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Characterisation of barley (*Hordeum vulgare* L.) varieties and breeding lines using RAPD and QTL associated PCR markers

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ABSTRACT

Random decamer primers were screened for their ability to produce polymorphic bands in a set of barley breeding materials. Twelve primers out of forty tested and two primer combinations produced stable polymorphic signals. Polymorphic data were used for diversity studies. Cluster analysis showed rather high relatedness among characterised genotypes, which confirmed limited genetic basis of the Czech malting barley germplasm. RAPD polymorphic markers found during the investigation provided an addition to classical morphological markers for germplasm identification and for estimation of genetic similarities among genotypes. Amplicon specific QTL associated PCR markers developed for American germplasm evaluation were tested for their ability to differentiate cultivars used for barley breeding in the Czech Republic. They discriminate among 24 mostly malting barley cultivars and lines. Maximum variability was found in *Amy1* and *Hor2* loci with diversity index value over 0.6. The markers identifying the two loci could be suitable candidates for marker-assisted selection for breeding programs, in which European malting barley cultivars and lines are used.

Keywords: barley; *Hordeum vulgare* L.; marker assisted selection; polymorphism; RAPD; breeding

Barley belongs to the most important cereal crops in the world. In the Czech Republic almost 70% of area is covered by spring barley, which is used mostly for malt production (30%) and animal feeding. Malting quality is therefore a highly important set of traits in this crop. Many successful cultivars have been introduced into the market using traditional breeding approaches, which perform a long-term, laborious and costly process. The results depend on a good choice of suitable parents and careful screening of numerous progenies. Several parameters such as hot water extract, Kolbach index, diastatic power, friability, ß-glucan and α-amylase contents are monitored to predict the malting quality along with other important traits like yield, disease resistance and stress tolerance. The malting quality parameters form a complex of closely related characters (Stoklasová 1993; Psota et al. 1995; Kosař et al. 1997; Špunarová and Prokeš 1998). To improve the malting quality, several approaches have been proposed including also transformation-based technologies (McElroy and Jacobsen 1995).

Molecular markers could play an important role in the breeding process. They can be used for the assessment of genetic distances among cultivars or breeding materials. Genetic basis of modern barley cultivars is narrow due to the environmental pressure and especially due to the necessity to maintain carefully balanced genetic background in modern cultivars. Assessment of the genetic diversity among cultivars and breeding lines plays an important role in the barley breeding process. The information can be used as a general guide in the choice of parents for crosses depending on the breeding strategy (Mannienen and Nissilá 1997; Friedt et al. 1999). Several methods have been used for barley profiling including RFLP, RAPD, SSR and AFLP (Saghai-Maroof et al. 1995; Powell et al. 1996; Russel et al. 1997; Han et al. 1997; Dávila et al. 1998). Among them RAPD technique is the easiest to be performed. RAPD requires only the basic laboratory equipment. It is a cost effective approach as it was proved in barley and other crops (Giese et al. 1994; Terzi 1997; Mignouna et al. 1998; Dávila et al. 1999).

The marker-assisted selection (MAS) is considered to be another approach useful for breeders. MAS makes selection of the progenies with required characters easier and faster. Several markers have been developed up to now. Markers for monogenic traits like disease resistance in barley (Mohler and Jahoor 1996; Ordon et al. 1997; Ford et al. 1998) were introduced. Their usefulness for MAS have been demonstrated (Friedt et al. 1999; Ovesná et al. 1999). Quantitative trait loci (QTL) are, however, more important when the high malting quality is the breeding goal. Development of QTL markers is expensive, because it is necessary to develop mapping populations (e.g. set of double haploid lines) and use a large set of different markers. RFLP markers are often used, but it is not easy to handle them. Several QTL markers associated with yield and malting quality parameters or disease resistance have been also published (Larson et al. 1996; Han et al. 1997; Toojinda et al. 1998; Romagosa et al. 1999). Lee and Penner (1997) converted some of RFLP probes associated with QTLs into amplicon specific markers. The applicability of QTL markers in MAS has been also discussed. However, low reliability across different populations has been found (Lee and Penner 1997; Spaner et al. 1999).

Assessment of genetic diversity among cultivars and lines involved in a breeding program of malting barley

using RAPD was the main objective of this study together with investigation of the applicability of amplicon specific-QTL associated markers prepared on Harrington/TR303 cross MAS in non-related germplasm.

MATERIAL AND METHODS

Plant materials. Cultivars and lines used as a breeding material in a malting barley breeding program were analysed using RAPD. Twenty-two cultivars and lines were used to verify the value of amplicon-specific primers for evaluation of European germplasm. For details see Table 1.

DNA preparation. Genomic DNAs were extracted from young leaves using the protocol of Saghai-Maroof et al. (1984). The genomic DNA of each accession was extracted from bulked leaves of at least twenty individual plants. Quality and concentration of DNA was detected electrophoretically and spectrophotometrically. DNA was diluted to obtain final concentration 50 ng/µl.

RAPD Assay. Decamer oligonucleotides used in this study were obtained from Advanced Biosystem Ltd. (England) series ABN and AB2. Stoeffel fragments (Perkin-Elmer) were used for RAPD amplification. The amplification reaction mixture (25 µl) contained 1× buffer (Perkin-Elmer, for Stoeffel fragment), 3 mM $MgCl_2$, 0.25 mM dNTP, 50 ng of primer, 50 ng of template DNA and 1 U of Stoeffel fragment. DNA amplification was performed using MJ Research 100 thermocycler programed for one cycle 94°C 1 min, 37°C 1 min, 72°C 1 min 30s and 35 cycles of 1 min 92°C, 1 min 37°C, 1 min 30s 72°C followed by 5 min at 72°C. The RAPD fragments were separated by

Table 1. List of barley cultivars and lines used in the present study including their names, pedigree information, place of origin and description of malting quality

Cultivar/Lines	Pedigree	Origin	Malting quality
Alexis	Breun1622/Trumf (Diamant/St.16029-64-6/Alsa/S3170// Hadm.11719/Union)	DEU	high malting
Amulet	Salome/Perun (HE1728/Karát)	Selgen, CZE	high malting
Atribut	KMV3-83 (Diamant in the pedigree)/BR2174	Selgen, CZE	high malting
Bitrana	Salome/HVS18709-78	DEU	malting
Blenheim	Trumf (Diamant/St.16029-64-6/Alsa/S3170//Hadm.11719/Union)/ Egmont (Yak/W1001//Vada)	GBR	high malting
CE785	not available	Cesea, CZE	feeding
CE790	HVS1589-84/SK2889	Cezea, CZE	feeding
Diamant	(M) Valtický	Morstar, CZE	high malting
Harrington	Klages/3/Gazelle/Betzes//Centennial	CAN	high malting
HE4886	HE1728/Karát//633-82BVU	Plantselect, CZE	high malting
Jubilant	SK1410/HE 868//Dera (Diamant in the pedigree)	*Hordeum*, SVK	high malting
Karát	Valtický/B2145//Carlsberg/F.Union/Diamant/1293-70	Kroměříž, CZE	high malting
KM1174	KM967/85 (Diamant in the pedigree) × HVS12115/80	Kroměříž, CZE	malting
KME1587	not available	Morstar, CZE	malting
Kompakt	Galan (SK2567-79/HE1428)/KMA10	*Hordeum*, SVK	high malting
Krona	Nebi (99991/70) × (Diamant × 14029/64) × 640 (Emir × S.487)/ 11827-80//29314-78/3/Gimpel	DEU	high malting
Krystal (HE950)	Korál [HE748 = Hana/4/F.Union/3/Alsa//Čel. Hanácký (Haisa// Starnovský Kneifel/Nolčův A)/125]/Rapid (HE598 = Voldagsen/ Kneifel//Diamant/Denár) [(M) Čel. Hanácký (Haisa//Starnovský Kneifel/Nolčův A/Bavaria)]	Plantselect, CZE	high malting
Maltine	Aramir//Zephyr (Heines2149/Carlsberg)/Sundance	FRA	malting
Marina	not available	Lochow-Petkus, DEU	malting
Novum	FUD II./Gerda//Ls.č.8/3/Krystal	*Hordeum*, SVK	high malting
Olbram	HVS1703-82/BR2174	Morstar, CZE	high malting
Otis	8020/Europa//Atem (L92/Minerva//Emir/3/Zephyr)	DEU	high malting
Perry	Mo.B400/Ludwig//Cartens	USA	feeding
Perun	HE1728/Karát	Plantselect, CZE	high malting
Primus	Jaspis ST6984/72/Opál [Ametyst (Voldagsen/3/Domen//Hanácký jubilejní) (Opavský/Selekční hanácký VIII)/Valtický/3/Diamant]/ Palestine 10//Sladár (Valtický/Slovenský Dunajský trh) [(S) Ackermann Danubia/Diosecký (S) Proskowetzova Haná pedigrée// Danubia/Dregerův]/E1197-85	Plantselect, CZE	feeding
Terno	HES170-74/Opál [Ametyst (Voldagsen/3/Domen//Hanácký jubilejní) (Opavský/Selekční hanácký VIII)/Valtický/3/Diamant]/Palestine10// Sladár (Valtický/Slovenský Dunajský trh) [(S) Ackermann Danubia/ Diosecký (S) Proskowetzova Haná pedigrée//Danubia/Dregerův]	Kroměříž, CZE	malting

agarose gel electrophoresis in 1× TAE buffer on 1.5% agarose (mol. biol. grade, Sigma-Aldrich). Separated DNA fragments were stained in water solution of ethidium bromide (3 μg/ml), visualised and photographed under UV light.

Amplicon QTL-specific PCR primers. Primers published by Lee and Penner (1997) were used. For sequences and reaction conditions see Table 2. Polymerase chain reaction (PCR) was carried out in the DNA Thermal cycler (MJ Research, PTC-100) in 25 μl reaction mixture: 10 μmol of each primer, 30 ng of genomic DNA, 200 μmol of each dNTP, Red Taq buffer with 1.1 mM concentration of $MgCl_2$ and 1 U Red Taq Polymerase (Sigma). Initial amplification conditions for each locus specific primer consisted of a temperature profile of 1 min/94°C, 1 min/60°C and 1 min/72°C for 35 cycles. Amplification products were separated on 1.6% agarose gels and stained with ethidium bromide, visualised and photographed under UV light.

Data analysis. For each accession, a binary matrix reflecting specific RAPD band presence (1) or absence (0) was generated. Pairwise distances between the accessions based on Jaccard (1907) similarity metrics were calculated with the use of RAPDALG program (The RAPDistance Package, Armstrong et al. 1994). UPGMA-clustering and principal component analysis was conducted using the statistical software package STATISTICA (StatSoft, Inc.).

The data obtained by the use of amplicon specific QTL's associated marker was analysed as proposed by Dahleen (1997). Amplification patterns were analysed as genotypes (single loci) using diversity index (DI) for each of the amplicon marker. DI was calculated using Nei's (1987) genetic diversity index:

$$DI_i = 1 - \Sigma p^2_{ij}$$

Where p_{ij} is the j^{th} pattern of amplification product i and the summation extends over all patterns. Barley cultivars and clones were assumed to be homozygous. If amplification products had been identical across all the analysed genotypes, DI had been considered 0, if amplification products differed in all the genotypes $DI = 1$.

RESULTS

RAPD primers survey

Forty decamer primers (kit AB2 and kit ABN Advanced Biosystem, England) were initially screened for their ability to produce polymorphic patterns using five accessions originating at different breeding companies. Twelve decamer primers and two primer combinations, which gave reproducible and distinct polymorphic amplification products, were selected for evaluation of diversity across all the accessions (Table 3). Each of the selected twelve decamer primers and two primer combinations varied in their ability to produce polymorphic fragments ranging from one to five per reaction. On average 3.9 polymorphic bands per reaction were recorded. Total of 55 different polymorphic amplification products were obtained from these decamer primers across eighteen accessions.

Cluster and principal component analysis

The observed polymorphism was generally low. Minimally four decamer primers were necessary for discrimination among all the genotypes. Polymorphic bands were used to estimate genetic similarity. Jaccard's metrics was used to process RAPD data and calculate the pair-wise

Table 2. Primer sequences of amplicon specific QTL associated primers and PCR conditions used for reactions

Locus	Malting quality traits	Primer sequences (5′–3′)	Annealing temperature	Concentration of Mg^{2+}
Nar1	fine/coarse difference ß-glucan	CACCATCAAGGGATACGC/ GTCATAAAAACAGTTTGTGTTC	60°C	1,1 mM
Nar1	fine/coarse difference ß-glucan	TGCTTGCTGACACAAGCTC/ CGTCACCTCCACTCGTGT	60°C	1,4 mM
ABG610	ß-glucan fine extract	TGCCTTGGTGATTCATAAGATG/ AGTCGACCTGCAGAAACAAAA	56°C	1,1 mM
ABG622	ß-glucan fine extract α-amylase soluble/total protein diactic power fine/coarse difference	TTTGGGAGGATACAATGGACGG/ GACTCGGAATCATCGTCGTCG	70°C	1,7 mM
Hor2	diastic power	CCACCATGAAGACCTTCCTC/ TCGCAGGATCCTGTACAACG	64°C	1,5 mM
Amyl	α-amylase diastic power	AAACACATGTCCCTCTCG/ GCGTCCAGGTCGTAGTA	58°C	1,5 mM

Table 3. List of decamer primers and their sequences (Advanced Biosystem, England) used for evaluation of diversity within the studied set of barley cultivars and lines

Primer	Sequence	Primer	Sequence	Contents of CG pairs
ABN-02	5′-ACC AGG GGC A-3′	ABN-04	5′-GAC CGA CCC A-3′	70%
ABN-07	5′-CAG CCC AGA G-3′	ABN-08	5′-ACC TCA GCT C-3′	
ABN-09	5′-TGC CGG CTG G-3′	ABN-13	5′-AGC GTC ACT C-3′	
ABN-14	5′-TCG TGC GGG T-3′	ABN-20	5′-GGT GCT CCG T-3′	
AB2-02	5′-GGT GCG GGA A-3′	AB2-09	5′-CTT CAC CCG A-3′	60%
AB2-10	5′-CAC CAG GTG A-3′	AB2-19	5′-ACG GCG TAT G-3′	
		ABN-13 × AB2-19		
		ABN-13 × AB2-10		

distances. Genetic similarities were estimated to be between 0.107 (HE4886 versus Amulet and HE4886 versus KM1174) and 0.643 (CE758 versus Marina). The calculated genetic distances indicate, that evaluated barley genotypes are closely related. Subsequently the calculated distances were used to generate dendrogram (Figure 1). Three main clusters were observed. The first group included six high malting quality cultivars. All of them have cv. Diamant in their pedigrees. The second group consists of five cultivars and lines, cv. Diamant is included in the pedigree of four of them. Only cv. Maltine according to the information available does not possess cv. Diamant. However, cv. Carlsberg appears in the pedigree of this cultivar and also in the pedigrees of other three members of the group. The third cluster consists of four cultivars and lines, only one of them has cv. Diamant in the pedigree. Three out-layers were identified by this approach. Unfortunately, no pedigree data are available for CE785. However, both CESEA lines are of a feeding quality. CE lines were included in the investigated collection because they are used in the breeding program as a donor of other valuable genes (e.g. disease resistance). CE790 and cv. Jubilant have SK lines in the pedigree, therefore, they might really contain similar genetic background in some extent.

Principal component analysis PCA (Figure 2) grouped the studied germplasm principally in the similar way as cluster analysis above. PC1 accounted for 34% of the total variation and separates genotypes produced by CESEA Ltd., which differ in their end-use quality from the rest of analyzed germplasm. PC2 and PC3 accounted for 25% and 12.5% of total variation, respectively, and separated three German cultivars – two without cv. Diamant in the pedigree (Otis, Bitrana) and one with unknown pedigree data. Unlike the cluster analysis, PCA drives Moravian malting cv. Krystal based on cv. Diamant to the group of high malting barley cultivars. Also the position of cv. Jubilant corresponds better to our a priori knowledge of its origin and quality parameters.

QTL markers survey

Together six pairs of PCR amplicon specific – QTL associated primers (Lee and Penner 1997) derived for Harrington/TR303 (North American materials) crosses were tested to verify their usefulness for characterization of European breeding material. Up to now their suitability for Harrington and TR303 related germplasm analysis have been proved (Lee and Penner 1997). *Nar1a* ampli-

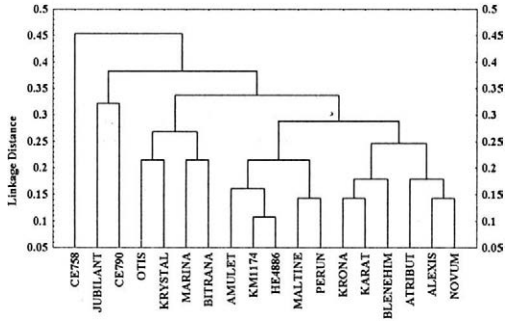

Figure 1. Association among barley cultivars and breeding lines revealed by cluster analysis performed on genetic similarity estimates calculated from 55 polymorphic products amplified 14 RAPD primers and their combinations

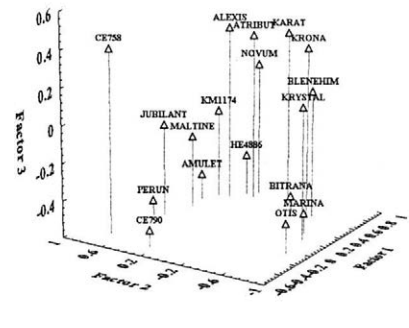

Figure 2. Association among barley cultivars and breeding lines revealed by principal component analysis performed on genetic similarity estimates calculated from 55 polymorphic products amplified 14 RAPD primers and their combinations

con, associated with good malting quality of cv. Harrington, was amplified only in cv. Perry, a cultivar of American origin. *Nar1b* amplicon was amplified only in cv. Alexis, which is a high malting quality material. Three different types of signals were recorded, when XABG610 primer pair was used. Only four cultivars with a good malting quality did not amplify any product as well as cv. Harrington. Two cultivars (Krona, Novum) amplified 148 bp long fragments, which did not correspond either to Harrington or TR303 products. In the other cultivars and lines TR303 corresponding signal was amplified. XABG622 primer pair amplified both Harrington and TR303 corresponding signals across examined materials and one more different signal (890 bp) was observed. Even the presence of two signals in one of the accessions was repeatedly observed. Five different amplification products were observed when *Amy 1* primer pair was used indicating high variability in this locus (Table 4). Diversity index (DI) varied from 0.123 to 0.643 (Table 4) showing different ability of tested markers to discriminate among European cultivars and lines.

