGAGTAGAAGGAGGATT R CCTTTGTCTTCTGCCTTTT	(GAA)5	98	CDS	V
STM 1064 (Legast)	F GTTCTTTGGTGGTTTCCT R TTATTTCTCTGTTGTTGCTG	(TA)12...(TG)4 GT (TG)5	204	INTRON1	II
STM 1069 (Lepdsge)	F ATGCTAAGTGGACACTTA R AGTCTCTCAGGAGGATTAC	(TCC)4	393	INTRON8	III
STM 1104	F TGATTCTCTGCCTACTGTAATCG R CAAAGTGGTGTGAAGCTGTGA	(TCT)5	168	3'–UTR	VIII
STM 2002	F AGAACCATTGATGCATATCCC R TTGTAAGTTGGGATGAAGCG	(TTG)3...(AC)3	159		
STM 2005	F TTTAAGTTCTCAGTTCTGCAGGG R GTCATAACCTTTACCATTGCTGGG	(CTGTTG)3	166		XI
STM 2020	F CCTTCCCCTTAAATACAATAACCC R CATGGAGAAGTGAACCGTCTG	(TAA)6	162		I
STM 3012	F CAACTCAAACCAGAAGGCAAA R GAGAAATGGGCACAAAAAACA	(CT)4...(CT)8	193		IX

Primer sets are listed in MILBOURNE *et al.* (1998), primers PP a PIG in SCHNEIDER and DOUCHES (1997) and primer PIP in PROVAN *et al.* (1996a); LG is linkage group

RESULTS AND DISCUSSION

Polymorphism among 30 potato cultivars has been studied using analysis of microsatellites. Firstly, conditions for the PCR reaction were optimised and then suitable primer sets for comparison of potato's varieties were preselected. Preliminary experiments were carried out on five selected cultivars – Vera, Impala, Dali, Karabela and Vladan. Twenty-two primer sets amplified fragments of sizes ranging from 98 to 500 bp. The SSR primer sets amplified consistently, with the number of bands ranging from 1 to 6, in total 76 bands (Table 2). Altogether, 56 polymorphic bands were amplified. Based on this information we could define the most suitable primer sets for the discrimination and the identification of potato genotypes. We chose the scale from 1 (convenient) to 5 (inconvenient) to evaluate the used primer pairs (Table 2). Of twenty-two sets of primers, fourteen were convenient for the cultivar identification (level 1, 2, 2–3). The final

products of these sets were characterised by high variability, good quality and were highly reproducible. Similar results were obtained for primer pairs tested by SCHNEIDER and DOUCHES (1997). On the other hand the products of the primer sets STM 2002, STM 1104, STM 1041, STM 1045 and STM 1056 were monomorphic.

This study demonstrated that variability exists for seventeen of twenty-two SSRs tested across the potato cultivars. On average, 2.5 band variants were observed per amplified SSR. A similar level of variation was observed by VEILLEUX *et al.* (1995) using DNA from anther-derived monploids and diploids of potato or by SCHNEIDER and DOUCHES (1997) in their study of polymorphism of North American potato cultivars. This level of polymorphism is substantially below that observed for other crop species. For example, nine alleles per locus were identified in rice (YANG *et al.* 1994) and for barley, 17,7 alleles per locus were observed by SAGHAI MAROOF *et al.* (1994). The disparity in the level of SSR variation found within het-

Table 2. Optimized conditions for MgCl₂ concentration and annealing temperature. Results of amplification and suitability for cultivar identification

Name	MgCl ₂ (mM)	T _{ann} (°C)	Bands scored		Suitability for identification
			total	polymorphic	
STM 0040	2.5	52.5	4	3	3
STM 0051	2	52.5	4	2	2
STM 1008	2.5	49.5	4	2	2–3
STM 1009	2.5	39.6	3	2	4
STM 1020 (II KIG)	2.5	46	6	4	1–2
STM 1045	2	42	2	0	4
STM 1050	2	46.5	2	1	3
STM 1056	2	49.5	3	0	4
STM 1102	2	48.5	4	3	1–2
PP (SSR 7)	2	54	3	3	1
PIG (SSR 2)	2	55	4	4	2
PIP	2	54	3	2	1–2
STM 0004	2.5	55	5	5	2–3
STM 1024	2	51	3	3	2–3
STM 1041	2.5	49	1	0	4
STM 1064	2.5	48	6	6	2–3
STM 1069	2.5	49.5	2	2	3
STM 1104	2	55	2	0	3
STM 2002	2.5	52.5	1	0	4
STM 2005	2.5	55	5	5	2–3
STM 2020	2	53.5	3	3	2–3
STM 3012	2	53.5	6	6	2