DISCUSSION

Genetic distance between parents can play an important role in the final performance of the progeny. On one hand, a carefully balanced genetic background of the malting quality germplasm should be maintained (Melchinger et al. 1994; Rasmusson 1992), on the other hand, a better performance is expected when non-related genotypes are crossed in new breeding lines. Besides pedigree analysis molecular markers offer another way of looking at genetic diversity. Moreover, no pedigree data are available in some cases. The results demonstrate that it is possible to discriminate between closely related elite barley genotypes using at least four decamer primers. Among molecular markers RAPD is considered to be fast and simple. Even when some special consideration should be taken in account (Lynch and Milligan 1994), in homozygous species like barley dominant markers can be used in the same way as co-dominant ones (Clark and Lanigan 1993). RAPD was in our study again proved to be a suitable and cost effective tool for genetic charac-

Table 4. Amplicon specific products recorded accross evaluated barley cultivars and lines; cv. Harrington and line TR306 were used as controls; the sizes of products are given in basepairs (bp), np = no products amplified; the values of diversity indexes (*DI*) calculated for each primer pair are given in the bottom line

Name of cultivars and lines	Hnar1a/ nar1a	Hnar1b/ nar1b	TABG610	ABC622	Hor2	Amy1
Harrington	326 bp	1537 bp	np	1198 bp	1031 bp	300 bp
TR306	np	np	138 bp	np	850 bp	np
Alexis	np	1537 bp	138 bp	np	850 bp	300 bp
Amulet	np	np	np	1198 bp	850 bp	800 bp
Atribut	np	np	np	np	850 bp	np
Bitrana	np	np	138 bp	np	890 bp	250, 900 bp
Blenheim	np	np	np	np	850, 1031 bp	300 bp
CE785	np	np	138 bp	np	850 bp	300, 1200 bp
CE790	np	np	138 bp	np	850 bp	300, 1200 bp
Diamant	np	np	138 bp	np	850 bp	300 bp
HE4886	np	np	138 bp	np	890, 1031 bp	300 bp
Jubilant	np	np	138 bp	np	850 bp	300 bp
Karát	np	np	np	np	890, 1031 bp	300, 800 bp
KM1174	np	np	138 bp	1198 bp	850, 1031 bp	300 bp
KME1587	np	np	np	1198 bp	850, 1031 bp	300 bp
Kompakt	np	np	np	np	850 bp	np
Krona	np	np	148 bp	np	850 bp	np
Krystal	np	np	138 bp	np	850 bp	300 bp
Maltine	np	np	138 bp	280, 380 bp	850 bp	300 bp
Marinka	np	np	138 bp	np	1031 bp	np
Novum	np	np	148 bp	np	1031 bp	300 bp
Olbram	np	np	138 bp	np	850 bp	300 bp
Otis	np	np	138 bp	np	850 bp	np
Perry	326 bp	np	138 bp	np	850, 1031 bp	np
Perun	np	np	np	np	890 bp	300 bp
Primus	np	np	138 bp	np	850, 1031 bp	300 bp
Terno	np	np	138 bp	np	1031 bp	300 bp
DI	0.15879	0.15879	0.49907	0.28733	0.62760	0.60113

terisation of cultivated species in addition to microsatellites, RFLP or AFLP (Graner et al. 1990; Terzi 1997; Dávila et al. 1998; Han et al. 1999).

We evaluated a set of cultivars and breeding lines used in the malting barley breeding program in our investigation. We found a low diversity within the inspected germplasm. According to the pedigree information, two thirds of the evaluated accessions had cv. Diamant in their pedigrees, which may explain these results. Diamant a short straw mutant of cv. Valtický has shown a significant impact on the European barley breeding (Granner et al. 1999). We found only two lines to be less related towards the rest of the evaluated group as certified by both the cluster analysis and PCA: line CE758 with unknown pedigree and line CE790, both of feeding quality. They were included in the breeding programs as donors of other valuable genes. The most similar accessions: HE4886 versus Amulet and HE4886 versus KM1174, both pairs with the same genetic distances towards the counterparts, are of the Czech origin. They possess cv. Diamant in their pedigrees and HE4886 and Amulet possess cv. Karát as parental and grandparental line, respectively. Generally, genetic distances varied between 0.107–0.643 within the studied set of genotypes. Similar results were obtained by Mannienen and Nissilá (1997). They used a comparable number of polymorphic RAPD markers to evaluate diversity among Finnish cultivars. According to their assumption and according to the data presented by Giese et al. (1994), this number of RAPD markers should cover all the linkage groups. We conclude, that the grouping of accessions is consistent with the pedigree information and the high genetic similarity reflects the effort of breeders in the entire Europe to pyramide the genes governing good malting quality.

For cultivated crops, like barley, several DNA markers for genes underlying agronomically important characters have been developed. In addition to diversity studies, these DNA markers can be used for aid controlled introgression of agronomically important diversity. Furthermore, knowledge regarding the agronomic architecture of related and unrelated germplasm at key regions of the genome should allow for systematic dissection of the basis of selection response in related germplasm.

We have shown in this investigation, that amplicon specific primers (STS-PCR) developed for evaluation of Harrington/TR303 progenies and related genotypes can differentiate among lines and cultivars used in our region. All the PCR markers detected polymorphism within examined genotypes. Diversity index varied from 0.158 to 0.628 with an average mean of 0.378 ± 0.215. This is a little bit lower value as compared with the data obtained by Dahleen (1997). She used RFLP markers for evaluation of 28 North American cultivars (average *DI* = 0.419). The American set of barley cultivars might contain higher diversity, because winter, spring, feeding and malting cultivars were included. Difference between restriction and amplified polymorphism must be taken in account as well. Four out of six tested markers could be used only in limited number of crosses, because of the low *DI* value (0.251 and less). These results support the observation, that it is difficult to develop QTL marker applicable across various genotypes (Romagosa et al. 1999). The highest variability was found in *Amy1* loci previously localised on 6H chromosome (Jones et al. 1991). *Hor2* and *Amy1* markers are the most informative among tested ones with *DI* 0.625 and 0.615 respectively. *Amy1* marker is associated with α-amylase activity with heritability between 30–65% (Foster et al. 1967). Both the recommended markers are associated with diastatic power, which is usually tested only in the F_4 generation. These two markers could be further used to facilitate the transfer of corresponding locus from parents into the progeny. They can be used in early generations like F_2, F_3 and can make the selection of progenies with desirable genes easier.

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Abbreviations

PCR = polymerase chain reaction, QTL = quantitative trait loci, RAPD = random amplified polymorphic DNA

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ABSTRAKT

Charakterizace odrůd a šlechtitelských linií ječmene (*Hordeum vulgare* L.) pomocí RAPD a PCR markerů specifických pro QTL

Testovali jsme RAPD primery s ohledem na jejich schopnost rozlišovat odrůdy a šlechtitelské linie sladovnického ječmene. Pouze 12 ze 40 testovaných primerů a dvou jejich kombinací produkovalo stabilní a polymorfní markery. Shluková analýza prokázala poměrně nízkou míru diverzity mezi studovanými genotypy, což potvrzuje úzkou genetickou bázi českých sladovnických ječmenů. Získané RAPD markery představují vedle morfologických znaků další možnost identifikace genotypů a odhadu genetické příbuznosti mezi odrůdami a liniemi ječmene. Pro charakterizaci zkoumaných genotypů byly použity dále amplikon-specifické PCR markery, které jsou ve vazbě s lokusy podmiňujícími znaky kvantitativního charakteru. Původně byly vyvinuty pro charakterizaci amerických ječmenů. Sledovali jsme možnost jejich využití pro rozlišení genotypů sladovnického ječmene využívaných v ČR. Testované markery rozlišovaly v různé míře sledované genotypy. Nejvyšší variabilita byla nalezena v lokusech *Amy1* a *Hor1*, kde index diverzity přesahoval hodnotu 0,6. Markery identifikující uvedené lokusy mohou být vhodnými kandidáty pro šlechtění sladovnického ječmene pomocí markerů ve šlechtitelských programech, do kterých vstupují některé z evropských linií a odrůd.

Klíčová slova: ječmen; *Hordeum vulgare* L.; selekce pomocí markerů; polymorfismus; RAPD; šlechtění

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Genetic analysis of grain yield in crosses of wheat genotypes with different Rht genes

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ABSTRACT

The objectives of this study were to investigate the variability, gene effects and mode of inheritance of grain yield in the crosses of wheat genotypes with different Rht genes. The parents were Siete Cerros (Rht-B1b), Aobakomughi (Rht-D1b), Tom Thumb (Rht-B1c), Ai-Bian 1 (Rht-D1c), Sava (Rht 8) and Bankuty 1205 (rht). In F_1 and F_2 generations, highest grain yields per plant and smallest coefficient of variation have been observed in crosses Bankuty 1205 × Aobakomughi, Bankuty 1205 × Sava and Sava × Ai-Bian 1 (in F_1) and Bankuty 1205 × Sava (in F_2). Individual and joint tests showed that the additive-dominance (AD) model was not adequate for accessing gene effects for any of the crosses. Using the model with six parameters, the presence of additive, dominant and epistatic gene effects have been determined and discussed for each cross. The data from F_1 generation showed that from crosses in which one parent was semidwarf (Aobakomughi or Sava) and other was tall (Bankuty 1205) or dwarf (Ai-Bian 1), genotypes with increased grain yield could be selected. For all crosses in F_2 generation, except for cross Bankuty 1205 × Aobakomughi, mode of inheritance of grain yield per plant is overdominance of parent with higher grain yield per plant. The obtained results showed that hybridization of genotypes with different Rht genes could, in some crosses, bring out good starting material for wheat breeding targeting increased grain yield.

Keywords: gene effects; grain yield; inheritance; Rht genes; variability; wheat

Over the past four decades global wheat production has risen dramatically predominantly due to introduction of semidwarf wheats (with different Rht genes), into breeding programs worldwide (Austin et al. 1980; Borojević 1983; Hesselbah 1985; Ledent and Stoy 1988; Damish and Wiberg 1991). At the moment 21 Rht genes have been determined, but only few of them had important impact to the development of high-yielding wheat varieties. The most frequently used Rht genes in breeding programs are Rht-B1b and Rht-D1b originate from cultivar Norin 10. Recent studies showed that, besides stem reduction, they have a positive pleiotropic effect on yield components (Gale and Youssefian 1985) and thus consequently led to higher wheat yields in India, Mexico (Borlaug 1968), Australia (Cooper 1979), U.K. (Austin et al. 1980), Israel, China (Li et al. 1998), Czech Republic (Šip et al. 1998), Germany (Borner et al. 1993) and U.S.A. (Gale 1984). On the contrary, in winter and spring breeding programs in South Europe and Mediterranean the most frequently used Rht gene is Rht 8 originate from Akakomughi, which on the similar bases as previous ones causes grain yield increase by 5–28% (Gale et al. 1982; Petrović et al. 1998; Worland et al. 2001). Besides the dwarfing genes mentioned above mostly investigated are Rht-B1c (cv. Tom Thumb) and Rht-D1c (cv. Ai-Bian 1). Experiments with isogenic lines showed that in some cases genotypes caring Rht-B1c could, despite the stem reduction of 50%, outyield genotypes with Rht-B1b and Rht-D1b genes (Flintham et al. 1997; Djunusova 1998). Comparing genotypes mentioned above, the lowest grain yields and shortest stems, were observed in the presence of Rht-D1c (Gale and Youssefian 1985).

MATERIAL AND METHODS

The parents used in the crosses were Bankuty 1205 (rht-tall), Siete Cerros (Rht-B1b), Aobakomughi (Rht-D1b), Tom Thumb (Rht-B1c), Ai-Bian 1 (Rht-D1c) and Sava (Rht 8). In the 1995 five crosses have been made: Bankuty 1205 × Aobakomughi, Bankuty 1205 × Sava, Siete Cerros × Tom Thumb, Siete Cerros × Ai Bian 1 and Sava × Ai-Bian 1. With standard breeding procedure the F_1, BC_1, BC_2 and F_2 generations were produced in 1997. By this, the most crucial requirement for application of the additive-dominance model – that all generations are tested in the same year is satisfied. The experiment was conducted at the experimental station of the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia. Sowing was done each year at the beginning of October, in 1.2 m^2 plot, with 6 cm space inside the row and 20 cm space between rows. A completely randomized design with three replications was used in the experiment. In order to produce enough plants for the analysis, sowing of F_2 was done for each replication in four subreplications. At full maturity, 30 randomly selected plants in P_1, P_2, F_1, BC_1 and BC_2 as well as 270 plants in F_2 generation were taken for analysis.

The means (x), minimum values (x_{min}), maximum values (x_{max}), standard errors (S_x), standard deviations (S) and

Table 1. Mean values (x), standard errors (S_x), standard deviation (S), coefficient of variation (CV) and heterosis (H) for wheat grain yield per plant in parents, F_1, BC_1, BC_2 and F_2 in 1997

	x (g)	S_x	S	CV (%)	H (%)
Bankuty 1205 (rht)	3.95	0.10	0.5	13.2	101.3**
BC_1	5.42	0.35	1.4	25.1	
F_1	7.19	0.34	1.9	25.9	
F_2	3.22	0.37	2.0	63.4	
BC_2	6.18	0.47	1.8	29.1	
Aobakomughi (Rht-D1b)	3.20	0.15	0.8	25.5	
Bankuty 1205 (rht)	3.95	0.10	0.5	13.2	114.0**
BC_1	6.38	0.45	1.8	27.4	
F_1	6.43	0.32	1.3	19.8	
F_2	5.26	0.28	2.7	50.5	
BC_2	2.95	0.36	1.4	46.9	
Sava (Rht 8)	2.06	0.16	0.9	41.5	
Siete Cerros (Rht-B1b)	3.60	0.16	0.9	24.0	−13.9*
BC_1	4.31	0.41	1.6	37.1	
F_1	2.54	0.24	0.9	36.8	
F_2	3.07	0.22	2.1	69.1	
BC_2	2.17	0.20	0.8	35.9	
Tom Thumb (Rht-B1c)	1.97	0.10	0.5	27.2	
Siete Cerros (Rht-B1b)	3.60	0.16	0.9	24.0	−49.3**
BC_1	3.04	0.15	0.6	19.2	
F_1	1.39	0.19	0.7	52.7	
F_2	3.02	0.17	1.6	53.2	
BC_2	1.98	0.15	0.6	30.0	
Ai-Bian 1 (Rht-D1c)	1.86	0.10	0.5	28.6	
Sava (Rht 8)	2.06	0.16	0.9	41.5	118.7**
BC_1	3.79	0.32	1.3	32.9	
F_1	4.28	0.43	1.7	39.1	
F_2	2.46	0.14	1.4	55.4	
BC_2	2.15	0.21	0.8	37.1	
Ai-Bian 1 (Rht-D1c)	1.86	0.10	0.5	28.6	

* significant for 0.05
** significant for 0.01

coefficients of variation (CV) were determined by computer program MSTATC (Crop and Soil Sciences Dep., Michigan St. Univ., USA, Ver. 1986). The estimation of the gene effects have been done using generation mean analysis with three (additive-dominance – AD model) or six parameter model as well as the best fit model (Mather and Jinks 1982), by computer program Programs for Quantitative Genetic Analysis, IDC-AGRO/CuSoft, Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia. The mode of inheritance have been determined by comparing F_1 and F_2 means to parents means for 0.05 significance level.

RESULTS AND DISCUSSION

In average, highest grain yield/plant and highest stem (unpublished data) have been determined for cv. Bankuty 1205 and the lowest grain yield/plant and shortest stem for cv. Ai-Bian 1 (Table 1). These results confirm earlier findings of Gale and Law (1977) and Law et al. (1978), that positive correlation occurred between these two traits. Grain yields found for genotypes Siete Cerros and Aobakomughi indicate that semidwarf wheats could also achieve high grain yields (Table 1), which supports the results of Borlaug (1968), Cooper (1979), Austin et al. (1980), Gale (1984), Borner et al. (1993), Li et al. (1998) and Šip et al. (1998). Lower grain yield per plant for short stature cultivar Tom Thumb was expected, since in Yugoslavian conditions this genotype is extremely late. Low yield of Yugoslavian cultivar Sava is probably result of unsuitable agroecological conditions during the growing season. This is confirmed by high value of coefficient of variation (45.0%) for this cultivar (Table 1).

The highest grain yields per plant and smallest coefficient of variation have been observed in crosses Bankuty 1205 × Aobakomughi, Bankuty 1205 × Sava and Sava × Ai-Bian 1 in F_1 generation and Bankuty 1205 × Sava in F_2 generation (Table 2). These results correspond to the

Table 2. Estimated values of mean (m), additive [d] and dominant [h] gene effects, individual (A, B, C), and joint (χ^2) test for grain yield per plant in wheat

	Bankuty 1205 (rht) × Aobakomughi (Rht-D1b)	Bankuty 1205 (rht) × Sava (Rht 8)	Siete Cerros (Rht-B1b) × Tom Thumb (Rht-B1c)	Siete Cerros (Rht-B1b) × Ai-Bian 1 (Rht-D1c)	Sava (Rht 8) × Ai-Bian 1 (Rht-D1c)
m	3.41	2.84	2.86	3.03	1.96
[d]	0.61	1.38	0.58	1.04	0.23
[h]	1.58	2.42	−0.36	−1.54	1.69
			Individual test		
A	−0.29	2.38*	3.13**	1.10*	1.25
B	1.97	−2.59**	−0.51	0.72*	−1.85**
C	−8.64**	−2.18	−1.40**	3.84*	−2.64*
$\chi^2_{(3)}$	114.2	78.0	42.8	26.1	33.7
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Table 3. Estimated values of mean (m), additive [d], dominant [h] and epistatic [i] – additive/additive, [j] – additive/dominant and [l] – dominant/dominant gene effects for grain yield per plant in wheat

	Bankuty 1205 (rht) × Aobakomughi (Rht-D1b)	Bankuty 1205 (rht) × Sava (Rht 8)	Siete Cerros (Rht-B1b) × Tom Thumb (Rht-B1c)	Siete Cerros (Rht-B1b) × Ai-Bian 1 (Rht-D1c)	Sava (Rht 8) × Ai-Bian 1 (Rht-D1c)
			Six parameter model		
m	−6.76**	5.39*	−1.07	4.75**	−0.09
[d]	0.38*	0.95**	0.65**	0.87**	0.10
[h]	25.96**	−1.56	10.25**	−3.57	5.82*
[i]	10.33**	−2.39	4.02**	−2.02	2.05
[j]	−2.26	4.97**	3.64**	0.37	3.10**
[l]	−12.01**	2.59	−6.64**	0.20	−1.45
			Best-fit model		
m	−5.38**	3.82**	−1.66	2.05**	2.34**
[d]	–	0.80**	–	0.58**	–
[h]	21.82**	–	12.03**	–	–
[i]	9.05**	–	4.02**	–	–
[j]	–	2.72**	4.93**	–	2.28*
[l]	−9.26**	–	−7.83**	–	–
χ^2	(2) 10.3	(3) 124.8	(1) 40.1	(4) 192.4	(4) 94.4
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

findings of Worland et al. (1990) and Flintham et al. (1997), who state that Rht genes present in cultivars Siete Cerros, Aobakomughi and Ai-Bian 1, in Mediterranean and South European conditions, have the negative pleiotropic effect on grain yield. It is interesting that for those crosses the biggest positive heterotic effect was also recorded. This is probably due to significant genotypic and phenotypic differences among them, as well as because for half of genes donors were cultivars Bankuty 1205 or Sava which are much more adapted to Yugoslavian agroecological conditions than other genotypes used in the study.

Individual and joint tests showed that the AD model was not adequate for assessing gene effects for any of the crosses.

Using the model with six parameters, it was found that grain yield is controlled by genes with additive (Bankuty 1205 × Sava and Siete Cerros × Ai-Bian 1) and additive/dominant gene effects (Table 3). This is of importance in wheat breeding, since additive genes could be fixed more easily than dominant ones. In the cross Siete Cerros × Ai-Bian 1 no epistasis have been determined, despite the fact that AD model suggest their existence. This is probably caused by the presence of polygenic epistasis (Mather and Jinks 1982), while in this study only duplicate epistasis has been examined. In crosses Bankuty 1205 × Aobakomughi, Siete Cerros × Tom Thumb and Sava × Ai-Bian 1 grain yield per plant was under much stronger control of dominant genes than additive ones (Table 3). From the breeding point, negative duplicate epistasis present in the crosses Bankuty 1205 × Aobakomughi and Siete Cerros × Tom Thumb is undesirable, since it suppresses the dominant gene effect, leading to a reduced trait expression. On the other side, according to Awamate and Behl (1995), epistasis additive/additive determined for those crosses increase possibility for selection towards genotypes with increased grain yield.

The best fit model indicated almost identical gene effects to those determined by the six-parameter model (Table 3). Estimated values of most important gene effects are given in Table 3.

In the F_1 generation grain yield per plant in cross Siete Cerros × Tom Thumb was inherited intermediately, despite the fact that significant additive gene effect, which determined this mode of inheritance have not been found (Figure 1). The presence of duplicate epistasis and significant additive/additive [i] epistasis effect suggested that these epistasis probably pull gene effects towards additivity. Negative heterotic effect of grain yield in cross Siete Cerros × Ai-Bian 1 seems to be the result of negative pleiotropic effect of Rht-D1c gene (Ai-Bian 1). The similar conclusion was obtained by Law et al. (1973), Kertezs et al. (1991) and Borner et al. (1993).