Scale from 1 (convenient) to 5 (inconvenient) to the evaluation of the primer pairs' suitability for cultivar identification

Table 3. Summary of the primer sets' combinations and the results of amplification

Combination	Bands scored		Products and their origin (bp)	Negative control (bp)
	total	polymorphic		
PIP - STM 1104	3	2	<i>457 200 158</i>	80
STM 2020 - STM 3012	1	1	<i>178</i>	80
STM 1069 - STM 1008	5	3	<i>680 500 400 300 160</i>	80
PIP - PIG	2	2	<i>646 562</i>	70
PIP - STM 0004	~	~		70
PIP - STM 2005	~	~		70

The products written in italics are polymorphic

erozygous potato cultivars and highly inbred self-pollinated crops is not fully explained, although a lot of loci are examined in potato and SSR variation is studied in more crop species. However, if SSR variation is a result of mismatch and unequal crossing-over, the reduced number of meiotic events in the breeding and maintenance of a vegetatively propagated crop may explain the lower allelic diversity compared to highly inbred, seed propagated crops (SCHNEIDER & DOUCHES 1997).

We observed polymorphism among 30 selected cultivars using any of 14 primer sets (Fig. 1). The primer sets STM 3012 and STM 2005 produced most polymorphism and permitted to differentiate 12 and 10 varieties, respectively. Together, the sets STM 3012 and STM 2005 distinguished definitely 20 cultivars. The polymorphism produced by PP, STM 0004 and STM 1020 distinguished 8 cultivars and the use of primer set STM 1064 enabled the identification of 7 cultivars. The primer sets STM

tial to satisfy all these criteria. Our study has shown that SSR variation across potato cultivars exists. But our preliminary results also proved that SSRs did not generate unique fingerprints for all examined cultivars in one step and further evaluation would be necessary.

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Abstrakt

POLZEROVÁ H., PTÁČEK J. (2001): **Využití analýzy mikrosatelitů k detekci polymorfismu DNA u brambor.** *Czech J. Genet. Plant Breed.*, **37**: 61–67.

Genetická diverzita 30 genotypů bramboru (*Solanum tuberosum*), reprezentující převážně české odrůdy, byla hodnocena pomocí analýzy SSRs (mikrosatelitů). Amplifikací s 22 páry primerů bylo vytvořeno 76 produktů, z toho 56 bylo polymorfních. Velikost fragmentů se pohybovala v rozmezí od 98 do 500 bp. Množství produktů u jednotlivých sad primerů se pohybovalo od 1 do 6 na danou odrůdu. Sady primerů s vhodností 1, 2, 2–3 (14 párů) byly použity ke studiu polymorfismu vybraných odrůd. Sady primerů STM 3012 a STM 2005 odlišily jednoznačně nejvíce odrůd, a to 12 pár STM 3012 a 10 pár STM 2005. Společně jmenované sady odlišily jednoznačně 20 odrůd. Dendrogram sestavený na základě podobnost-

ni matice ukazuje, že čtrnáct vybraných sad je schopno rozlišit zvolené odrůdy. Některé odrůdy (Dali, Zlata, Kobra) nebylo možné jednoznačně rozlišit použitím jedné sady primerů, ale bylo nutné provést další PCR reakce s různými sadami primerů. Odrůda Dali byla jednoznačně určena kombinací PCR reakcí s primery PIP a STM 1020. Takovýto postup je však zdlouhavý, poměrně pracný a pro potřeby rychlého posouzení nevhodný. Pokusili jsme se tento problém překonat použitím mnohonásobné PCR. Ukázalo se, že by bylo nutné navrhnout nové sady primerů, aby došlo ke zvýšení efektivity mnohonásobné PCR. Naše výsledky ukazují, že mikrosatelity jsou cenné genetické markery, které se vyznačují dostatečnou variabilitou a vysokou reprodukovatelností, jejich možnost využití je diskutována.