Positive heterotic effect was present in crosses where one parent was with semidwarf stature (Aobakomughi or Sava) while the other was extremely tall (Bankuty 1205) or with dwarf stature (Ai-Bian 1), (Figure 1). This suggests that from those crosses semidwarf genotypes with increased grain yield could be selected. In the F_2 generation for all crosses, except cross Bankuty 1205 × Aobakomughi, grain yield is inherited by partial, full or over dominance of the parent with higher grain yield per plant.

The obtained results showed that hybridization of genotypes with different Rht genes could, in some crosses, bring out good starting material for wheat breeding targeting increased grain yield.

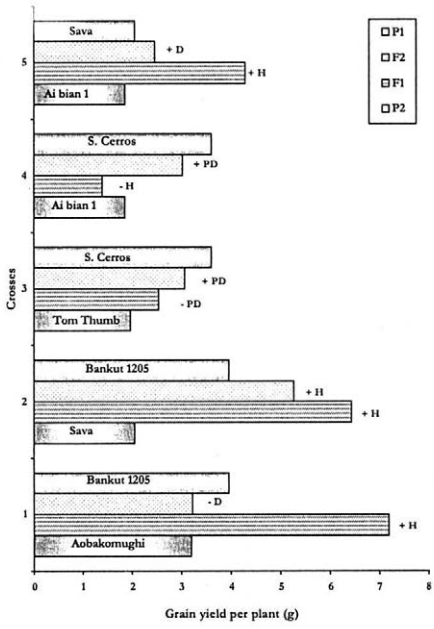

Figure 1. Mode of inheritance of grain yield per plant in crosses of wheat genotypes with different Rht genes

D – dominance
PD – partial dominance
H – heterotic effect (overdominance)

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ABSTRAKT

Genetická analýza výnosu zrna u kříženců genotypů pšenice s rozdílnými geny Rht

Byla sledována variabilita, genové efekty a způsob dědičnosti výnosu zrna u kříženců genotypů pšenice s rozdílnými geny Rht. Rodičovské komponenty představovaly odrůdy Siete Cerros (Rht-B1b), Aobakomughi (Rht-D1b), Tom Thumb (Rht-B1c), Ai-Bian 1 (Rht-D1c), Sava (Rht 8) a Bankuty 1205 (rht). Nejvyšší výnosy zrna na rostlinu a nejnižší variační koeficient v generaci F_1 a F_2 jsme zaznamenali u kříženců Bankuty 1205 × Aobakomughi, Bankuty 1205 × Sava a Sava × Ai-Bian 1 (v F_1) a Bankuty 1205 × Sava (v F_2). Individuální a společné testy naznačily, že pro stanovení aditivních genových efektů model aditivní dominance (AD) nebyl vhodný u žádného z těchto kříženců. Přítomnost aditivních, dominantních a epistatických genových efektů jsme určili za použití modelu o šesti parametrech a u každého křížence jsme provedli jejich interpretaci. Údaje získané v generaci F_1 naznačily, že z kříženců, kdy jeden rodič je odrůda polozakrslá (Aobakomughi nebo Sava) a druhý je odrůda vysoká (Bankuty 1205) nebo zakrslá (Ai-Bian 1), lze vyšlechtit genotypy se zvýšeným výnosem zrna. Způsobem dědičnosti výnosu zrna na rostlinu u všech kříženců v generaci F_2 s výjimkou křížence Bankuty 1205 × Aobakomughi je superdominance rodičovského komponentu s vyšším výnosem zrna na rostlinu. Získané výsledky ukázaly, že u některých hybridů může hybridizace genotypů s rozdílnými geny Rht poskytnout vhodný počáteční materiál pro šlechtění pšenice, jehož cílem je vyšší výnos zrna.

Klíčová slova: genové efekty; výnos zrna; dědičnost; geny Rht; variabilita; pšenice

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The effect of rotational and continuous grazing on sward

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ABSTRACT

The effect of rotational (RG) and continuous (CG) grazing of heifers on swards was studied in an experimental pasture in upland of the Jizerské hory mountains (lat. 51°20' N, long. 15°02' E) from 1993 to 1997. The number of grass tillers, white clover stolon growing points (sgp), and forbs were measured 3–4 times during the grazing season in each treatment. Clover stolon growing density averaged over time was significantly increased ($P < 0.001$) by continuous grazing. There was a general trend for sgp to gradually increase during the grazing season. The grazing system did not influence the number of grass tillers. Contrary to the white clover sgp, the number of grass tillers was highest at the beginning of the grazing season and after that it decreased. The average number of forbs tended to be greatest with CG with high variation at both treatment sites during the grazing seasons without significant differences. The number of forbs peaked in the first and last grazing cycle. The seasonal fluctuation of the present species was the main effect on the number of clover sgp, grass tillers and forbs.

Keywords: grassland; rotational grazing; continuous grazing; sward characteristics

Europe grasslands will be more intensively and effectively exploited in the near future with low-cost, low input systems of animal production (Frankov-Linberg and Frame 1997). White clover (*Trifolium repens*) is the most important legume component of the temperate grasslands and contributes to high quality forage. By means of its symbiotic relationship to rhizobia bacteria, it also fixes atmospheric N for its own growth and for that of the grass. The feeding value of grass-clover mixtures is better than grass alone, because white clover forage is low in fibre and is highly digestible, nutritious and palatable.

Maintaining the right proportion of clover in swards is important for grazing management. Defoliation during period of maximum grass growth favours white clover by reducing grass competition. A frequent mistake in white clover-grass pasture management is to allow grass to shade the clover. Clover content declined in a lightly stocked sward, even though the initial clover content was high, whereas clover content increased in a sward with a low initial content (Gibb et al. 1989). Varying grazing intensity in spring did not affect white clover content (Gibb and Baker 1989). Laidlaw et al. (1995) noted that the clover content under continuous cattle grazing was not greatly affected over five years by different sward heights during the grazing seasons. Similarly, Gibb et al. (1989) found similar clover contents in a four-year study in which the sward had begun with markedly different levels of clover, but had been continuously stocked with cattle. On the other hand, selective sheep grazing decreased clover content from year to year (Orr et al. 1990).

The number of grass tillers under different grazing system was studied for sheep by Hunt (1989) and Brock et al. (1996) and for cattle by Ernst et al. (1980) and Schlepers and Lantiga (1985). Generally, higher numbers of grass tillers were found under continuous grazing than rotational.

Most young cattle grazing in the Czech Republic is rotational rather than continuous. Knowledge of the behaviour of plants under such conditions is important for grazing management. The experiment described here was carried out at the Liberec Grassland Research Station to study the response of stolon growing points of white clover and the tiller density of grasses under rotational and continuous grazing over five years.

MATERIAL AND METHODS

Experimental site and management

The experiment was carried out from 1993 to 1997 in an experimental pasture in upland of the Jizerské hory mountains (lat. 51°20' N, long.15° 02' E), where the same soil condition and botanical composition were. The altitude of the experimental pasture is 420 m and the average annual precipitation for the area is 803 mm (Table 1). The mean annual temperature is 7.2°C. The dominant species are common bent grass (*Agrostis capillaris*), ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) and dandelion (*Taraxacum officinale*). No fertilizers had been applied since 1992.

There were two treatments: rotational grazing (RG) and continuous grazing (CG), each of them on 1.0 ha. Each area was grazed by four young Czech Pied heifers in 1993, by six young Friesian × Czech Pied heifers in 1994–1996

Table 1. Monthly rainfall (mm), mean daily air temperature (°C) and their 30-year average total or mean

	Rainfall					Air temperature				
	1993	1994	1995	1996	1997	1993	1994	1995	1996	1997
January	36.5	98.4	103.7	6.3	12.6	0.20	1.70	−2.1	−4.9	−4.6
February	57	26	67.3	6.8	58.5	−1.9	−1.5	2.9	−4.9	1.5
March	50	102.1	68	53.3	40	1.4	4.7	1.9	−1.6	3.4
April	39	57.4	56.1	29.6	96.9	9.2	6.8	7.4	7.2	3.8
May	44.6	71	104.8	162.8	93	14.6	11.7	11.6	10.9	12.1
June	63.3	63.1	131.1	68.9	114.8	14.5	14.5	13.7	14.9	15.3
July	223.5	22.1	40.8	159.6	224.4	15.3	21	19.6	14.8	15.8
August	96.9	155.4	153.7	102	55.9	15.5	17.1	17	16.3	18
September	104.9	70.9	114.3	97.9	23.4	11.7	13.2	11.8	9.2	12.1
October	45.1	54.2	12.5	56.4	76.5	7.6	6.4	10.7	9.2	5.7
November	76.6	66	77.6	44.4	39.8	−0.1	5.2	0.2	4.5	3.1
December	124.9	94.6	54.7	38.9	98.8	1.6	1.4	−2.9	−5.4	0.7
Total or mean	962	881	985	827	935	7.5	8.5	7.7	5.9	7.2
30-year average total or mean			803					7.15		

and by four young crossbreed Czech Pied × Charolle or Friesian heifers in 1997. The heifers were weighed each month during the grazing season. The average initial weights of the heifers on RG and CG during the experiment are shown in Table 3. No feeding supplementation was used.

The area of RG was divided into six paddocks, each measuring about 0.166 ha, and heifers were moved from one paddock to another, when the sward height after grazing was 7 cm. The sward with CG was maintained at a height of 5.0–7.5 cm by varying the grazing area available for treatment and by reducing the number of heifers in late summer. The grazing season lasted from the beginning of May to the end of September or the middle of October.

Sward measurements

The sampling sites during the whole experiment were paired blocks in the first paddock of the RG sward and in the first part of the grazing area (0.166 ha) in the CG sward. The time of sampling was after grazing in the first paddocks with RG treatment. The number of grass tillers, clover stolon growing points (sgp) and forbs were calculated from the ten randomized quadrats (20 × 5 cm) 3–4 times (according to the beginning of the grazing cycles at the RG treatment) during the grazing season in each treatment. In these quadrats the white clover was cut to obtain all surface and buried stolons. Samples of stolons were stored frozen, then measured for the number of sgp. In spring and late summer in each sampling site the percentage of the ground surface covered by all plant species within a 10 × 10 m area were determined. Sward height was measured using the first contact method twice weekly on grazed plot (100 per plot) at CG and before and after the plots were grazed (50 per plot) at RG.

Statistical analysis

The clover sgp, grass tiller and forbs measurements were analyzed by ANOVA-like generalized linear model (GLM) using program S plus 4.5.

RESULTS AND DISCUSSION

Monthly records of rainfall and mean temperatures collected at the meteorological station in Liberec (5 kilometres from experimental sites) are shown in Table 1, and demonstrate a period of several weeks in each year, during July (especially 1994, 1995), when rainfall was much lower than the long term average. The sward heights for each treatment are given in Table 2.

Animal performance

There was no significant difference in the weight gain of heifers over the grazing season between the treatments (Table 3). This confirms the conclusions of other studies (e.g. Ernst et al. 1980; Hardy et al. 1989; Volesky et al. 1994), that using rotation or continuous grazing system has a minor influence on the performance of grazing animals. The average stocking rate was higher with CG (Table 3), but this was due to the quite high stocking rate in spring (more than 2000 kg.ha^{-1}) for maintaining the target height of the sward. At the end of the grazing season the stocking rate was very similar at both treatments.

Clover stolon growing points

At the start of the experiment, the clover sgp densities were similar on both treatments, 700 in RG and 620 in CG

Table 2. Mean sward height under rotational (RG) and continuous (CG) grazing

		1993	1994	1995	1996	1997
RG sward height (cm)	before grazing	11.73	15.64	11.28	13.15	14.11
	after grazing	6.89	8.60	6.99	6.09	6.55
CG sward height (cm)		6.90	6.63	6.03	5.83	6.69

Table 3. Animal performance under rotational (RG) and continuous (CG) grazing

	1993		1994		1995		1996		1997	
	RG	CG	RG	CG	RG	CG	RG	CG	RG	CG
Initial live weight (kg)	177	179	140	136	137	138	139	139	212	212
Final live weight (kg)	313	298	226	215	230	229	211	249	343	343
Mean stocking rate (kg.ha^{-1})	1 205	1 610	1 395	1 735	1 370	1 745	1 229	1 611	1 459	1 654
Live-weight gain (g.day^{-1})	781	718	542	528	556	549	481	723	961	987

per m^2 (Figure 1). During the grazing season in 1993, the number of sgp for treatment CG increased until September 1993. In July and August 1994 the long term water deficiency (Table 1) decreased sgp on both treatments, and this lower number lasted until May 1995, when the number of sgp at CG started increasing again. The number of sgp at RG remained relatively constant from the beginning of this experiment to August 1996, and varied from 340 to 790 sgp.m^{-2}. After that the shape of the curve was similar to CG treatment. The lowest number of sgp was at the beginning of the grazing season (657 sgp.m^{-2}) and increased through the grazing seasons, when it peaked in the fourth grazing cycle to 1271 sgp.m^{-2} with significant differences between grazing cycles ($P < 0.001$). Also, Gibb and Baker (1989) noted that sgp densities under beef cattle grazing were increased during the later part of the season. The average number of sgp fell in 1994 and 1995, probably due to lower rainfall, and, after that, gradually increased. Low density was mainly influenced by higher number of measured quadrats without clover

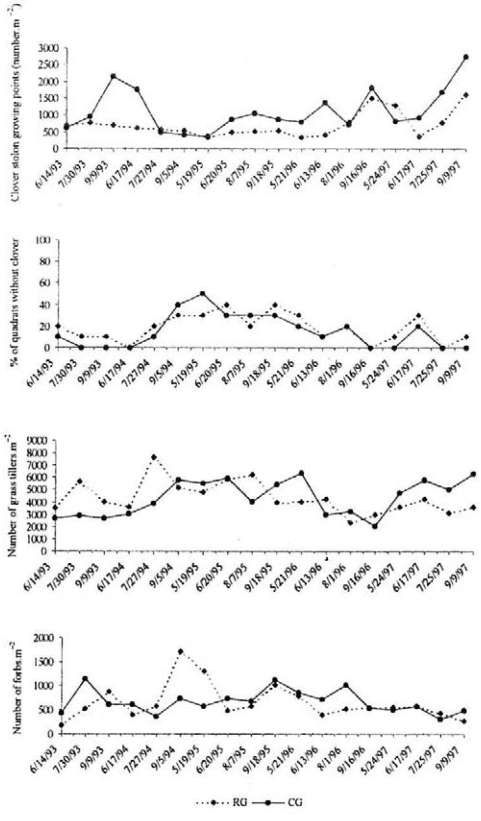

Figure 1. Population density of white clover, grass and forb under rotational (RG) and continuous (CG) grazing

Table 4. Mean plant density of grass (tillers m^{-2}), white clover (growing points m^{-2}) and forbs (plants m^{-2}) under rotational (RG) and continuous (CG) grazing

Treatment	Grasses		White clover		Forbs	
	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
RG	4 377	204	707	58	648	46
CG	4 367	198	1 135	97	663	44
Significance	NS		***		NS	
1993	3 588	237	982	128	623	74
1994	4 858	456	735	115	728	101
1995	5 239	288	634	115	810	76
1996	3 539	289	967	114	669	59
1997	4 563	268	1 254	148	460	41
Significance	***		**		**	
Grazing cycle 1	4 852	307	657	100	763	87
Grazing cycle 2	4 210	248	815	97	509	38
Grazing cycle 3	4 420	318	834	82	606	54
Grazing cycle 4	4 199	255	1 271	147	788	74
Significance	NS		***		***	

NS = not significant
** $P < 0.01$
*** $P < 0.001$

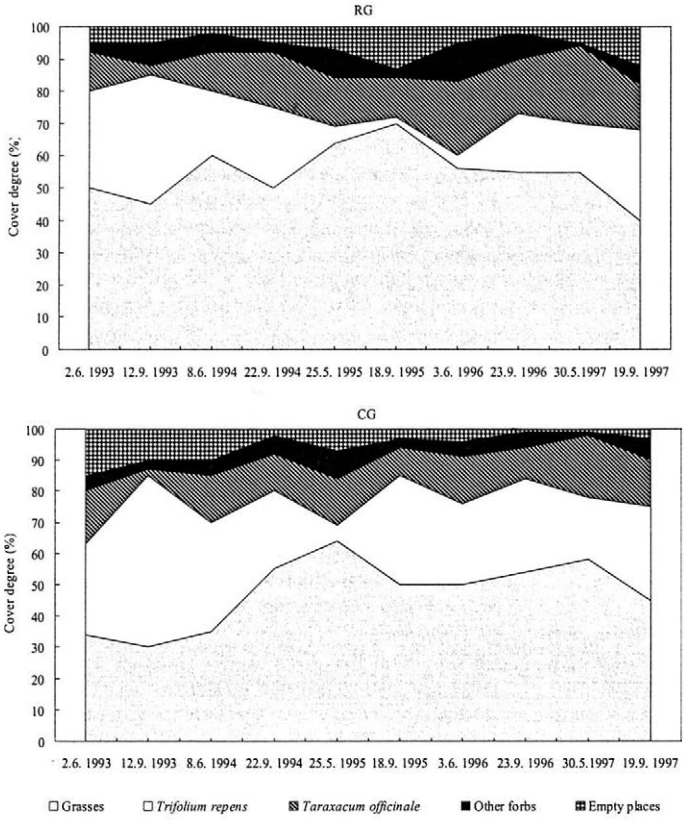

Figure 2. Development of botanical composition of the sward under rotational (RG) and continuous (CG) grazing

at both treatments, rather than lower density of sgp in each quadrat (Figure 1). Sgp density averaged over all measurements each year was significantly higher ($P < 0.001$) in the frequently-grazed treatment CG than in the RG treatment (Table 4). Similarly Laidlaw and Steen (1989) found, at the end of a three-year steer grazing experiment, that a lower stocking rate had significantly fewer growing points per unit area than a frequently-grazed treatment with higher stocking rate. There were also significant differences between years ($P < 0.01$), which could be caused by seasonal variation of weather.

Grass tillers

Figure 1 presents the mean number of grass tillers during grazing experiments. At the start of this experiment the mean number of tillers was 3540 in the RG sward and 2700 in the CG sward per m^2. With lower summer precipitation in July (1994, 1995), the grass tillers increased at both treatment sites, especially with RG. It seems that the taller sward with RG protects the grass tillers more from summer drought. After that they were about 2700 tillers.m^{-2} at both treatments without significant statistical differences. However a higher density of grass tiller was found by Hunt (1989), Brock et al. (1996) in continuous sheep grazing, and by Ernst et al. (1980), Schlepers and Lantiga (1985) with continuous cattle grazing, rather than with rotational grazing. Those experiments were done on a simple grass/white clover mixture or grass monoculture and without forbs, unlike this experiment. Contrary to the white clover sgp, the number of grass tillers was highest at the beginning of the grazing season 4852 per m^2 (grazing cycle 1). In consecutive grazing cycle they fell to 4210 (grazing cycle 2), 4420 (grazing cycle 3) and 4199 (grazing cycle 4) tillers per m^2 without significant statistical differences (Table 4). There were significant differences between years ($P < 0.001$), with higher numbers of grass tillers generally found in years with higher summer temperatures. The average number of grass tillers was lower than in the work published from seaside areas and was close to results recorded by Fiala (1990), which were studied only on lawn grassland in the Czech Republic.

Forbs

The average number of forbs (Table 4) tended to be slightly greater in CG, with high variation at both treatment sites during the grazing seasons (Figure 1). Significant differences were found between years ($P < 0.01$) and grazing cycles ($P < 0.001$). We observed an increase in the number of forbs in September 1994 at RG. It could be probably explained by the shortage of rainfall in July of

that year. After this water deficiency (Table 1) there was heavy rain and *Taraxacum officinale* and *Cerastium holosteoides* started to colonize in the open canopy. The maximum number of forbs occurred in the first grazing cycles, when many of forbs were at their growing peak, and in the last grazing cycle when grass growth rates are much lower. The percentage of total number was *Taraxacum officinale* 38% and 36%, *Cerastium holosteoides* 22% and 26%, *Veronica* sp. (*Veronica chamaedris*, *Veronica serpilifolia*), *Stellaria media* 12% and 14% and others forbs 10% and 5% for RG and CG treatments, respectively. There was a very high variation in the number of forbs because several species with a different habitus were counted as one plant. However, large plants were mostly depressed by grazing. The exceptions were some plants with based rosette, e.g. *Taraxacum officinale* and *Leontodon autumnalis*, which are more tolerant to grazing than others. Comparable data about forbs density do not exist for either cut or grazed sward.