Klíčová slova: brambory; polymorfismus DNA; SSRs; mikrosatelity; identifikace odrůd

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Conferences and Seminars

The 9th Plant Breeders Seminar at the Mendel University of Agriculture and Forestry in Brno

The already traditional 9th Plant Breeders Seminar took place on Tuesday the 30th January 2001 at the Mendel University of Agriculture and Forestry in Brno. The seminar was organised by the Commission of Genetics, Plant Breeding and Seed Production of the Academy of Agricultural Sciences together with the University and was presided by Prof. O. CHLOUPEK, DrSc., and Ing. V. ŠÍP, CSc. The seminar was devoted to the presentation of results of plant breeding projects, supported by the National Agency of the Agricultural Research. Ninety nine participants, most of them plant breeders (41), research scientists from the branch of agriculture of the Academy (35), university students and teachers (15), members of the Central Institute for Supervision and Testing in Agriculture (6) and others.

Of the fourteen contributions dealt 8 with cereals (5 with wheat and 3 with barley), 3 with *Brassicaceae* and one contribution each covered potatoes, flax and poppy, respectively.

The presented contributions contained basic information about the projects and their results, including detailed information on the utilisation in breeding programs. The lecturers also informed about their intended projects, to enable possible future co-operation with breeders. Although the presentations were short, concise and the time for discussion was limited, the participants obtained a useful survey on the research project, because the contributions were well prepared and well presented. It is regrettable that at least brief summaries have not been published.

Most of the research was devoted to studies of genetic resources and methods of pre-breeding (DNA markers, fingerprinting, *in vitro* cultures, doubled haploids etc.). Many contributions indicated, that well defined pre-breeding resources had been developed. Dr. ŠÍP demonstrated a direct utilisation of genetic markers in breeding on the example of the registered winter wheat cultivars Šárka and Vlasta, carrying the dwarfing gene *Rht2*. Especially this form of pre-breeding, together with the development of various sources of resistance and quality, utilising DNA markers, is very useful and effective in breeding and should be extended in future. Dr. KOŠNER referred about chromosomal manipulations, *in vitro* cultures and molecular methods in wheat. Contributions by Dr. L. KUČERA and Dr. BARTOŠ were devoted to pre-breeding of sources of disease resistance, whereas Dr. ŠÍP presented a contribution on the development of resources for breeding of low input wheat varieties. This project was finished in 2000. Dr. OVESNÁ concentrated on the preparation of new DNA markers and fingerprinting in breeding barley for resistance to powdery mildew, BYDV and malting quality. Dr. ŠPUNAR informed about the development of new sources for resistance to the BaYMV complex in winter barley. This soil-borne disease has a high incidence in several European countries (Great Britain, France, Germany), but has not yet been found in the Czech Republic. The disease can be controlled by growing resistant cultivars. Dr. KRAUS reported on DNA fingerprinting of barley cultivars for the needs of breeders and the malting industry. Several contributions dealt with *Brassicaceae*, particularly winter rape. Dr. ČURN and Dr. SÁKOVÁ reported about genome analysis of *Brassica napus* using molecular fingerprinting for the identification of cultivars and of breeding material. Dr. V. KUČERA presented information on the utilisation of doubled haploids to produce new breeding material in *Brassica napus*. Drs. KOLOVRAT and HAVEL referred about the variability of indolylglucosinolates in the oil of of *Brassica* seeds. Transformation of potatoes by genes controlling the glycid metabolism and tuber forming was presented by Dr. L. KUČERA. Dr. TEJKLOVÁ informed about anther and microspore cultures in flax, and Drs. HAVEL and KOLOVRAT about a method of screening genetic resources of poppy with a high content of the bain, which is an important alkaloid for the pharmaceutical industry.

Finally, the organisers deserve an appreciation for the high standard of the seminar and it's value for plant breeders and a for the utilisation of genetic research in plant breeding in this country.