Botanical composition

Changes in species composition through the season are shown in Figure 2. White clover generally increased as the grazing season progressed in all years in both treatments. Averaged over years, white clover comprised about 15% with RG and 23% with CG at the start of grazing in spring, and increased gradually to 23% in RG and 35% in CG in September. The covers of grasses and dandelion (*Taraxacum officinale*) were generally highest in spring and then declined in September. The proportion of other forbs varied from 1% to 15% over the grazing experiment. In years of summer drought, white clover may be replaced by dandelion and other forbs. However, white clover seems to recover to its average percentage in years with a regular distribution of precipitation.

CONCLUSION

In the present study, the tiller densities and number of clover stolon growing points were considerably lower than results from seaside areas, where conditions are more suitable for grassland growing, than in the Czech Republic. The causes are more heterogeneity (about 20 species) and an open sward under different soil and weather conditions than at seaside areas. The grazing system had no effect on animal performance. The continual grazing significantly extended the number of clover sgp, but did not influence either grasses or forbs. The number of clover sgp was gradually increased during the grazing season in both treatments. On the contrary, the number of grass tillers peaked in spring and after that decreased. Maximum number of forbs occurred in the first and last grazing cycles. The number of clover sgp, grass tillers and forbs were mainly influenced by plant responses to rainfall.

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ABSTRAKT

Vliv rotační a kontinuální pastvy na travní porost

Byly studovány některé charakteristiky travního porostu při kontinuálním (CG) a rotačním (RG) systému pastvy jalovic. Pokusy probíhaly v letech 1993 až 1997 na podhorské pastvině v Jizerských horách. Pokusné plochy tvořily 2 ha mezofilního travního porostu, rozdělené na dvě části, každá o rozloze 1 ha, na kterých byla uplatňována rotační (RG) a kontinuální (CG) pastva mladých jalovic. Pastvina s rotační pastvou byla rozdělena na šest oplůtků (0,166 ha) a jalovice byly přeháněny do dalšího oplůtku, přičemž výška porostu po spasení byla 7 cm. Výška pastevního porostu u kontinuální pastvy byla v průběhu celého experimentu udržována v rozmezí 5,0 až 7,5 cm. Počet travních odnoží, růstové body jetele plazivého a počet ostatních dvouděložných bylin byly sledovány pomocí párových ploch (0,05 m × 0,2 m) v deseti opakováních v každé variantě 3× až 4× v průběhu pastevní sezony. Průměrné denní přírůstky jalovic u obou pastevních systémů byly obdobné a kolísaly od 500 do 1000 g za den v závislosti na počáteční živé hmotnosti. Počet růstových bodů jetele plazivého se významně zvýšil ($P < 0,001$) při kontinuální pastvě (1135 ks.m^{-2}) ve srovnání s rotační pastvou (707 ks.m^{-2}). Počet růstových bodů jetele plazivého se u obou variant v průběhu pastevní sezony postupně zvyšoval (obr. 1) a vrcholu dosahoval na konci pastevní sezony, kdy se růstová aktivita trav snižuje. Naopak počet travních odnoží byl nejvyšší na začátku pastevního období a postupně klesal. Nebyly nalezeny významné rozdíly mezi variantami v počtu travních odnoží a počtu bylin. Počet bylin měl vrchol na začátku a na konci pastevního období. Sezonní fluktuace zastoupených druhů měla hlavní vliv na sledované charakteristiky travinného porostu (tab. 4).

Klíčová slova: travní porosty; rotační pastva; kontinuální pastva; charakteristiky porostu

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Bread-making quality and stability of winter wheat grown in semiarid conditions

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ABSTRACT

The objective of the paper was to determine the effects of the cultivar, year, and cultivar × year interactions on the expression of several technological quality characters in winter wheat grown under semiarid conditions of the Vojvodina province. Twenty-three winter wheat cultivars were grown over a seven-year period (1990–1996). The harvested grain was milled and the flour was analysed for protein content, wet gluten, bread yield, and loaf volume. The significance of cultivar × year interactions was determined using two parameters of stability – the coefficients of regression and deviation from regression. In protein content, wet gluten, and bread yield, the contribution of the year to total variance was higher than that of the cultivar, while for loaf volume the situation was the opposite. The parameters of stability showed that a number of cultivars exhibited stability for some of the quality indicators under investigation. However, only the cultivar Proteinka had both a high phenotypic value and high environmental stability for all the traits involved. In order to reduce the negative effects of cultivar × environment interactions on the technological quality of winter wheat, we need to develop cultivars that have a high potential for yield and technological quality as well as tolerance to soil and air droughts at grain fill, so that they are suitable for growing under semiarid conditions.

Keywords: wheat (*Triticum aestivum* L); cultivar; genotype × environment interaction; stability; protein content; wet gluten; bread yield; loaf volume

Wheat cultivars differ significantly as to their grain and flour quality. These differences follow from the actions of various genes that control the characters responsible for the technological quality of bread (Payne 1987). However, environmental conditions, too, play a large role in the expression of a genotype's characters (Bassett et al. 1989; Lukov and McVetty 1991; Peterson et al. 1992). Because of this, it is essential to understand how the various indicators of bread quality change under different environmental conditions. The knowledge of the effects of the genetic background, environmental conditions, and various interactions provide us with a fuller insight into the variability of these characters. Breeding cultivars, that are adapted throughout a reasonably large geographical area and that show some degree of stability from year to year, are a major problem facing plant breeders. Quality characteristics of wheat are important to the plant breeder because the product must have a marketable value to the miller and the baker. High quality flours and baked goods begin with good quality wheat. Most of the modern wheat breeding programmes lay major emphasis on quality standards, particularly those concerned with high quality prime wheat for the export market.

In the early days of wheat breeding in Yugoslavia, domestic cultivars were modelled on Italian cultivars that had short stems, productive spikes, and a shorter growing season. Efforts were made to incorporate into these cultivars genes for resistance to low temperatures, a better grain and flour quality, and resistance to diseases (Borojević 1990). With the introduction of the cultivar Bezostaja-1 into the breeding program, the quality of wheat improved considerably. Using various methods for the hybridization of genetically divergent parents, genes that control all major agronomic characters were combined to produce cultivars with a yield potential of over 10 t/ha and satisfactory to excellent technological quality (Mišić and Mladenov 1998).

In order to be a good raw material for the production of bread, wheat has to possess a series of morphological, physiological, and biochemical traits, which, in addition, must be stable in different production conditions (Baenziger et al. 1985; Lukow and McVetty 1991; Peterson et al. 1992). Traits that determine the quality of the grain are controlled by polygenes and are largely dependent on the agroecological conditions. In most of the production areas, there is a certain number of limiting factors that prevent genotypes from realizing their full genetic potential. It is estimated that only 10% of the total agricultural acreage in the world are free from biotic and abiotic stress (Solh 1993). Of the areas exposed to stress, 26% are affected by drought and 15% by low temperatures (Christiansen 1982), while Monti (1987) estimates that drought is a limiting factor in agricultural production on as much as one third of agricultural land worldwide. Due to the global change of climate, drought is a frequent occurrence both in southern parts of central Europe and in the Balkan Peninsula, especially from 1980 onwards (Jovanović et al. 1996). According to the drought index for July and August, 74% of years in Yugoslavia are either semiarid or arid. Thanks to such climatic conditions, southern Europe and southern parts of central Europe are

Table 1. Coordinates, soil type and climatic data for Novi Sad

Coordinates	N	45°33'
	E	19°85'
Elevation	(m)	82
Mean precipitation	(mm/month)	
October–March		47.5
April–June		63.0
Mean temperature	(°C)	
October–March		
Low		0.8
High		8.6
April–June		
Low		10.1
High		21.5
Soil classification		Chernozem limeless

considered suitable for the production of bread wheat (Borghi et al. 1991; Mišić and Mladenov 1998; Panayotov 1998; Bedo et al. 1998). In order to minimise the negative effects, the environment has on the quality of wheat grain, it is necessary for the growers to follow strictly the production technology recommendations that have been made for the area in question (Przulj et al. 1998; Mladenov et al. 1999). In addition to this, producers have to make the right choice of cultivars, that is to say, they must choose several cultivars with different levels of adaptability and stability in order to reduce the negative effects of variations occurring in environmental factors (Borojević 1990).

The objective of this study was to: (i) determine the respective contributions of the cultivar, year, and cultivar × year interactions to the variation of some indicators of winter wheat quality; and (ii) estimate the reaction and stability of the cultivars studied.

MATERIAL AND METHODS

Analyses were performed on grains taken from a small-plot trial carried out at an experiment field of the Institute of Field and Vegetable Crops in Novi Sad between 1990 and 1996. Long-term period data, collected from the meteorological station located close to the experimental field, are presented in Table 1. Weather conditions in the experimental years (1990–1996) are given in the details in our previous paper (Mladenov and Przulj 1999).

Used in the study were 23 winter wheat cultivars having diverse parentage and representing much of the current elite and historic germplasm grown in Yugoslavia (Table 2). The cultivars represented a broad spectrum of baking qualities. Wheat cultivars were planted in a randomized complete block design with three replications. Plots of 5 m^2, with ten rows spaced 10 cm apart, were seeded at a rate of approximately 230 kg ha^{-1}. Quality tests were performed on the harvested seed of each cultivar for each year.

Table 2. Release date, pedigree, and technological level of cultivars studied

Cultivar	Release date	Pedigree	Technological level
Balkan	1979	Bačka/Bez.1//Miron.808/3/NS435/4/Skorospelka35	excellent bread
Danica	1990	NS2773/Partizanka//Sremica	excellent bread
Dicna	1992	NSrana1/Tisa//N.Banatka/3/Macvanka	very good bread
Divna	1994	NS646/Bez.1//Partizanka/3/NS3187	very good bread
Evropa 90	1990	Talent/NSrana2	satisfactory bread
Jednota	1987	San.Past/Purdue5369A612//Bez.1/3/Aur./4/Partizanka	very good bread
Kosovka	1988	NS2705/Partizanka	excellent bread
Lasta	1987	Stepnjačka30/NS736//Bez.1/3/Lutescens32/4/ Aurora/5/Miron.808/6/Jubile.50/7/NS1481/8/Panonija	satisfactory bread
Milica	1992	Zelengora/Macvanka//Partizanka	excellent bread
NS rana 5	1991	NSrana1/Tisa//Partizanka/3/Macvanka	excellent bread
Partizanka	1973	Bezostaja1/NS116	excellent bread
Prima	1995	Tob.66/Kav.//N.Banat/3/NS3142/4/R.niska/5/NS3985	very good bread
Proteinka	1990	NS2767/Balkan//Sremica	excellent bread
Rana niska	1990	Tobari66/Kavkaz//Bačvanka/3/NSrana1	excellent bread
Rodna	1988	NS646/Bez.1//Aurora/3/Partizanka	excellent bread
Sara	1998	Partizanka/Jedina//Evropa	excellent bread
Somborka	1986	NS2153/Aurora//NovaBanatka	excellent bread
Sonja	1998	NinMai/NS2853//Posavka/3/NS2897/NS3142	very good bread
Stepa	1993	S.Pas./Purdue5369A123//Bez.1/3/Aurora/4/Partizanka	very good bread
Tanjugovka	1988	Jugoslavija/Partizanka	excellent bread
Tera	1995	NS2879/NS3000//Rananiska	satisfactory bread
Varadinka	1991	NSrana2/Aurora//Partizanka/3/Sremica	very good bread
Zlatka	1997	Lozničanka/NS-3000//NS-3014/3/NS2853/Zg1-628/77	very good bread

Table 3. Mean squares for the analysis of variance and percentage of total variance for each source of variation of bread making quality

Source of variation	df	Protein content		Wet gluten		Bread yield		Loaf volume	
		MS	%	MS	%	MS	%	MS	%
Rep. (year)	14	1.37		22.23		23.46		484	
Year (Y)	6	70.86**	53.2	605.21**	44.5	243.22**	52.2	27289**	10.6
Cultivar (C)	22	4.48*	5.6	44.54*	4.0	17.67**	7.1	32946**	43.6
C × Y	132	2.30**	40.9	28.89**	51.3	7.91**	40.3	4252**	44.9
Error	308	0.01	0.3	0.04	0.2	0.03	0.4	28	0.9

The following indicators of technological quality were analysed: grain protein content (N × 5.7; 0% moisture basis) of whole meal – determined according to Kjeldahl by the ICC method 105/2 (1980, 1994); wet gluten (%) – determined according to ICC approved methods 106/2 (ICC 1972, 1992); bread yield in g/100 g of flour; and loaf volume in ml/100 g of flour – determined after baking according to ICC approved methods 131 (ICC 1972, 1992). The bake formula included flour, water, salt (2.0%), and yeast (2%).

Analysis of variance and estimates of the components of variance were calculated according to Comstock and Moll (1963). The year was treated as a separate environment for statistical analyses. Cultivars and environments (years) were treated as random effects. The significance of mean squares for cultivars, years, and cultivar × year interaction was tested by using mean squares for cultivar × year and pooled error, respectively. The percentage contribution of each variance component was estimated by summing the appropriate terms to give an estimate of total variance and then dividing the specific variance component by the total variance (Comstock and Moll 1963). Ratios of variance components were calculated as per Peterson et al. (1992). Stability analyses were determined using regression methods as outlined by Eberhart and Russell (1966). Environmental index values were calculated by subtracting the grand mean from the mean in each environment. Cultivar response to differing environments (b_i) was estimated by regressing the cultivar mean on the seven environmental index values. The b_i-values were tested for differences from $b_i = 1.0$ using t-tests. The variance of the deviations from regression was estimated by S^2_d. An approximate F-test was employed to determine whether the deviations from regression were significant.

RESULTS AND DISCUSSION

All the main effects (cultivar and year) were significant, meaning that they significantly contributed to the variation of each parameter of quality studied (Table 3). The differences between the years were large and significant (data not shown), hence the strong interactions between the cultivar and the year ($P < 0.01$). The components of variance for all the quality characters have been expressed as percentage to illustrate the relative contribution of each variation source to total variance. The contribution of the year to total variance for protein content, wet gluten, and bread yield was larger than that of the cultivar, meaning that these characters varied more according to the year than according to the genotype. The large variance for the year and cultivar × year interactions resulted from differences in cultivar response to the varying environmental conditions.

These results are in agreement with the findings for semidwarf spring wheats (Lukow and McVetty 1991), hard red winter wheats (Baenziger et al. 1985; Peterson et al. 1992), and soft white winter wheats (Bassett et al. 1989). Fowler and De La Roche (1975), on the other hand, reported relatively insignificant cultivar by environment interactions for the qualitative traits of hard red spring wheat. The environmental range used by these authors was apparently quite narrow, reducing the likelihood of significant cultivar by environment interactions. The main reason for the different cultivar responses was their different genetic background (Lukow and McVetty 1991; Mišić and Mladenov 1998). Because of this, the same cultivar had different phenotypic values in different years. Genotype × environment interactions can be either positive or negative (Talbot 1993; Mladenov and Przulj 1999), therefore the production should involve cultivars of both narrow and wide adaptability so as to be able to exploit to the maximum different agroecological and cultural practice conditions, i.e. to capitalize on positive cultivar × environment interactions and minimize the effects of negative ones.

The strong influence of genetic factors on loaf volume was confirmed by the large variances for the genotype (43.6%) and cultivar × year interactions (44.9%). Technological quality is comprised of a number of traits (Finney 1985), so it is very hard to make a combination of genes that will have positive values for all parameters of quality. For this reason, breeding efforts should be primarily focused on improving those parameters of quality that can easily be changed in genetic terms and that at the same time cannot be easily improved by using various technological procedures.

The relative effects of cultivar, year, and their interactions on quality parameters were studied using variance ratios (Table 4). For protein content, wet gluten, and bread yield, year variance (non-genetic factors) was higher than the cultivar one, while the loaf volume was affected more by genetic factors. The variance ratios indicate that the

Table 4. Ratios of variances estimated for year and cultivare mean effects and their interaction for various traits in wheat

	Protein content	Wet gluten	Bread yield	Loaf volume
Year/cultivar	9.6	12.2	7.3	0.2
Cultivar/cultivar × year	0.1	0.1	0.2	1.0

year had a greater effect on quality parameters than the cultivar, and the variation ranges in Table 5 also confirm this. For loaf volume, the variance of cultivar × year interactions was close to the genetic variance, whereas in the case of protein content, wet gluten, and bread yield it was higher. The relatively high cultivar × year interaction for protein content, wet gluten, and bread yield (more than 20% of cultivar variance) indicates that these parameters should be studied in several different environments if we want to determine with certainty the genetic potential of the genotype concerned. Cultivars are usually evaluated for at least three successive years. In the present study, although the cultivar × year interaction effect was significant for all the qualitative traits, the effect of the environments was confusing in the estimates obtained, therefore it is not possible to comment on the optimum number of years and sites desirable for quality testing. Bhatt and Derera (1975) argue that it is better to do the testing over a large number of sites in a few years than to do several years of testing over a few sites each year. Bassett et al. (1989), on the other hand, emphasize that seasonal effects are usually greater than site effects.

A cultivar's value depends not only on its genetic potential for particular characters but also on its ability to realize this potential in actual production and under different environmental conditions (Dotlačil and Toman 1991; Mladenov and Przulj 1999). Adaptability is the genetic ability of a genotype to produce high and stable phenotypic values in different environmental conditions. According to the Eberhart and Russell (1966) method, the b_i-value parameter of stability is the linear regression of a genotype relative to the mean value of all genotypes in a given environment. The other stability parameter of a genotype (S^2_d) is its deviation from linear regression. In the present study, the values of stability parameters for protein content, wet gluten, bread yield, and loaf volume differed from year to year, confirming that the cultivars responded differently to year variations. For each quality trait, two or more cultivar regression coefficients (b_i) were significantly larger or smaller than the mean b_i (Table 6). These differences in cultivar response indicate cultivar × year interactions.

Partizanka had the highest and Lasta the lowest protein content. The high protein content of Partizanka, Somborka, Milica and Proteinka was expected, as excellent bread wheats are developed for high protein content. Very few cultivars (namely Dicna and Lasta) had regression coefficients significantly ($P < 0.05$) different from one. Few deviations from regression were significant, which indicate that most of the cultivars can be classified as stable for protein content.

Milica had the highest and Lasta the lowest wet gluten. The low values for Lasta and Tera were expected, as they are satisfactory bread wheat and should have lower wet gluten than excellent bread wheat. Seven cultivars had regression coefficients significantly ($P < 0.05$) different from one. Most of the other cultivars had significant deviations from regression.

Proteinka had the highest bread yield and yielded 2.8% more bread than Evropa 90, which had the lowest bread yield. Only four cultivars had regression coefficients different from one. Many of the deviations from regression were small, which indicated that most of the cultivars were stable for bread yield.

Sara had the highest loaf volume and yielded 31.5% more than Lasta, which had the lowest loaf volume. The regression coefficient (b) of the 23 cultivars used in this study ranged from 0.2 to 1.9 for loaf volume. Ten cultivars had regression coefficients different from one, while many of the cultivars had large and significant deviations from regression, indicating that they should be classified as unstable.