New Varieties

Nové odrůdy

Winter wheat Sulamit

Registered: Czech Republic 2000

Breeders rights: SELGEN a.s., Prague, Czech Republic

Breeder and maintainer: SELGEN a.s., Plant Breeding Station Stupice

Parentage: crossing ST252-88 × Alidos. Breeding line ST252-88 = Hana × Zdar/2

Breeding method – pedigree: The parents used for crossing had good or top baking quality. The breeding goal was a top baking quality. Since F_2 generation, screening for baking quality was performed, using sedimentation value (SDS) and protein percentage as indicators. Since F_6 , gluten analyses (percentage and swelling) were performed. From F_7 on, the quality was evaluated by mixograph and baking tests, using a Panasonic Automatic Bread Maker. Single plant selection started in F_2 and continued in F_3 and F_4 . In F_5 two-row microplots were harvested. Preliminary yield trials started in F_6 (1 location, 2 replications), and in F_7 the line SG-S1393-95 was tested in company trials (6 locations, 2 treatments). Diseases (yellow, brown and stem rust, mildew) were evaluated under natural and artificial infection from F_1 till the end of the breeding. Greenhouse seedling tests for mildew resistance were performed from F_6 on and resistance to *Septoria nodorum* was evaluated in a field disease nursery from F_7 on. Maintenance breeding has started since F_7 . The line SG-S1393-95 was tested in Czech Official Trials from 1997 to 1999 and registered as the variety Sulamit in the generation F_{11} .

Disease resistance: field resistance to powdery mildew is good. Sulamit shows medium resistance (MR) to powdery mildew in seedling tests in the greenhouse. The variety is resistant to yellow and stem rusts, and is medium susceptible (MS) to brown rust. Resistance to *Septoria nodorum* and to foot diseases is medium.

Grain quality: In Official Tests Sulamit was found to belong to the highest bread making quality class E (elite). All quality parameters are within the limits of this class and are well stable. The glutenin subunits: n, 17 + 18, 5 + 10 indicate high baking quality: Good sprouting resistance, high falling number and high level of seed dormancy are important for the end-use quality. The grain is red, TKW is medium (45–50g) and test weight is high (80–82 kg/hl).

Frost resistance was determined in field and laboratory tests. Sulamit performed very good and results over years show good to high level of frost resistance (class 5–6).

Other traits: Maturity is medium late, straw length is medium (90–95cm), resistance to lodging is high. The ear is white with short scurs and pyramidal shape. The ear glucosity is very strong (9). Grain coloration in the phenol test is dark to very dark (8).

Pšenice ozimá Sulamit

Registrována: Česká republika 2000

Šlechtitelská práva: SELGEN a.s. Praha, Česká republika

Šlechtitel a udržovatel: SELGEN a.s., Šlechtitelská stanice Stupice

Rodokmen: kříženec linie ST252-88 × Alidos. Šlechtitelská linie ST252-88 = Hana × Zdar

Metoda šlechtění – rodokmenová: Rodičovské odrůdy použité pro křížení měly dobrou až špičkovou pekařskou jakost. Šlechtitelským cílem byla špičková – elitní pekařská jakost. Výběr byl zaměřen od generace F_2 na ukazatele pekařské jakosti – sedimentační test (SDS) a procento bílkovin, od generace F_6 na rozbory lepku (obsah a bobtnání). Od generace F_7 bylo hodnocení jakosti rozšířeno o mixografické hodnocení a pekařský test v pekařském automatu Panasonic. Výběr jednotlivých rostlin byl zahájen v generaci F_2 a pokračoval i v generacích F_3 a F_4 . V generaci F_5 byly

sklizeny dvouřádkové mikroparcely. Předběžné výnosové zkoušky byly zahájeny od generace F_6 (1 lokalita ve 2 opakováních), od F_7 byla linie SG-S1393-95 testována ve firemních pokusech (6 lokalit, dva systémy ošetření). Choroby (rzi plevová, pšeničná a travní, padlí travní) byly hodnoceny v podmínkách přirozené i umělé infekce od generace F_7 až do ukončení šlechtění. Od generace F_6 byla kombinace testována na padlí travní i ve skleníkových testech na mladých rostlinkách a braničnatka plevová (*Septoria nodorum*) od F_7 v polní fytoškolce s umělou infekcí.