The ideal cultivar according to Eberhart and Russell (1966) would have a high mean performance over a range of environments, a regression coefficient of one, and zero deviation mean square from regression. Becker et al. (1982) regarded mean square for deviation from regression to be the most appropriate criterion for measuring phenotypic stability in an agronomic sense because this parameter measures the predictability of genotypic reaction to environments. According to Breese (1969), cultivars with regression coefficients greater than 1.0 would be adapted to more favourable environments, while those

Table 5. Ranges of cultivar and environment mean values for quality characteristics of 23 winter wheat cultivars grown in seven years in the 1990–1996 growing seasons

Trait	Ranges				Mean	*CV*% $n = 161$
	cultivars $n = 23$	*CV*%	year $n = 7$	*CV*%		
Protein content in % of dry matter	12.5–14.5	3.4	12.1–15.0	7.5	13.5	9.7
Wet gluten in %	29.2–35.6	4.5	28.2–35.7	9.2	32.2	13.0
Bread yield in g/100 g of flour	137.5–141.4	0.7	137.0–142.6	1.3	138.7	1.8
Loaf volume in ml/100 g of flour	409–538	8.5	440–494	4.2	468	11.7

Table 6. Means, regression coefficients, and deviations from regression for protein content, wet gluten, bread yield, loaf volume of 23 winter wheat cultivars grown across seven years in 1990–1996

Cultivar	Protein content (% of dry matter)			Wet gluten (%)			Bread yield (g/100 g of flour)			Loaf volume (ml/100 g of flour)		
	M	b_i	S^2_d	M	b_i	S^2_d	M	b_i	S^2_d	M	b_i	S^2_d
Balkan	13.7	1.2	0.6	32.0	1.6*	2.9	138.5	1.2	7.9**	521	1.7	44
Danica	13.5	1.5	1.0*	32.3	1.4	7.3*	138.7	1.0	2.2	446	1.5	145*
Dicna	13.4	1.7*	0.6	32.1	1.5	11.4**	138.1	1.6	1.0	428	0.8	91
Divna	13.2	0.9	0.4	31.2	0.7	7.3*	138.6	1.2	1.9	503	1.8*	419**
Evropa 90	13.4	1.0	0.4	31.2	0.8	8.9*	137.5	0.8	0.8	414	0.6	103
Jednota	13.5	1.0	0.9*	33.0	1.4	2.9	138.4	1.0	1.7	473	1.9*	53
Kosovka	13.2	0.9	0.7	33.3	0.8	3.0	138.2	1.2	0.8	516	1.9*	232**
Lasta	12.5	0.2*	1.9**	29.2	0.3*	22.4**	140.2	1.1	1.1	409	1.0	139*
Milica	14.1	1.2	0.3	35.6	1.4	2.3	137.9	1.3	2.2	501	0.3*	124*
NS rana 5	13.8	1.1	0.4	34.4	1.1	6.9*	138.3	1.5	3.0	447	1.0	263**
Partizanka	14.5	1.2	1.5**	31.1	1.0	26.3**	138.5	1.3	1.0	504	1.9*	131*
Prima	12.8	0.6	0.6	32.1	0.3*	8.7*	138.9	0.1*	3.1	443	0.2*	17
Proteinka	14.1	1.4	0.2	32.9	1.2	1.8	141.4	1.3	3.7	522	0.9	57
Rana niska	13.3	0.6	0.6	31.1	0.7	8.1*	138.2	0.1*	4.9*	417	0.5	161*
Rodna	13.2	1.2	0.3	32.9	1.5	3.9	138.2	0.9	1.8	472	1.9*	169*
Sara	13.9	1.2	0.6	32.9	1.1	1.4	139.8	1.6*	1.5	538	0.5	124*
Somborka	14.1	1.0	1.1**	32.4	0.7	12.1**	138.1	1.1	3.1	436	0.3*	62
Sonja	13.5	0.6	0.7	31.2	0.0*	2.9	138.3	0.5	2.1	499	0.3*	244**
Stepa	13.1	1.3	0.6	30.7	1.6*	6.6*	138.0	1.2	1.3	448	1.0	186*
Tanjugovka	13.0	1.1	0.4	32.2	1.5	2.3	138.3	0.6	1.0	472	0.9	59
Tera	13.4	0.5	0.9*	29.9	0.5	5.6*	139.4	1.1	1.8	415	0.3*	48
Varadinka	13.6	1.4	0.7	33.4	1.8*	5.9*	138.9	0.9	0.9	457	0.7	126*
Zlatka	14.0	0.4	0.6	34.1	0.1*	11.2**	140.3	0.4*	1.3	492	1.2	42
Mean	13.5	1.0		32.2	1.0		138.7	1.0		468	1.0	
$LSD_{0.05}$	0.5			1.3			3.1			32		
$LSD_{0.01}$	0.6			1.7			4.2			42		

with coefficients lower than one would be relatively better adapted to less favourable growing conditions. The stability parameters from our study show that a number of cultivars exhibited stability for some of the quality indicators but that only Proteinka had both a high phenotypic value and high environmental stability for all the traits involved. The b_i-values of Proteinka indicated that it was relatively better in favourable environments but less well adapted to low favourable growing conditions. This indicates that some traits behave differently with respect to environmental stability, i.e. those genes that control certain quality parameters express themselves only under particular conditions, which often need to be highly specific. Therefore, for the selection of a particular technological character to be successful, we must determine under which particular agroecological conditions the extent of the character's expression will be high.

The majority of wheat cultivars used in the study were semi-dwarf ones (gene *Rht8* present) with an average plant height of 80–85 cm. The study also included several dwarf (plant height under 75 cm) cultivars (Rana niska, Prima, Tera, Sonja, Divna and Zlatka) with genes *Rht1* and *Rht2* (Jošt and Jošt 1989; Petrović and Worland 1992; Worland et al. 1998; Mišić and Mladenov 1998). The dwarf cultivars had high phenotypic values for some indicators of quality but were also very unstable in this regard. Such behaviour may have been due to the pleiotropic effects of genes *Rht* and particularly GA I (gibberellic acid insensitive), since high temperatures and low relative humidity at heading and flowering have an unfavourable effect on the latter gene (Petrović and Worland 1993).

Temperature, an uneven distribution of precipitation, and water balance variations are known to be the main factors of wheat yield instability in the semiarid conditions of the Vojvodina province (Przulj et al. 1998; Mladenov and Przulj 1999). Temperature (Corbellini et al. 1998), soil type (Moss et al. 1983), precipitation (Flood et al. 1996), and grain fill duration (Wardlaw and Moncur 1995) are other environmental factors that affect the technological quality of wheat. Breeding for increased tolerance to soil and air drought at grain fill can greatly improve the stability of quality indicators.

Cultivar × environment interactions are the main reason for the variation of phenotypic values and their sta-

bility. These interactions, which can be attributed to the fact that cultivars respond differently to different environmental conditions, can severely hamper the progress of breeding (Borojević 1990). The breeding of winter wheat produced cultivars with a high potential for grain yield and grain technological quality as well as dwarf and semi-dwarf cultivars with high values of quality indicators (McGuire et al. 1980; Mišić and Mladenov 1998).

In order to reduce the negative effects of the cultivar × environment interactions on winter wheat traits, it is necessary to develop cultivars that are tolerant to environmental variations. The phenotype should be able to reflect the genetic basis of the cultivar as much as possible under different growing conditions. Stability parameters can be used in breeding and choosing wheat cultivars for particular production conditions. By developing cultivars with stable quality indicators, the production in semi-arid conditions would become more profitable for producers, millers, and bakers alike, while the consumer would get bread of a more uniform quality.

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ABSTRAKT

Pekařská jakost a stabilita ozimé pšenice pěstované v semiaridních podmínkách

Byl sledován vliv odrůdy, ročníku a interakce odrůda × ročník na projevy několika znaků technologické jakosti ozimé pšenice pěstované v semiaridních podmínkách Vojvodiny. Během sedmiletého období (1990 až 1996) jsme pěstovali 23 odrůd ozimé pšenice. Sklizené zrno jsme umleli a provedli jsme analýzu mouky na obsah bílkovin, mokrého lepku, pekařskou výtěžnost a objemovou vydatnost mouky. Významnost interakcí odrůda × ročník jsme stanovili pomocí dvou parametrů stability – regresních koeficientů a odchylky od regrese. Pokud jde o obsah bílkovin, mokrého lepku a pekařskou výtěžnost, byl podíl ročníku na celkovém rozptylu vyšší než podíl odrůdy, zatímco pro objemovou vydatnost mouky platil opačný vztah. Ukazatele stability naznačily, že řada odrůd vykazuje stabilitu některých ze sledovaných ukazatelů jakosti. Avšak pouze odrůda Proteinka dosáhla u všech sledovaných znaků jak vysokou fenotypovou hodnotu, tak vysokou ekologickou stabilitu. Abychom dosáhli snížení negativních vlivů interakcí odrůda × prostředí na technologickou jakost ozimé pšenice, je třeba vyvinout odrůdy s vysokým potenciálem pro výnos i technologickou jakost, jakož i pro odolnost k půdním a atmosférickým přísuškům v době nalévání zrna, které budou vhodné pro pěstování v semiaridních podmínkách.

Klíčová slova: pšenice (*Triticum aestivum* L.); odrůda; interakce genotypu × prostředí; stabilita; obsah bílkovin; mokrý lepek; pekařská výtěžnost; objemová vydatnost mouky

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Identification of common barley varieties by parallel electrophoresis of hordeins and esterases

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ABSTRACT

Usability of parallel electrophoretic analysis of hordein (SGE) and esterase (PAGE) proteins – genetic markers of barley was tested in a collection of registered varieties of spring barley and barley of winter habit with respect to their genetic structure evaluation and identification in a seed sample. The proportion of hordein polymorphic varieties of spring barley was demonstrated to be significantly higher than that of esterase polymorphic varieties. No such a difference was determined in winter varieties. Identity classes of hordein electrophoretic spectra of spring and winter barleys were defined to differentiate the varieties or protein lines of varieties-populations from each other. Identity classes of esterase electrophoretic spectra of spring and winter barleys were established analogically. Usability of protein genetic markers of barley, especially hordein proteins, to identify changes in the genetic structure of barley varieties of the type populations during their maintenance and reproduction was proved.

Keywords: barley; hordeins; esterases; electrophoresis; polymorphism; genotype identification; varietal stability

Protein genetic markers of barley, i.e. hordein proteins of barley grain and some enzymatic proteins of germinating seed, can be used not only for markering of some commercial traits, characteristics (Černý et al. 1999) but also for identification of the different registered varieties of spring and winter barley.

As polymorphism of the two above-mentioned genetic markers is rather low, possibilities of parallel identification of registered barley varieties by hordein and esterase electrophoresis should be verified.

MATERIAL AND METHODS

Electrophoretic analyses of hordeins and esterases were carried out in standard seed samples of registered spring and winter barley varieties from harvests 1992 to 1998 received from the Varietal Testing Department of Central Institute for Supervising and Testing in Agriculture (CISTA).

Fifty grains and 25 grains from bulk samples of breeders seed were analyzed to determine electrophoretic patterns of hordeins and esterases, respectively.

Methods of hordein electrophoretic analysis and definition of allelic hordein blocks were described in a paper by Šašek et al. (1990a, b) while the method of esterase electrophoretic analysis and identification of the esterase alleles was published by Sýkorová, Šašek (1996).

Statistical evaluation of the level of protein polymorphism of hordeins and esterases was based on *t*-test of qualitative traits.

RESULTS AND DISCUSSION

HORDEIN AND ESTERASE ELECTROPHORETIC SPECTRA OF BARLEY VARIETIES (CR 1999)

Polymorphism of hordein patterns

Table 1 shows a description of hordein electrophoretic spectra of spring and winter barley varieties expressed by sets of majority and minority allelic blocks of zones or by individual allelic zones.

The collection of spring barley varieties comprises 40 varieties: among them, 11 varieties consist of two hordein lines and one variety (Scarlett) of four hordein lines. The total proportion of hordein polymorphic varieties in the collection of spring barley varieties amounts to 30%.

The collection of winter barley varieties contains 15 varieties while one variety consists of two hordein polymorphic lines. The proportion of polymorphic varieties is 6.6% only.

Statistically insignificant proportions of lines were detected in spring barley varieties Atribut, Pejas and in winter barley variety Luxor (Černý, Šašek 1998). These lines are given in brackets in Table 1 and allegedly, they can be mechanical admixtures.

Polymorphism of esterase patterns

The alleles of esterase loci of spring and winter barley varieties are also shown in Table 1.

Table 1a. Hordein and esterase genetic markers of spring barley varieties registered in the CR in 1999

Variety name	Hordein markers						Esterase markers				
	hordein line		HRD allelic blocks of zones			minority Hrd gene	esterase lines	alleles of esterase loci			
	n	%	A	B	F			Est-1	Est-2	Est-4	Est-5
Akcent	A	100	12	21	1		e 1	Ca	Dr	Su	Pi
							e 2	Ca	Fr	Su	Pi
Amulet	A	100	12	47	1	E, C	e 1	Ca	Dr	Su	Pi
Atribut	A	98	21	25	1		e 1	Ca	Dr	Su	null
	(B)	2	12	47	1	E, C					
Ditta	A	100	32	21	1		e 1	Pr	Dr	Su	Pi
Famin	A	100	12	21	1		e 1	Ca	Dr	Su	Pi
Forum	A	100	N1	8	2		e 1	Pr	Fr	Su	Pi
Galan	A	100	2	19	1		e 1	Pr	Fr	Su	Pi
Heran	A	100	2	47	1	E	e 1	Ca	Fr	At	Pi
Heris	A	100	2	47	1	E	e 1	Pr	Fr	Su	Pi
Jarek	A	100	2	19	1		e 1	Ca	Fr	Su	Ri
Jubilant	A	6	N2	29	3		e 1	Ca	Fr	Su	Te
	B	94	23	29	3						
Kompakt	A	79	2	19	1		e 1	Pr	Fr	Su	Pi
	B	21	2	3	2						
Krona	A	100	23	29	3	E*	e 1	Ca	Fr	Su	Pi
Krystal	A	100	32	21	0		e 1	Ca	Un	Nz	Pi
Ladik	A	100	23	21	1	G*	e 1	Ca	Fr	Su	Pi
Lumar	A	100	5	17	3		e 1	Ca	Fr	At	Ri
Madeira	A	100	23	29	3		e 1	Ca	Fr	Su	Pi
Madonna	A	100	23	29	3	E*	e 1	Ca	Dr	Su	Pi
Malvaz	A	100	2	17	3		e 1	Ca	Fr	Su	Pi
Maridol	A	100	N3	N2	1		e 1	Ca	Fr	Su	Pi
Nordus	A	100	23	29	3	E*	e 1	Ca	Dr	Su	Pi
Novum	A	100	32	21	0		e 1	Ca	Un	Nz	Pi
Olbram	A	90	4	21	1	G	e 1	Ca	Dr	Su	Me
	B	10	5	17	2						
Orbit	A	100	21	25	1		e 1	Ca	Fr	At	Ri
Orthega	A	100	N4	29	3		e 1	Ca	Fr	At	Pi
Pax	A	100	2	19	1		e 1	Pr	Fr	Su	Pi
Pejas	A	92	2	47	1	E	e 1	Ca	Fr	At	Pi
	B	6	2	47	1						
	(C)	2	2	25	1						
Perun	A	78	2	47	1		e1	Ca	Fr	Su	Pi*
	B	22	2	47	1	E					
Primus	A	43	21	25	1		e 1	Ca	Fr	Su	Ri
	B	57	2	25	1		e 2	Ca	Fr	Su	Pi
Profit	A	100	2	25	1		e 1	Ca	Fr	Su	Pi
							e 2	Pr	Fr	Su	Pi
							e 3	Ca	Fr	At	Pi
Rubín	A	100	4	45	3		e 1	Ca	Fr	Su	Pi
Prosa	A	100	2	19	1		e 1	Ca	Fr	At	Pi
Scarlett	A	35	2	8	2	D	e 1	Pr	Fr	Su	Pi
	B	42	2	N1	1						
	C	17	N1	8	2						
	D	6	21	N1	1						
Signal	A	100	N3	N2	1		e 1	Ca	Fr	At	Pi
Sladko	A	58	12	21	1	G*	e 1	Ca	Dr	Su	Ri
	B	42	12	21	1	E*, G*					
Stabil	A	83	2	47	1		e 1	Ca	Fr	At	Pi
	B	17	N2	47	1		e 2	Ca	Dr	Su	Ri
Svit	A	94	2	47	1	E	e 1	Ca	Fr	Su	Pi
	B	6	2	47	1						
Terno	A	75	21	(17)	2		e 1	Ca	Fr	Su	Pi
	B	25	2	(17)	2						
Tolar	A	100	2	19	1		e 1	Ca	Fr	Su	Pi
Viktor	A	75	2	17	3		e 1	Ca	Fr	At	Pi
	B	25	2	17	3	E	e 2	Ca	Fr	Su	Pi

* very weak expression of the allele

Table 1b. Hordein and esterase genetic markers of winter barley varieties registered in the CR in 1999

Variety name	Hordein markers						Esterase markers			
	hordein line		HRD allelic blocks of zones			minority Hrd gene	esterase lines	alleles of esterase loci		
	n	%	A	B	F			Est-1	Est-2	Est-4	Est-5
Winter varieties – multirow											
Borwina	A	100	14	3	2		e 1	Ca	Fr	Su	null
Kamil	A	100	14	3	2		e 1	Ca	Fr	Su	Pi
Kromir	A	100	3	N1	1		e 1	Ca	Fr	At	null
Kromoz	B	100	3	N1	1	C*	e 1	Ca	Fr	At	null
Lunet	A	100	14	3	2		e 1	Ca	Fr	Su	Pi
Luran	A	100	3	N1	1		e 1	Pr	Fr	Su	null
Luxor	A	98	3	N1	1		e 1	Pr	Fr	Su	null
	(B)	2	3	3	2						
Okal	A	100	3	N1	1		e 1	Pr	Fr	Su	null
Sigra	A	89	3	N1	1		e 1	Pr	Fr	Su	Pi
	B	11	3	N1	1	C*					
Winter varieties – two-rowed											
Agrilo	A	100	3	N1	1		e 1	Pr	Fr	Su	Pi
Babylone	A	100	21	3	2		e 1	Pr	Fr	Su	Pi
Marinka	A	100	3	3	2		e 1	Ca	Fr	Su	Pi
Marna	A	100	3	N1	1		e 1	Ca	Dr	Su	Te
Monaco	A	100	3	3	2		e 1	Ca	Dr	Su	Pi
Tifanny	A	100	3	3	2		e 1	Pr	Fr	Su	Pi

* very weak expression of the allele

Five out of the total number of 40 spring barley varieties show polymorphism of the electrophoretic patterns of esterases, i.e. 12.5%. The lower esterase polymorphism in comparison with hordein polymorphism is apparently constituted by higher sensitivity to mutation changes of esterase genes, expressed by higher lethality of mutants.

The level of hordein and esterase polymorphism in sprig varieties was evaluated statistically by *t*-test of ra-

Table 2. Identity groups of hordein electrophoretic spectra of spring barley varieties

Class	HRD allelic blocks of zones	Varieties and/or HRD lines
1	2–3–2	Kompakt–B
2	2–8–2–D	Scarlett–A
3	2–17–2	Terno–B
4	2–17–3	Malvaz, Viktor–A
5	2–17–3–E	Viktor–B
6	2–25–1	Primus–B, Profit
7	2–47–1	Pejas–B, Perun–A, Stabil–A, Svit–B
8	2–19–1	Galan, Jarek, Kompakt–A, Pax, Prosa, Tolar
9	2–47–1–E	Heran, Heris, Pejas–A, Svit–A, Perun–B
10	2–N1–1	Scarlett–B
11	4–21–1–G	Olbram–A
12	4–45–3	Rubín
13	5–17–2	Olbram–B
14	5–17–3	Lumar
15	12–21–1	Akcent, Famin
16	12–21–1–G	Sladko–A
17	12–21–1–E, G	Sladko–B
18	12–47–1–C, E	Amulet
19	21–17–2	Terno–A
20	21–25–1	Atribut, Orbit, Primus–A
21	21–N1–1	Scarlett–D
22	23–21–1–G	Ladik
23	23–29–3	Jubilant–B, Madeira
24	23–29–3–E	Krona, Madonna, Nordus
25	32–21–0	Krystal, Novum
26	32–21–1	Ditta
27	N1–8–2	Forum, Scarlett–C
28	N2–47–1	Stabil–B
29	N2–29–3	Jubilant–A
30	N3–N2–1	Maridol, Signal
31	N4–29–3	Orthega

Capital letters after the variety name designate HRD lines (A, B, C, D)
N1, N2, N3, N4 – not catalogued allelic blocks of zones and/or alleles
C, D, E, G (column 2, i.e. allelic blocks) – minority hordein components

Table 3. Identity groups of esterase electrophoretic spectra of spring barley varieties

Class	Alleles of esterase loci				Varieties and/or esterase lines
	Est-1	Est-2	Est-3	Est-4	
1	Ca	Dr	Su	Pi	Akcent–e1, Amulet, Famin, Madonna, Nordus
2	Ca	Dr	Su	null	Atribut
3	Ca	Dr	Su	Me	Olbram
4	Ca	Dr	Su	Ri	Sladko, Stabil–e2
5	Ca	Fr	Su	Pi	Akcent–e2, Krona, Ladik, Madeira, Malvaz, Maridol, Perun, Primus–e2, Profit–e1, Rubin, Svit, Terno, Tolar, Viktor–e2
6	Ca	Fr	At	Pi	Heran, Orthega, Pejas, Profit–e3, Prosa, Stabil, Viktor–e1
7	Ca	Fr	At	Ri	Lumar
8	Ca	Fr	Su	Ri	Jarek, Primus–e1
9	Ca	Fr	Su	Te	Jubilant
10	Ca	Un	Nz	Pi	Krystal, Novum
11	Pr	Dr	Su	Pi	Ditta
12	Pr	Fr	Su	Pi	Forum, Galan, Kompakt, Pax, Profit–e2

Esterase lines are designated by letter e and numerals 1, 2 or 3

tios. The value $t = 1.69^*$ indicates a significantly higher proportion of hordein polymorphic varieties.

Evaluation of the level of hordein and esterase polymorphism in winter varieties by *t*-test of ratios is useless since esterase polymorphism is null.

Identity of electrophoretic hordein and esterase spectra

As hordein polymorphism is lower than the polymorphism of wheat gliadins (Šašek, Černý 1988), it is more difficult to determine the trueness and purity of barley varieties because many varieties have identical hordein spectra. Therefore it is advisable to use another genetic marker for verification of varieties with identical hordein patterns, for example esterases. To evaluate a possibility of differentiation between the hordein identical varieties of spring barley on the basis of esterase patterns, the varieties are included in identity groups of hordein (Table 2) and esterase (Table 3) patterns.

Differentiation between the hordein identical varieties Galan and Jarek with the identical patterns of hordein alleles A2-B19-F1 can be used as an example. Galan variety is described by electrophoretic patterns of esterases expressed by a set of alleles Pr-Fr-Su-Pi while Jarek variety has alleles Ca-Fr-Su-Ri.

Analogically, it is possible to differentiate between the winter barley varieties with identical electrophoretic patterns of hordeins (Tables 4 and 5). E.g. the variety of winter malting barley Tiffany has an identical hordein electrophoretic spectrum with the variety of fodder type Monaco. But they are different from each other in the alleles of loci Est-1 and Est-2 (Monaco – Ca-Dr-Su-Pi; Tiffany – Pr-Fr-Su-Pi).

Hordein genetic markers can be used to differentiate two-rowed malting barleys from winter two-rowed and multirow barleys of fodder type. None of the spring barley varieties possesses hordein allele HRD A-3 or HRD A-14, which are typical of winter varieties. Winter barley variety Babylone is characterized by the presence of allele HRD A-21, which has also been identified in spring

Table 4. Identity groups of hordein electrophoretic spectra of winter barley varieties

Class	HRD allelic blocks of zones	Varieties and/or HRD lines
1	3–3–2	Tifanny, Monaco, Marinka
2	3–N1–1	Kromir, Luran, Luxor, Okal, Sigra–A
3	3–N1–1–C	Agrilo, Marna, Kromoz, Sigra–B
4	14–3–2	Borwina, Kamil, Lunet
5	21–3–2	Babylone

Capital letters after the variety name designate HRD lines (A, B, C, D)
N1 – not catalogued allelic block
C (column 2, i.e. HRD allelic blocks) – minority hordein component

Table 5. Identity groups of esterase electrophoretic spectra of spring barley varieties

Class	Alleles of esterase loci				Varieties and/or esterase lines
	Est-1	Est-2	Est-3	Est-4	
1	Ca	Dr	Su	Pi	Monaco
2	Ca	Dr	Su	Te	Marna
3	Ca	Fr	At	null	Kromir, Kromoz
4	Ca	Fr	Su	Pi	Kamil, Lunet, Marinka
5	Ca	Fr	Su	null	Borwina
6	Pr	Fr	Su	Pi	Tifanny, Sigra, Agrilo, Babylone
7	Pr	Fr	Su	null	Luran, Luxor, Okal

Table 6. Evaluation of the stability of barley varieties – populations by hordein marker genes

Variety name	Standard samples of CISTA analyzed in 1992–1997						Standard samples of CISTA from 1998 crop, analyzed in 1999					
	Hrd line		Hrd allelic blocks				Hrd line		Hrd allelic blocks			
	n	%	A	B	F	minority loci	*n*	%	A	B	F	minority loci
						Spring varieties						
Heran	A	70	2	47	1	E	A	100	2	47	1	E
	B	23	2	47	1		–					
Jubilant	A	92	N2	29	3		A	6	N2	29	3	
	B	8	23	29	3		B	94	23	29	3	
Kompakt	A	58	2	19	1		A	79	2	19	1	
	B	42	2	3	2		B	21	2	3	2	
Olbram	A	65	4	21	1	G	A	90	4	21	1	G
	B	35	5	17	2		B	10	5	17	2	
Pejas	A	86	2	47	1	E	A	92	2	47	1	E
	B	14	2	47	1		B	6	2	47	1	
	–						(C)	2	2	25	1	
Primus	A	66	21	25	1		A	43	21	25	1	
	B	28	2	25	1		B	57	2	25	1	
	C	6	2	19	1		–					
Perun	A	75	2	47	1		A	78	2	47	1	
	B	25	2	47	1	E	B	22	2	47	1	E
Scarlett	A	70	2	8	2	D	A	35	2	8	2	D
	B	30	2	N1	1		B	42	2	N1	1	
	–						C	17	N1	8	2	
	–						D	6	21	N1	1	
Signal	A	84	N3	N2	1		A	100	N3	N2	1	
	B	16	N3	29	3		–					
Sladko	A	89	12	21	1	G*	A	58	12	21	1	G*
	B	11	12	21	1	E*, G*	B	42	12	21	1	E*, G*
Stabil	A	49	2	47	1		A	83	2	47	1	
	B	13	N2	47	1		B	17	N2	47	1	
	C	27	21	25	1		–					
	D	8	2	25	1		–					
Svit	A	40	2	47	1	E	A	94	2	47	1	E
	B	60	2	47	1		B	6	2	47	1	
Terno	A	57	21	(17)	2		A	75	21	(17)	2	
	B	35	2	(17)	2		B	25	2	(17)	2	
Viktor	A	63	2	17	3		A	75	2	17	3	
	B	37	2	17	3	E	B	25	2	17	3	E
						Winter varieties						
Kromir	A	83	3	N1	1		A	100	3	N1	1	
	B	8	3	3	2		–					
	C	8	2	3	2		–					
Kromoz	A	53	3	N1	1		–					
	B	37	3	N1	1	C*	B	100	3	N1	1	C*
Sigra	A	93	3	N1	1		A	89	3	N1	1	
	B	7	3	N1	1	C*	B	11	3	N1	1	C*

barley varieties Atribut, Orbit, Primus and Terno. But it is differentiated from the mentioned spring barleys by alleles of locus HRD B and/or HRD F as well as by electrophoretic patterns of esterases.

Stability of hordein polymorphic barley varieties

It is possible to check on the basis of hordein marker genes whether the stability of the genetic constitution of multiline varieties of populations is conserved in the course of maintenance of registered barley varieties.

Electrophoretic patterns of hordein polymorphic varieties of spring and winter barley are compared in Table 6. Electrophoretic spectra of hordeins were determined in 14 spring barley varieties and 3 winter barley varieties by evaluating standard seed samples of the above-mentioned varieties (Table 6) received from the Varietal Testing Department of CISTA in Brno. Standard seed samples from 1992–1997 were the first experimental variant, stan-

dard samples of the same varieties from 1998 crop, supplied in 1999, were the second variant. Genetic structure stability markered by hordein marker genes was proved only in 10 out of 17 polymorphic varieties over the period of evaluation (from 1992 and/or 1997 to 1998): Jubilant, Kompakt, Olbram, Pejas, Perun, Sladko, Svit, Terno, Viktor, Sigra, i.e. 59%. One or two hordein lines of the original genetic structure disappeared in six cases in the evaluated polymorphic varieties (Heran, Primus, Signal, Stabil, Kromir, Kromoz). Additional two lines appeared in one case in the original patterns of hordein lines (Scarlett).

Using electrophoretic analysis of hordeins it is possible to detect not only qualitative but also quantitative differences indicated by percentage ratios of the hordein lines during several cycles of maintenance of varieties – populations.

Variety Jubilant registered in 1991 can be given as an example. The variety was evaluated by hordein electrophoresis in 1990 as a new breeding SK 2777-11, and on the basis of relative frequency line A (77%) was determined as a major line and line B (23%) as a secondary one (Šašek et al. 1992). The ratio changed in 1993, when line B became a minor line (8%) (Šašek et al. 1995). But the inverse ratio of both lines (A 6%, B 94%) was demonstrated by hordein analysis of this variety from 1998 crop (Table 1).

The described changes in the structure of varieties – populations and/or in ratios of the different lines document un(intentional) interventions in the genetic structure of barley varieties – populations during several cycles of maintenance of the varieties concerned.

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ABSTRAKT

Identifikace odrůd ječmene pomocí souběžné elektroforézy hordeinů a esteráz

Bílkovinné genetické markery ječmene, tj. hordeinové bílkoviny ječného zrna a některé enzymové bílkoviny klíčících semen, lze využít nejen k markerování některých hospodářsky významných znaků – vlastností, ale rovněž k identifikaci jednotlivých registrovaných odrůd jarního a ozimého ječmene. K elektroforetickým analýzám hordeinů a esteráz byly použity standardní (etalonové) vzorky semen registrovaných odrůd jarního a ozimého ječmene ze sklizní 1992 až 1998, předané Odborem odrůdového zkušebnictví ÚKZÚZ. Metody elektroforetické analýzy hordeinů a vyčlenění alelických hordeinových bloků byly publikovány, rovněž tak použitý postup elektroforetické analýzy esteráz a identifikace jednotlivých esterázových alel. Sortiment odrůd jarního ječmene se skládá ze 40 odrůd. Z toho je 11 odrůd dvouliniových a jedna odrůda (Scarlett) se skládá ze čtyř linií; pět odrůd je esterázově polymorfních. Stupeň polymorfismu hordeinů a esteráz byl statisticky hodnocen pomocí *t*-testu podílů a získaná hodnota $t = 1{,}69^*$ prokazuje významně vyšší podíl hordeinově polymorfních odrůd. Sortiment odrůd ozimého ječmene obsahuje celkem 15 odrůd, z toho jedna odrůda se skládá ze dvou hordeinově odlišných linií; esterázový polymorfismus nebyl prokázán. K verifikaci odrůd s identickou skladbou hordeinů byl použit další genetický marker – esterázy. K posouzení možnosti rozlišit hordeinově identické odrůdy ječmene pomocí esteráz jsou hodnocené odrůdy zařazeny do skupin podle identity hordeinových a esterázových spekter (tab. 2 až 5). Pomocí hordeinových signálních genů je možné kontrolovat, zda udržování registrovaných odrůd ječmene zachovává stálost genetické skladby víceliniových odrůd populací. Byla stanovena elektroforetická spektra hordeinů 14 odrůd jarního ječmene a tří odrůd ozimého ječmene hodnocením etalonových vzorků osiv, dodaných Odborem odrůdového zkušebnictví ÚKZÚZ v Brně. První variantu představují etalonové vzorky z let 1992 až 1997, druhou pokusnou variantu pak etalonové vzorky týchž odrůd ze sklizně 1998, dodané v roce 1999. Ze 17 polymorfních odrůd vykázalo za posuzované období (od roku 1992, resp. 1997 do roku 1998) stálost genetické struktury, markerované hordeinovými signálními geny, pouze 10 odrůd (Jubilant, Kompakt, Olbram, Pejas, Perun, Sladko, Svit, Terno, Viktor, Sigra). V šesti případech došlo ke ztrátě jedné či dvou hordeinových linií původní genetické struktury hodnocených polymorfních odrůd (Heran, Primus, Signal, Stabil, Kromir, Kromoz). V jednom

případě byla původní skladba hordeinových linií o dvě linie rozšířena (Scarlett). Elektroforetická analýza hordeinů umožňuje stanovit nejen kvalitativní, ale i kvantitativní rozdíly, které spočívají v procentuálním zastoupení jednotlivých hordeinových linií v průběhu několika cyklů udržování odrůd – populací. Jako příklad lze uvést odrůdu Jubilant registrovanou v roce 1991. Tato odrůda byla hodnocena pomocí elekroforézy hordeinů již v roce 1990 jako novošlechtění SK 2777-11. Bylo zjištěno, že tato odrůda je populace složená ze dvou linií A (77 %) a B (23 %). V roce 1993 bylo elektroforézou hordeinů prokázáno vysoké zastoupení linie A (92 %). Avšak analýzou hordeinů této odrůdy ze sklizně 1998 bylo naopak určeno vysoké zastoupení linie B (94 %). Pozorované změny ve skladbě odrůd – populací, resp. v podílu jednotlivých linií svědčí o záměrných, či neúmyslných zásazích do genetické struktury odrůd – populací ječmene během několika cyklů udržování zmíněných odrůd.

Klíčová slova: ječmen; hordeiny; esterázy; elektroforéza; polymorfismus; identifikace genotypů; stálost odrůd

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Vegetative and agronomic characteristics of wild populations of orchard-grass (*Dactylis glomerata* L.) from Mediterranean and Atlantic regions grown in Central European conditions

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ABSTRAKT

Field trials with 43 collection items and the assortment of Czech cultivars of orchard-grass (7) and one cultivar of forest cocksfoot (*Dactylis polygama* Horvat.) were established at two trial sites and conducted in the period 1997 till 1999 harvested three times a year. All kinds of orchard-grass well tolerate continental winter in the Czech Republic, however no ecotype shows active growth during warmer winters without snow. Significant differences among tested items were also found out for alternativity, earliness in heading, plant height in heading, average number of panicles per plant, length of leaf blade of sterile offshoots, length of blade of stem offshoots, number of functional leaves on stem, rust resistance, grass mildew resistance, dry matter yield in the 3rd harvest, speed of regrowth after the 1st harvest. Forage productivity of diploids (*Dactylis glomerata* ssp. *galiciana*, *D. g.* ssp. *lusitanica*, *D. polygama*) was in average by 16% lower compared to tetraploid ecotypes and cultivars of *Dactylis glomerata*. It shows a possibility of practical use of orchard-grass ecotypes from maritime region for breeding of new cultivars of orchard-grass.

Keywords: grasses; orchard-grass; cultivars; ecotypes; breeding; leaf diseases; photoperiod; genetic resources

Family of orchard-grass has very variable morphological and physiological traits (Domin 1943). Extensive interspecific variability is in clear contrast to relatively narrow genetic basis of current assortment of cultivars of orchard-grass (*Dactylis glomerata* L.). One of the possibilities to extend the genetic basis for breeding is the use of adapted population from foreign resources that were thoroughly observed in field trials and evaluated genetically and biologically. Simmonds (1993) uses the expression incorporation for this procedure, Duvick (1990) uses expressions genetic enhancement or prebreeding.

Due to recent collections a comprehensive gene pool collection of orchard-grass was assembled in various European institutions. Some items exhibit even more production of matter throughout the year (Mousset 1989; Mousset et al. 1992), or prolonged vegetation period till the beginning of winter, higher level of fungal diseases resistance, etc. Considering that current orchard-grass cultivars in climatic conditions of Central Europe suffer from obvious growth stagnancy in the second half of vegetation and from high occurrence of leaf diseases, the use of these ecotypes in breeding programmes might be useful.

The aim of the project is the evaluation of behaviour of orchard-grass natural populations from maritime environment in the conditions of Central Europe with a special view to the distribution of forage production throughout the year and leaf diseases resistance.

MATERIAL AND METHODS

Field trials with 43 collection items (from the gene bank INRA Lusignan, France) and the assortment of the Czech cultivars of orchard-grass (7) and one cultivar of forest cocksfoot (*Dactylis polygama* Horvat.) were established according to unified methodology in the spring 1997 at two experimental sites (Table 1).

Only the materials, which Mousset (1989) suggested as suitable for verification in the conditions of Central Europe, were selected from the gene bank and used into the trials:

No. 1–11 *Dactylis glomerata* ssp. *glomerata* ($2n = 28$), collections from Brittany, France;
No. 12–20 *Dactylis glomerata* ssp. *glomerata* ($2n = 28$), collections from Normandy, France;
No. 21–34 *Dactylis glomerata* ssp. *glomerata* ($2n = 28$), only items 30 and 31 *Dactylis glomerata* ssp. *galiciana* ($2n = 14$), generally collections from northwestern Spain;
No. 35–43 *Dactylis glomerata* ssp. *glomerata* ($2n = 28$), only No. 37, 39, 40 *Dactylis glomerata* ssp. *galiciana* ($2n = 14$), and 41, 42, 43 *Dactylis glomerata* ssp. *lusitanica* ($2n = 14$), generally collections from northern Portugal; these were natural populations of orchard-grass collected by C. Mousset from natural grasslands in 1968 till 1982;
No. 44–50 *Dactylis glomerata* ssp. *glomerata* ($2n = 28$), cultivars Velana, Dana, Zora, Vega, Milona, Lada, Niva;
No. 51 *Dactylis polygama* Horvat. ($2n = 14$), cultivar Tosca.

Table 1. Characteristics of experimental sites

Feature	Unit	Červený Dvůr	Jevíčko
Altitude	m	430	335
Long-term average			
annual temperature	°C	7.1	7.5
temperature in vegetation period (IV.–X.)	°C	13.5	13.0
annual rainfall	mm	591	629
rainfall in vegetation period (IV.–X.)	mm	382	397
Top-soil depth	m	0.19	0.34
Soil type		sandy loam	loam
Genetic soil type		cambisol, var. acidic on gneiss	stagnogleic fluvisol
pH/KCl		5.9	6.7
Available P_2O_5 content	$mg.kg^{-1}$	64	85
Available K_2O content	$mg.kg^{-1}$	108	118
Available MgO content	$mg.kg^{-1}$	57	140

From each ecotype or cultivar a sufficient number of plants was precultivated in the planting plates in the greenhouse and in May 1997 these were planted in the field (Červený Dvůr), in the mead (Jevíčko), resp., at both experimental sites in two planting alternatives, in two replications:

a) in the spacing 0.45 × 0.45 m (30 plants) in one row; next symbolised by I (individuals);
b) in three rows with spacing 15 cm, plants in the row distant 8 cm from each other (36 plants), simulating conditions of a normal cover; next symbolised by H (dense cover).

Soil preparation and fertilizing were in accordance with the methodology to conduct the State Varietal Trials (Schmidt et al. 1972) of the Central Institute for Testing and Supervising in Agriculture.