Udržovací šlechtění bylo zahájeno od F_7 . Linie SG-S1393-95 byla zkoušena v registračních pokusech ÚKZÚZ v letech 1997–1999 a povolena jako odrůda s názvem Sulamit v generaci F_{11} .

Odolnost k chorobám: Sulamit má dobrou polní odolnost k padlí travnímu a střední (MR) reakci ve skleníkových testech na mladých rostlinách. Je odolná ke rzi plevové a travní, ke rzi pšeničné ale středně náchylná (MR). Středně odolná je k braničnatce plevové a k chorobám pat stébel.

Jakost: Podle výsledků registračních zkoušek byla odrůda Sulamit zařazena do skupiny s nejvyšší pekařskou jakostí E. Všechny sledované ukazatele jakosti odpovídají třídě E a mají také dobrou stabilitu. Vysokou pekařskou jakost signalizují i gluteninové podjednotky: n, 17 + 18, 5 + 10. Pro konečnou jakost výrobků je důležitá i její vysoká odolnost k porůstání v klase, vysoké číslo pádu a vysoký stupeň dormance zrna. Zrno je červené, se střední HTS (45–50 g) a vysokou objemovou hmotností (80–82 kg/hl).

Mrazuvzdornost: Byla stanovena pomocí polně laboratorních a laboratorních testů. Sulamit vykazuje ve víceletých výsledcích velmi dobrou odolnost k poškození mrazem (třídy 5–6).

Ostatní vlastnosti: Délkou vegetační doby patří do skupiny středně pozdních odrůd, délka stébla je střední (90–95 cm) a má vysokou odolnost k poléhání. Klas je bílý, Jehlancovitý, s krátkými osinkami. Klas je silně ojiněný (9). Zbarvení zrna ve fenolovém testu je velmi tmavé (8)

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Potato Kornelie

Registered: Czech Republic, 2001

Breeders rights: Sativa Keřkov a.s.

Pedigree: mother parent: Karin (Rita × Hera)

pollen parent: A 509/28 (II.69.766 × I.65.751/132)

Tested under the designation KE 36/71.

Breeding method: Single cross and clonal selection. Crossing was carried out in 1987. The parents were chosen with the intention to breed an early, high-quality table variety resistant to the potato root eelworm

Seedlings from hybrid seeds were grown in the greenhouse in 1988. The seedling generation of the cross (J 36) comprised a total of 31 860 plants. The harvested tubers were grouped according to size. In the following year 8555 tubers larger than 2 cm in diameter were planted in the field as a bulk. From this part of materials, the variety Katka (KE 36/33) was selected, released in 2000, one year earlier than the variety Kornelie, whose breeding was delayed by repeating the D generation in 1995.

The bulk generations were negatively selected in 1989 and 1990 under field conditions. Plants with unsuitable types of foliage and tubers were discarded during the growing season and after harvest, respectively.

Clonal generations: 817 hybrids were individually planted in the 1st clonal generation. The hybrid KE 36/71 was planted in the 3rd, 4th and 5th clonal generation in station trials, and in the 6th and 7th generation, in inter-station trials, in which apart of yield also quality characteristics and resistance to pests and diseases. were evaluated. Provocative tests were conducted in 1992–1998 for resistance to potato wart and to the potato root eelworm.

The variety was registered under the name Kornelie in 2001 after 3-years of testing in official trials. Maintenance breeding started in 1998 by traditional clonal selection. Rapid meristem propagation from recovered virus-free plants was introduced in 2000.

Vegetation period – early (132 days), maturity class of Karin, but faster emergence.

Resistance to diseases – resistant to potato wart biotype D1 and to the potato root eelworm pathotype Ro1. Intermediate resistance to common scab, black leg and early blight, intermediate resistance to foliar late blight, resistant to diseases caused by leafroll and mosaic viruses.

Consumer quality – average starch content of 16%. Suitable for direct consumption during the whole storage period. Cooked potatoes are medium mealy, with medium crude texture, moister and tasty. The boiling type is BC, satisfying color of French fries (6), slow raw flesh darkening

Tuber yield – intermediate to high, yielding ability of market tubers is high. The yield in Czech official trials in 1998–2000 by Central Institute for Supervising and Testing in Agriculture reached 105.7% of control varieties. Tubers are of medium size and of very good uniformity, tuber setting under the shrub is intermediate.

Other characteristics: Tubers are oval with shallow eyes, attractive appearance, with light yellow to yellow flesh and resistant to mechanical damage. Sprouts are conical with red-violet anthocyanin base coloration. Plants are of intermediate type with large leaves and with sporadic white flowers.

Brambor Kornelie

Registrována: Česká republika, 2001

Šlechtitelská práva: Sativa Keřkov a.s., šlechtitelská stanice Keřkov

Rodokmen: mateřský komponent: Karin (Rita × Hera)

otcovský komponent: A 509/28 (II.69.766 × I.65.751/132)

Zkoušena pod označením KE 36/71.

Metoda šlechtění: pohlavní křížení bylo provedeno v roce 1987. Výběr rodičů byl zaměřen na získání kvalitní rané konzumní odrůdy odolné hádřátku.

Semenáče – výsadba ve skleníku v roce 1988. Od této kombinace (J 36) bylo vypěstováno 31 860 jedinců. Sklizeň hlízek byla rozdělena podle velikosti. Hlízky (8550) větší než 2 cm v průměru byly následující rok vysazeny do volné půdy na pole. Z této části materiálu posléze vzešla také odrůda Katka (KE 36/33), která byla povolena v roce 2000, tedy o rok dříve než odrůda Kornelie, která se zpozдила opakovaním D generace v roce 1995.

Ramšové generace – hodnoceny v letech 1989–1990 v polních podmínkách. Selektce za vegetace i po sklizni byla vedena proti nevhodným typům natě i hlíz neodpovídajícím šlechtitelskému cíli.

Klonové generace – v první klonové generaci bylo individuálně vysázeno 817 kříženců. Ve 3. a 4. klonové generaci byl pak již kříženec KE 36/71 vysázen ve staničním pokuse, který ještě v 5. generaci opakoval a teprve v 6. a 7. generaci absolvoval mezistaniční předzkoušky, v nichž obstál i při podrobném hodnocení dalších vlastností – výnosových, kvalitativních i v odolnostech chorobám a škůdcům. V letech 1992–1998 byla v provokačních zkouškách hodnocena odolnost rakovině brambor a háďátku bramborovému.

V roce 2001 byla po tříletém zkoušení v registračních zkouškách odrůda registrována pod jménem Kornelie. Udržovací šlechtění započalo v roce 1998 tradičním způsobem – klonovým výběrem. V roce 2000 bylo udržovací šlechtění rozšířeno o meristémové rychlé rozmnožování z ozdravených viruprostých rostlin.

Vegetační doba – raná odrůda (132 vegetačních dní), zralostní typ jako odrůda Karin, rychleji vzhází.

Odolnost k chorobám: Je vzdorná rakovině bramboru biotypu D1 a odolná háďátku bramborovému patotypu Ro1. Je středně odolná až odolná napadení obecnou strupovitostí, černání stonku a hnědé skvrnitosti, středně odolná proti napadení plísní bramborovou v nati, je odolná virovým chorobám svinutkového i mosaikového typu.

Konzumní jakost: Odrůda vykazuje v průměru 16 % škobnatosti. Je vhodná pro přímý konzum během celého skládovacího období. Vařené hlízy jsou středně moučnaté, středně hrubé struktury, příjemně vlhké a chutné. Je zařazena do varného typu BC, barva hranolků je uspokojující (6), tmavnutí za syrova je pomalé.

Výnos hlíz: – středně vysoký až vysoký, výtěžnost tržních hlíz je vysoká. Podle výsledků ÚKZÚZ dosáhla v průměru let 1998–2000 105,7 % ve srovnání s kontrolními odrůdami. Hlízy jsou průměrné velikosti, velmi dobře velikostně vyrovnané, nasazení hlíz pod trsem je střední.

Ostatní vlastnosti: Hlíza je oválná s mělkými očky, vzhledná, se světle žlutou až žlutou dužninou, značně odolává mechanickému poškození, klíček je kuželovitý s červenofialovým anthokyanovým zabarvením báze, rostlina je přechodného typu s velkými listy, sporadicky kvete bíle.

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