Evaluated features

AL: alternativity, i.e. grass heading in the year of sowing, the average number of stems per plant is evaluated in the period of three months after sowing;
PZ: over-wintering, the number of vital plants after winter related to the number before winter, on the nine-point breeding scale, where 9 = the highest expression of a positive feature, i.e. 9 = the best over-wintering and 1 = the worst over-wintering;
ZR: active growth in winter during warm periods (without snow cover), evaluated on the 9–1 scale;
ZL: proportion of dry leaves at the end of winter, evaluated on the 9–1 scale; (9 = the fewest dry leaves);
RM: earliness in heading expressed by number of days after 1st April until at least two panicles appear on the plant;
VM: height of plant in the period of heading;
LR: average number of panicles per a plant;
DC: blade length of sterile offshoots, average of 10 leaves measurements;
SC: blade width of sterile offshoots in mm, average of 10 leaves measurements;
DP: leaf sheath length of stem leaves, average of 10 measurements;
SP: leaf sheath width of stem leaves in mm, average of measurements;
PL: number of functional leaves on a stem, average of 10 stems (specification of functional leaves by Míka et al. 1997);
BL: leaf colour, on the 9–1 scale;
DL: leaf harshness, on the 9–1 scale (9 = smooth leaf);
OR: rust complex resistance (*Puccinia graminis* Pers., *P. coronata* Cda; *Uromyces dactylidis* Otth.), on the 9–1 scale;
OF: fusarium resistance (*Fusarium* sp.);
OP: grass mildew resistance (*Erysiphe graminis* DC.), on the 9–1 scale;
OV: virus diseases resistance, on the 9–1 scale;
VT: sod protrusion, on the 9–1 scale (1 = very protruding sod);
Q1: dry matter yield in 1st harvest [$g.m^{-2}$];
Q2: dry matter yield in 2nd harvest [$g.m^{-2}$];
Q3: dry matter yield in 3rd harvest [$g.m^{-2}$];
PL: leaf proportion in harvested matter in 1st harvest (g of dry matter out of 100 g of dry matter), determined with NIRS (Míka et al. 1998);
O1: regrowth speed after 1st harvest, evaluated 14 days after harvest on the 9–1 scale;
O2: regrowth speed after 2nd harvest, evaluated 14 days after harvest on the 9–1 scale.

Evaluation period is given in Table 2. The measurement results were processed by a method of the principal components analysis (PCA) and correlation of observed features in the relation to PCA axes (relationship of populations, or cultivars, resp.). Variance analysis was also used for the evaluation of production features.

In addition 1000-seed weight was determined (TSW), but about a third of ecotypes seed samples had less than thousand seeds available for determination. With regard

Table 2. Evaluation period

Year	H (dense cover) month	H (dense cover) evaluated feature	Year	I (individuals in field garden) month	I (individuals in field garden) evaluated feature
0. (1997)	IX.	Q1	0. (1997)	VII.	AL
	X.	ZR		IX.	DC, SC, DP, SP
				X.	OR, OP, OV
1. (1998)	I.–II.	ZR	1. (1998)	IV.	BL
	III.	PZ, ZL, OF, DC, SC, DP, SP		V.	RM, VM, LR, PL
	IV.	OR, OP, OV			
	V.	DL, Q1, PL			
	VI.	O1, OF			
	VII.	Q2			
	VIII.	O2, OR, OP, OV			
	IX.	DL, Q3, VT			
	XII.	ZR			
2. (1999)	II.	ZR	2. (1999)	IV.	BL
	III.	PZ, ZL, OF		V.	RM, VM
	IV.	OR, OP, OV		IX.	OR, OP, OV
	V.	DL, PL, Q1			
	VI.	O1, OF, VT			
	VII.	Q2, OR, OP, OV			
	VIII.	O2			
	IX.–X.	Q3			
3. (2000)	I.–II.	ZR	3. (2000)		
	III.	PZ, OF, ZL			

to possible inaccuracy, this feature was not evaluated by the PCA method and it only served to compare TSW groups of di- and tetraploid orchard-grasses.

RESULTS

Plant covers at both sites established well and they were equal before the first evaluation. In the period of three years, there was no interference so significant that it could cause any doubt on the authenticity of the experiment.

There was summer drought in the second and third years, otherwise the weather resembled the long-term average.

Principal components analysis (PCA) of mentioned morphological and agronomic traits for 51 experimental items (ecotypes and varieties) at two experimental sites covered 46% of variability in coordinates 1 and 2 and 9% in coordinates 1 and 3 (Figure 1).

Axis 1: negative correlation with blade length ($r = -0.906^{**}$) and leaf width ($r = -0.850^{**}$), positive correlation with leaf colour ($r = +0.801^{**}$) and sod protrusion ($r = +0.779^{**}$).

Axis 2: positive correlation with earliness in heading ($r = +0.764^{**}$).

Axis 3: positive correlation with rust resistance ($r = +0.631^{**}$).

However, leaf blade length and width are mutually correlated features ($r = +0.812^{**}$), leaf colour and sod protrusion as well ($r = +0.445^{*}$). Rust resistance did not correlate with any of evaluated features (linear correlation).

PCA demonstrated that discreet groups of di- and tetraploid varieties are not created in evaluated features along axis 1. Therefore, there is not presented any graph demonstration. Ecotypes and orchard-grass varieties from five geographically distant regions differed in earliness in heading, dry matter yield in the 3rd harvest, regrowth speed after the 1st harvest and rust resistance.

All ecotypes ($n = 43$) and cultivars ($n = 8$) at both sites over-wintered well and after the 3rd winter they are still well established. During three winters if there were any warmer periods without snow (with temperatures > 5°C), no orchard-grass started vegetation, just some things slowed down as leaf tips drying, blades withering away and their consequent decomposition. If the DM-weight of 10 leaf blades before freezing was set to 100%, then at the end of winter the weight was 63% for cultivar Milona, 71% for tetraploid ecotype No. 35 from Portugal, 65% for diploid ecotype *Dactylis glomerata* ssp. *galiciana* No. 39 from Portugal and 60% for *Dactylis polygama* cv. Tosca. But these are only results from the 3rd winter and that does not allow making serious conclusions.

There were not found any significant differences among evaluated experimental items in terms of over-win-

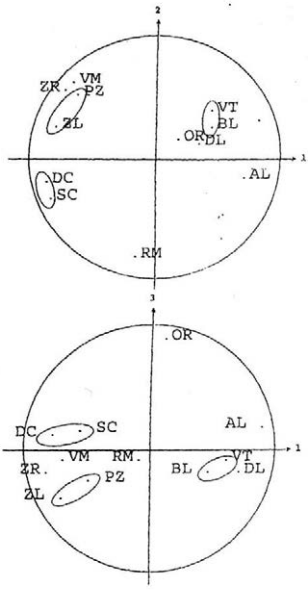

Figure 1. Correlation among variables with PCA axes marking AL = alternativity; PZ = over-wintering; ZR = active growth in winter during warm periods (without snow cover), ZL = proportion of dry leaves at the end of winter; RM = earliness in heading; VM = height of plant in heading; DC = blade length of sterile offshoots; SC = blade width of sterile offshoots; BL = leaf colour; DL = leaf harshness; OR = rust resistance; VT = sod protrusion

tering ($F = 2.06$, $F_{\alpha 0.95} = 3.64$, $n = 51$). Relatively best overwintering was demonstrated by Czech cultivars *Dactylis glomerata*, where No. 48 (Milona = control) was evaluated 7.3 ± 1.9, similarly to diploid *Dactylis polygama* cv. Tosca (7.3 ± 0.8) – Figure 2. Out of evaluated ecotypes the best was No. 8 (7.4 ± 1.0). Also there were found no significant differences among groups of *Dactylis glomerata* according to geographical origin (Table 3). Significant differences were found in leaf dying out after winter ($F = 8.42^{**}$). Ecotypes *Dactylis glomerata* from Spain and Portugal had fewer dried leaves than ecotypes from France or Czech Republic (Table 3). The average of point evaluation of tetraploid orchard-grass was 6.5 ± 1.8, of diploid orchard-grass 6.7 ± 1.1. The differences between trial sites were insignificant ($F = 2.05$, $F_{\alpha 0.95} = 2.77$, $n = 2$).

No ecotype had summer dormancy, neither at Červený Dvůr nor at Jevíčko.

Significant differences among experimental items were found also for alternativity ($F = 7.04^{**}$), earliness in heading ($F = 23.12^{**}$), plant height in heading ($F = 16.09^{**}$), average number of panicles per plant ($F = 6.79^{**}$), blade length of sterile offshoots ($F = 3.86^{*}$), blade length of stem offshoots ($F = 2.91^{**}$), number of functional leaves on stem ($F = 3.74^{**}$) – Figure 3, rust resistance ($F = 4.72^{**}$), grass mildew resistance ($F = 6.87^{**}$), dry matter yield in the 3rd harvest ($F = 3.75^{*}$), regrowth speed after the 1st harvest ($F = 12.42^{**}$).

Lesser extent of leaf dying out positively correlated with earliness ($r = +0.303^{*}$, $n = 51$), plant height in the period of heading ($r = +0.280^{*}$), regrowth speed after

Table 3. Averages of selected morphological features and agronomic qualities of *Dactylis glomerata* groups ($2n = 28$) according to origin

Evaluated features and qualities		Natural spontaneous populations of orchard-grass from region				Czech cultivars ($n = 7$)	*s.d.*
		Normandy ($n = 9$)	Brittany ($n = 11$)	Northwestern Spain ($n = 12$)	Northern Portugal ($n = 3$)		
ZR	active growth in winter	2.0	1.9	2.0	2.2	1.8	N.S.
PZ	wintering	7.9	7.6	7.9	7.0	8.3	N.S.
ZL	dry leaves proportion at the end of winter	6.2	6.6	7.4	7.5	6.5	*
RM	earliness in heading	33.6	31.5	29.2	28.0	35.7	*
VM	plant height in heading	0.62	0.60	0.59	0.56	0.64	N.S.
Q1	dry matter yield in 1st harvest	28.7	26.5	21.9	24.3	31.7	N.S.
Q2	dry matter yield in 2nd harvest	14.6	15.7	12.3	10.5	17.1	N.S.
Q3	dry matter yield in 3rd harvest	9.6	12.8	14.3	13.5	9.0	*
O1	regrowth speed after 1st harvest	6.0	6.9	8.0	8.3	7.2	*
OR	rust resistance	6.8	7.6	6.4	7.0	6.8	*
OF	fusarium resistance	6.2	7.1	6.0	4.8	7.5	**
OP	grass mildew resistance	7.0	7.4	6.9	6.9	7.1	N.S.
OV	virus diseases resistance	7.6	7.2	7.5	7.3	7.5	N.S.

N.S. insignificant differences
* differences significant on $P_{0.95}$ level
** differences significant on $P_{0.99}$ level

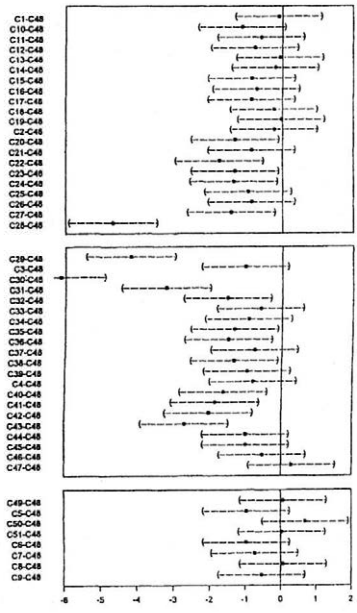

Figure 2. Point evaluation of orchard-grass wintering, evaluated by Dunnett's method with marked out 95% confidence interval, compared to control variety No. 48 (cv. Milona)

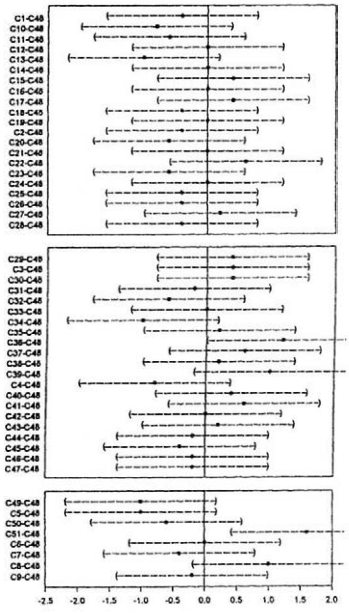

Figure 3. The number of functional leaves on orchard-grass stem, evaluated by Dunnett's method with marked out 95% confidence interval, compared to control variety No. 48 (cv. Milona)

1st harvest (r = +0.296*) and negatively correlated with average number of panicles per plant (r = –0.316*).

In the 1st harvest *Dactylis glomerata* provided in average of experimental items, harvest years and trial sites 50.5% out of annual dry matter production, 26.6% in the 2nd harvest and 22.9% in the 3rd harvest (Table 4). Significant differences among groups of orchard-grass according to geographical origin showed only in the 3rd harvest. Czech cultivars have in the 3rd harvest lower proportion of yield out of annual production compared to ecotypes from Spain ($P_{0.95}$) and Portugal (closely below the level of $P_{0.95}$). In fact groups at both trial sites were in the same order: Spearman coefficient value of rank correlation is r_s = 0.900*, however at Jevíčko the proportion of dry matter yield in the 3rd harvest out of annual production was lower than at Červený Dvůr (t = 3.22*).

Table 4. Dry matter yield proportion of ecotypes and cultivars of *Dactylis glomerata* in particular harvests out of annual total (%)

Groups according to origin	Harvest		
	1.	2.	3.
Normandy	54.2	27.6	18.2
Brittany	48.2	28.6	23.2
Northwestern Spain	45.1	25.4	29.5
Northern Portugal	50.4	21.7	27.9
Czech Republic	54.9	29.6	15.5

The lowest significant difference among groups on $P_{0.95}$ level = 13.0 and on $P_{0.99}$ level = 18.8

DISCUSSION

Orchard-grass is a very abundant species in natural plant stands of Atlantic and Mediterranean regions and a broad gene pool was made here with many significant morphological features and agronomic qualities (Mousset 1989), diversified by altitude and latitude. The evaluation of ecotype collection assembled on purpose (with supposed suitability for Central European conditions) confirmed remarkable variability at two sites in the CR with possible application in breeding programmes. If the ecotypes, that are adapted to lower latitudes (short days), are grown in our conditions (longer days), these are usually quite early (by 3–8 days earlier than Czech cultivars), they have longer and wider leaves on stems and sterile offshoots, they are of more erected growth, the sod shape is rather compact, they have good regrowth ability after harvest, and according to their condition during summer drought they are more drought resistant. Similar conclusions are made by Mousset (1989), Buxton, Falles (1994) and Míka et al. (1997).

In contrast to common opinion (Buxton and Falles 1994), southern ecotypes demonstrated good to very good persistence. This was probably helped by a careful choice of ecotypes made by C. Mousset based on precedent comprehensive evaluation with INRA Lusignan (Mousset 1989). At the same time we also have to consider relatively short period of the experiment: breeding practice shows that one- or two-year-old individuals of orchard grass often have excellent winter hardiness. However, in the next years their susceptibility to longer periods of dryfrost grows, esp. to late spring frosts, likely

as the expression of plant individuals ageing (Míka 1979) and decrease of their vitality. The long-term evaluation is for the effective plant breeding therefore necessary.

Higher ecotypes productivity towards lower latitudes is not definite though and it varies for factual collection items. Considering polyfactor nature of quantitative traits, as yield is, the influence of other factors can be determined, as was proved by Moussset et al. (1992) in the study of wild spontaneous populations of *Dactylis glomerata* ssp. *hispanica* form Corsica: Ecotypes coming from locations with rising altitude exhibited lower dry matter yield in spring ($P_{0.99}$) and in autumn ($P_{0.99}$) when they were grown in standard conditions. Also other grass species have a tendency of lower production ability with rising altitude (Míka et al. 1997).

Forage productivity of diploid orchard-grass (*Dactylis glomerata* ssp. *galiciana, D. g.* ssp. *lusitanica, D. polygama*) was in average by 16% lower compared to tetraploid ecotypes and cultivars of *Dactylis glomerata*. In the same sense Hertzsch (1959) indicates the increase of yield ability of euploids with increasing chromosome number (from 14 to 35). Nevertheless, diploid varieties exhibit in particular features great variability, e.g. *Dactylis polygama* cv. Tosca had the longest blades out of all 51 experimental items (292 ± 26 mm), but one of the most narrow (5.8 ± 0.5 mm), despite the usual close positive relation between grass leaf blade length and width. While the average number of functional leaves on a stem for the whole cocksfoot collection is 3.9 ± 0.8 leaves, cv. Tosca had the highest number 4.6 ± 1.0. Other heterogeneity of diploid orchard-grass group was exhibited by earliness in heading, sod protrusion, leaf colour and harshness. Detailed analysis will become the subject of the next report (Míka et al. 2001). All diploid orchard-grass exhibited significantly lower TSW (0.651 ± 0.0 33 g) compared to tetraploid *Dactylis glomerata* (1.290 ± 0.100 g, $t = 18.59^{**}$).

A very significant finding is that some ecotypes of *Dactylis glomerata* from Brittany exhibit significantly higher grass mildew resistance that is the most important orchard-grass disease in the ČR. It was also found that evaluation in different periods has about the same expression value, and therefore this feature can be evaluated whenever the disease occurs during vegetation. Ecotypes and cultivars, susceptible in youth growth stage, were even susceptible when evaluated later.

Beside grass mildew resistance some orchard-grasses mostly from Brittany exhibited higher rust resistance, first (according to economic importance) grass rust, crown rust and finally orchard-grass rust. The evaluation period for rust resistance is not important as it is for grass mildew resistance. On the contrary, crown rust resistance must be evaluated at the end of vegetation period and orchard-grass rust resistance (it occurred rarely) at the beginning of vegetation period.

Scoletotrichum graminis Fckl. (syn. *Cercosporidium graminis*) occurrence was not recorded during the 3 years of the orchard-grass experiment in the CR, whereas in France it is as common as rust (Mousset and Gallais 1974). It was found in the same period at adjacent trials with grass varieties of ryegrass at Červený Dvůr in wet spring 1999. Ecotypes from eastern France, northern Europe and America were generally very susceptible to leaf diseases (Mousset 1989).

Several orchard-grass ecotypes exhibited higher fusarium resistance ($P_{0.99}$). This feature showed probably the greatest variability among experimental items (Table 3). Fusarium occurrence on orchard-grass could be seen already during wet winter (without snow cover), but it was evaluated in a standard way at the beginning of vegetation. It shows up by reddish or rosy colour of withering sods in spring. Just only few of the diseased sods are able to overgrow. Mortality is about 80%, the mycelium coating remains rather long on the remaining 20% sods and these sods overgrow very slowly.

The possibility of real use of orchard-grass gene pool from maritime region in breeding is demonstrated by Welsh Plant Breeding Station in Aberystwyth (Great Britain). Some collections from northwestern Spain exhibited suitable characteristics for grazing and were used as the basis for new varieties (Cooper 1973). Some ecotypes from Normandy and Brittany show the possible grazing period extension in early spring and late autumn. Moreover, Brittany collections are more resistant against leaf diseases. Their use resulted (after two selection cycles) in registration of cultivars Luly (1977), Lude and Lutetia (1978), that exhibit lower disease attack by 15–20% compared to the control cultivar Prairial (at the time of registration in France). They are also by 7–10 days in spring (in heading) earlier and for 7–10 days in autumn they grow longer (Mousset 1989).

The study implies that collection and comprehensive description of particular items (e.g. with the use of electrophoretic and other methods) is very needful for conservation of genetic resources of variability that create gene pool. For practical application it is also necessary to process maximum amount of information about them (e.g. with multivariance analysis), to learn the relations of particular requested features among groups and at the very beginning to observe the qualities of populations in nature and to estimate their suitability for one's breeding aim (Mousset 1989; Chloupek 2000).

Acknowledgement

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ABSTRAKT

Růstové a agronomické charakteristiky planých populací srhy říznačky (*Dactylis glomerata* L.) z mediterránní a atlantické oblasti, pěstovaných ve středoevropských podmínkách

Polní pokusy se 43 sběrovými položkami a sortimentem českých odrůd srhy říznačky (7) a jednou odrůdou srhy hajní (*Dactylis polygama* Horvat.) byly založeny na dvou pokusných místech a vedeny v období 1997 a 1999 při třech sečích za rok. Všechny odrůdy dobře snášejí v ČR kontinentální průběh zimy, ale žádný ekotyp během teplejších období za bezsněžné zimy aktivně nepřirůstá. Významné rozdíly mezi pokusnými členy byly zjištěny též pro alternativnost, ranost v metání, výšku rostliny v metání, průměrný počet lat na rostlinu, délku čepele sterilních výhonů, délku čepele stébelných výhonů, počet funkčních listů na stéble, odolnost vůči rzem, odolnost vůči padlí travnímu, výnos sušiny ve třetí seči, rychlost obrůstání po první seči. Pícninářská produktivnost diploidních odrůd (*Dactylis glomerata* ssp. *galiciana*, *D. g.* ssp. *lusitanica*, *D. polygama*) byla v průměru o 16 % nižší ve srovnání s tetraploidními ekotypy a odrůdami *Dactylis glomerata*. Je poukázáno na možnost praktického využití ekotypů srhy z maritimní oblasti při tvorbě nových odrůd srhy.

Klíčová slova: trávy; srha; odrůdy; ekotypy; šlechtění; listové choroby; fotoperioda; genetické zdroje

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Potato glycoalkaloids and their significance in plant protection and human nutrition – review

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ABSTRACT

Among secondary metabolites potatoes contain especially glycoalkaloids that are important in their defence mechanism against different pathogens, such as viruses, bacteria, fungi and insects. On the other hand, these glycoalkaloids are significant toxins in human as well as animal nutrition. In the potatoes the most present glycoalkaloids are α-solanine and α-chaconine forming as high as 95% of total glycoalkaloids. The other glycoalkaloids found are β- and γ-solanines and chaconines, α- and β-solamarines and aglycones demissidine and 5-β-solanidan-3-α-ol, and in wild potatoes leptines, commersonine, demissine and tomatine. Potato glycoalkaloids are cholinesterase inhibitors and cause poisoning leading to accumulation of acetylcholine in nerve tissue. New varieties have to contain less than 200 mg glycoalkaloids.kg^{-1} fresh wt. and appreciated are varieties containing 20 to 130 mg glycoalkaloids.kg^{-1} fresh wt. The major factors affecting glycoalkaloid content in potatoes are variety, greening, maturity and tuber damage. Because of the significance of these compounds in protection of plants against pathogens, crossing between wild potatoes and standard varieties leads to new hybrids containing less amounts of glycoalkaloids but with their broader structural composition allowing synergistic effect against pathogens.

Keywords: potato; glycoalkaloids; solanines; chaconines; minor glycoalkaloids; glycoalkaloid contents; plant protection; toxic effect; hybridization

Numerous plant species in the *Solanaceae* contain various glycoalkaloids which are considered to be natural toxins (Schreiber 1968a). Among widely cultivated food crops, eggplant or aubergine as it is more commonly known worldwide (*Solanum melongena*), potato (*S. tuberosum*) and tomato (*Lycopersicon esculentum*) are their major sources (Schreiber 1968b, 1970). Despite its status as a food of global importance, the potato tuber contains toxic glycoalkaloids that cause sporadic outbreaks of poisoning in humans (Smith et al. 1996). Each year the world produces approximately 350 million tons of potatoes. The U.S. per capita consumption of potatoes is about 61 kg per year. Potatoes serve as a major food source as well as an inexpensive source of energy and good quality protein. Potatoes are grown mainly for human consumption but they are also widely used as food for livestock.

The glycoalkaloids identified to date (Duan and Feng 1992) are composed of a C-27-steroidal alkaloid along with various sugar moieties usually composed of a di-, tri- or tetrasaccharide. Also certain food plant by-products, such as potato peels, have an occasion found their way into animal foods, which in turn can cause health problems and even possible death to livestock. In the case of the potato, which is a major plant food crop, these concerns have resulted in hundreds of scientific investigations, attempting to identify specific glycoalkaloids with the aim to minimize their content.

The potato plant has an ability to produce its own protective chemicals which can make it lethal to insects, animals and fungi which attack them. These protective glycoalkaloids are at high levels in the leaves, stems and sprouts of the potato plant and are normally at very low levels in potato tubers (Olsson 1996a; Gelder et al. 1988; Gelder 1991). However, on exposure to light the potato tuber will produce elevated levels of these protective glycoalkaloids, with the highest levels being in the sprouts as they emerge from the tuber. Potatoes will also produce high levels of glycoalkaloids in response to bruising, cutting and other forms of physical damage, as well as to rotting caused by fungi or bacteria. In these instances high levels of glycoalkaloids are present in the potato.

Steroidal glycoalkaloids (SGAs) of potatoes may be detrimental to the health of humans and animals, best they are beneficial to the plants. The SGAs are thought to be a component of the certain varieties resistance to insects. They provide protection from the potato beetle, potato leafhopper, and wireworms. At a low pH level some SGAs have shown to have antifungal effects. In addition, low levels of glycoalkaloids are required in order to produce a desired flavour. More bitter varieties contain SGAs and are more toxic as well.

Potato glycoalkaloids

Potato alkaloids are found in plants in the form of glycosides of alkaloids. In these glycosides a noncarbohydrate moiety, the aglycone, is joined by an ester bond to a carbohydrate moiety. In solanum-type glycoalkaloids, the aglycone is a steroid alkaloid. Solanine and chaco-

nine cause poisoning in potatoes (Potus and Adrian 1995). They have the same aglycone, solanidine, but the structure of their carbohydrate side chains is different (Maga 1994).

A compounding factor usually associated with greening is the formation and accumulation of naturally occurring glycosidic steroidal alkaloids, such as solanine, which are commonly called glycoalkaloids. All normal potatoes are known to contain low levels of various glycoalkaloids, but numerous genetic environmental and stress factors physiologically induce accelerated glycoalkaloid production.

Glycoalkaloids are composed of three structural entities: namely a polar, water-soluble oligosaccharide portion composed of a monosaccharide unit of varying number and composition that is attached at C-3; a nonpolar lipophilic steroid and a nitrogen-containing heterocyclic. Potato alkaloids are composed of two steroidal bases: namely solanidine and spirosolane. Solanidine bases contain an indolizidine system as typified by the structures of solanines, chaconines, solanidine, leptines, leptinines, and demissidine. On the other hand, spirosolane bases possess an oxa-azaspirodecane structure as typified in tomatidenol and α- and β-solamarines (Schreiber 1957). In the case of the common cultivated potato, the major glycoalkaloids representing up to 95% are α-solanine and α-chaconine (Duke 1992a), both of which have the same alkaloidal aglycone base – solanidine. The structural relationships of these compounds are shown in Figure 1. In addition, β- and γ-forms of both solanine and chaconine are also present. The structural relationships of these compounds to α-solanine and α-chaconine are shown in Table 1. They differ only in their sugar moieties. The β- and γ-forms can be present during biosynthesis or hydrolysis during plant growth and metabolism. It was concluded that aglycone synthesis can proceed at a rapid rate in potatoes, whereas its conversion into a glycoalkaloid is much slower and dependent on the availability of sugar components (Zitnak 1961).The trisaccharide associated with α-solanine is known as solatriose, or O-α-L-rhamnopyranosyl-(1→2 gal)-O-β-D-glucopyranosyl-(1→3 gal)-β-D-galactopyranose, while the α-chaconine glycoside is known as

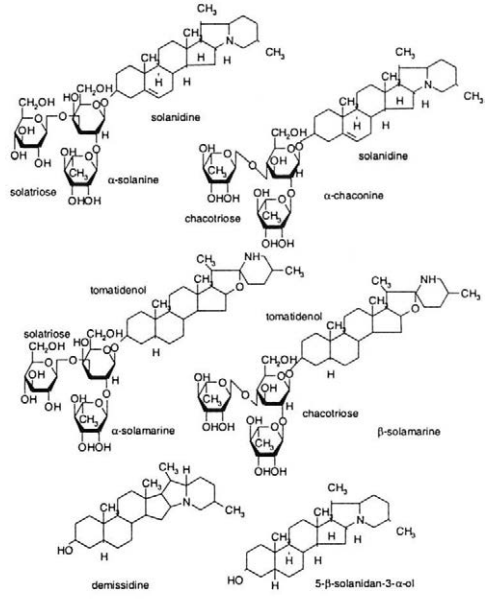

Figure 1. Structural relationship of some potato glycoalkaloids

β-chacotriose or O-α-L-rhamnopyranosyl-(1→2 glu)-O-α-L-rhamnopyranosyl-(1→4 glu)-β-D-glucopyranose (Boll 1962). In turn, β-solanine is composed of the disaccharide β-solabiose, or O-β-D-glucopyranosyl-(1→3)-β-D-galactopyranose, while β-chaconine is composed of the disaccharide β-chacobiose, or O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranose. Data have demonstrated that γ-solanine, or 3-β-D-galactosidosolanidine, and γ-chaconine, or 3-β-D-glucosidosolanidine, are bound with D-sugars via a β-glycosidic bound. Commersonine has been shown to be closely related to tetraglycosides in that the terminal unit is glucose.

Other minor glycoalkaloids found in potatoes include α- and β-solamarine, whose aglycone base is tomatidenol (Duke 1992a). Their structure was elucidated by Boll (1962, 1963) who isolated them from *Solanum dulcamara*. Other minor aglycones found include demissidine and

Table 1. Glycoalkaloids of *S. tuberosum*

Compound	Aglycone	Saccharide
α-solanine	solanidine	solatriose: galactose + glucose + rhamnose
β-solanine	solanidine	solabiose: galactose + glucose
γ-solanine	solanidine	galactose
α-chaconine	solanidine	chacotriose: glucose + rhamnose + rhamnose
β-chaconine	solanidine	chacobiose: glucose + rhamnose
γ-chaconine	solanidine	glucose
α-solamarine	tomadidenol	solatriose: galactose + glucose + rhamnose
β-solamarine	tomadidenol	chacotriose: glucose + rhamnose + rhamnose
	demissidine	
	5-β-solanidan-3-α-ol	

Table 2. Glycoalkaloids in wild potatoes

Compound	Aglycone	Saccharide
leptine I	O (23)-acetylleptidine	glucose + rhamnose + rhamnose
leptine II	O (23)-acetylleptidine	galactose + glucose + rhamnose
leptine III	O (23)-acetylleptidine	–
leptine IV	O (23)-acetylleptidine	–
leptinine I	leptidine	glucose + rhamnose + rhamnose
leptinine II	leptidine	galactose + glucose + rhamnose
leptinine III	leptidine	–
leptinine IV	leptidine	–
commersonine	demissidine	galactose + glucose + glucose + glucose
demissine	demissidine	galactose + glucose + glucose + xylose
dehydrocommersonine	demissidine	–
tomatine	tomatidine	–
– not determined		

5-solanidan-3-ol (Maga 1994). The structures of these compounds are shown in Figure 1.

In addition to α-, β- and γ-solanines and chaconines other numerous glycoalkaloids and aglycones have been identified in wild potatoes, such as *S. chacoense*. The structural relationships of these compounds are shown in Table 2. Some of the novel steroidal alkaloids found in wild potato species are shown in Table 3. In addition, an extensive number of acetoxy and hydroxy derivatives of solanidine have been identified in wild potatoes (Table 3) as referred by Schreiber (1968a, b). The free steroidal alkaloid tomatidenol had been found in potato sprouts and can serve as the base for the formation of the glycoalkaloids α- and β-solamarines (Schreiber 1957). Shih and Kuc (1974) identified solamarines only in the commercial potato variety, which would indicate that one of its parents had fairly high levels of the compound present. Solamarines in potato has confirmed also Duke (1992a). Another interesting trace glycoalkaloid is the compound commersonine, which was found in certain wild potato species by Osman et al. (1976). Leptinines I and II have been shown to be 23-hydroxy-α-chaconine and 23-hydroxy-α-solanine, respectively, while leptidine is 22-β-hydroxysolanidine. All natural solanidanes have been shown to possess a 20S:22R:25S:NS configuration. It was found that α-solanine had only half as much specific activity as α-chaconine (Guseva et al. 1960). Tomatidenol, or tomatid-5-en-3-β-ol, is C (5,6)-dehydrotomatidine. The minor glycoalkaloids found are tomatidine, tomatine, solanidine, solanthrene, and solasodine (Laurila et al. 1999; Väänän et al. 2000).

Glycoalkaloid content in potatoes and factors influencing it

Most potatoes sold for consumption have about 75 mg total of both chaconine and solanine in 1 kg of potato tubers. Potatoes with solanine levels greater than 140 $mg.kg^{-1}$ are bitter in taste.

The types of glycoalkaloids can also vary widely in the potato plant and tuber. In the case of potato sprouts, α-solanine represented 40% and α-chaconine 60% of total glycoalkaloids (Guseva et al. 1960), whereas in tubers the amount of α-solanine ranged from 28 to 57%, dependent upon variety (Morris and Petermann 1985).

Table 3. Some steroidal alkaloids and solanidine derivatives identified in wild potatoes

acetylleptinidine	5-tomatidenol
23-hydroxydemissidine	solasodine
23-acetyldemissidine	solanidadienol
tomatidine	methylsolanidenol
solanidanediol	3β-acetoxy-27-norsolanida-5,16,20 (22),23,25-pentaene
3β-acetoxy-5β-solanidane	3β-acetoxy-5α,16,17,20,22,27-norsolanid-X-ene A
23β-acetoxy-(5α)-solanidane	23β-acetoxy-(5β)-solanidane
23β-acetoxy-5α-solanidan-3β-ol	3-acetoxy-solanida-3,5-diene
3β,23β-diacetoxysolanid-5-ene	3β-acetoxy-25α-H-solanid-5-ene
3β, 3β-diacetoxy-5α-solanidane	3β-acetoxysolanid-5-en-23β-ol
23β-acetoxysolanid-5-en-3β-ol	3α-acetoxy-5α-solanidane
3β-acetoxy-5α-solanidane	3α-acetoxy-5β-solanidane

Glycoalkaloids, and phenolics are primarily responsible for bitter flavour in potatoes (Sinden et al. 1976). Total glycoalkaloid content of 13 different potato varieties varied from 7 to 580 mg.kg^{-1}, and phenolic content from 170 to 590 mg.kg^{-1}. Duke (1992a) and Friedman and Dao (1992) refer the contents of the major glycoalkaloid potato constituents as α-solanine, α-chaconine and solanosolone (Table 4).

Solanine levels above 140 mg.kg^{-1} are bitter in taste. Cultivars with greater than 200 mg.kg^{-1} cause a burning sensation in the throat and mouth (Cheeke 1998). The second most abundant steroidal glycoalkaloid in potatoes is α-chaconine. The solanine content of tubers varies depending on numerous factors. Most commercial varieties contain less than 120 mg.kg^{-1} and are normally between 20 and 130 mg.kg^{-1} (Concon 1988). Of the many factors affecting solanine content in potatoes variety, greening, and maturity seem to cause the largest variation. The conditions which vary the solanine content differ depending on what particular tuber is being discussed. A large level of variability in solanine content of potatoes can be attributed to differences in variety of the tuber. A certain variety may increase or decrease in solanine content with maturation, and increase or decrease or remain unaffected by fertilization. Other varieties may be affected greatly or slightly by storage conditions. Some varieties have shown increased solanine content upon storage (Concon 1988). Hamouz et al. (1999) have not confirmed significant influence of soil and environmental conditions of higher and lower situated regions of the Czech Republic on glycoalkaloid content. Only in 1995 trial significantly higher glycoalkaloid content in potato tubers from higher regions was determined (155 mg.kg^{-1}) in comparison with lower regions (89.1 mg.kg^{-1}). In all investigated years of cultivation could be observed evident unambiguous trend of higher glycoalkaloid content in potatoes cultivated in ecological way, but differences were not significant. Hamouz et al. (1999) determined average glycoalkaloid content in the Karin variety as high as 82.9 mg.kg^{-1}. This is in accordance with the results obtained by Čepl and Zrůst (1996) who found in the Czech variety Karin the SGAs content higher by 87% than in other varieties. Also nitrogen fertilization increased the SGAs content, but significantly only in the Karin variety. The strongest effect was found in tuber mechanical damage and in year. Damaged tubers had SGAs content higher by 89% than the tubers that were not damaged. During 1994, when the climate was unfavourable and dry, the SGAs content was found higher by 71% than the content in 1995. But regarding the temperature, Papathanasiou et al. (1996) reported that cool growing conditions had no evident effect on glycoalkaloid concentration of tubers during early development and also in tubers of comparable size at the final harvest. Levy et al. (1993) found that SGAs contents were higher in tubers from the spring and the summer seasons as compared to tubers from the winter season. In the greenhouse experiment, SGAs content was higher in tubers harvested from the "hot" chamber as compared to tubers harvested from the "cool" chamber. Physiologically young tubers reacted more than older ones and light-stress seemed to be the most effective method for discarding stress-sensitive clones (Olsson 1996b). Rogozińska (1999) found that mineral potassium fertilization significantly decreased solanine concentration. In comparison with K_2SO_4, a lower concentration of glycoalkaloids was found in experiments when it was fertilized with KCl. Storage caused an increase of SGAs level (range from 16 to 40%). Frydecka-Mazurczyk and Zgórska (1996) found that content of nitrates and SGAs was strongly dependent on the variety and climatic conditions during the growing period. Place of cultivation had a little effect on glycoalkaloid content. Panovská et al. (1994, 1997) monitored glycoalkaloid levels in different potato cultivars collected from three regions during two years. With the exception of Karin, one of the most widely grown cultivar in the Czech Republic, none of the cultivars used for human consumption exceeded the levels considered safe. SGAs contents for tested cultivars ranged from 31 to 166 mg.kg^{-1} fresh wt. There were no

Table 4. Content of major potato constituents in different potato organs

Content [mg.kg^{-1}]	Glycoalkaloid	Organ
0.5–635 (Duke 1992a)	α-chaconine	tuber
5–125 100 (Duke 1992a)	α-solanine	tuber
10 000 (Duke 1992a)	α-solanine	fruit
6000–7000 (Duke 1992a)	α-solanine	leaf
600 (Duke 1992a)	α-solanine	tissue culture
20–100 (Duke 1992a)	solanines	tuber
2.2 (Duke 1992a)	solanosolone	tuber
147 (Friedman and Dao 1992)	α-chaconine + α-solanine	tuber
320 (Friedman and Dao 1992)	α-chaconine + α-solanine	main stems
456 (Friedman and Dao 1992)	α-chaconine + α-solanine	small stems
860 (Friedman and Dao 1992)	α-chaconine + α-solanine	roots
1 450 (Friedman and Dao 1992)	α-chaconine + α-solanine	leaves
9 970 (Friedman and Dao 1992)	α-chaconine + α-solanine	sprouts

Table 5. Content of total steroidal glycoalkaloids (SGAs) in potato products

Potato product	SGAs [mg kg^{-1}]
Potato peel wedges, frozen	76–120 (Easton 1998)
Potato peel slices, frozen	66–71(Easton 1998)
Potato chips, commercial	24–720 (Easton 1998); 24–109 (Friedman and Dao 1992)
French fries, fresh	0.8–58 (Easton 1998); 0.8–8.4 (Friedman and Dao 1992)
Potato wedges, fresh	44 (Easton 1998)
Potato skins, baked	31–63 (Easton 1998)
Potato skins, fried	55–242 (Easton 1998); 31–203 (Friedman and Dao 1992)
Potato pancake powders	45–65 (Friedman and Dao 1992)

differences between SGAs levels in tubers from different regions. The highest content of total steroidal glycoalkaloids were found in industrial cultivar Oreb (126.5 mg.kg^{-1}). Zrůst et al. (2000) found in raw unpeeled tubers of the Czech varieties the SGAs content higher than in the foreign varieties: in the very early varieties by 24% and in the medium late to late varieties by 55%. After peeling the SGAs content decreased by 51.5% in the Czech varieties and by 55.1% in the foreign varieties. After mechanical damage the content of α-solanine in Czech varieties increased more than that of α-chaconine (in three years on average by 78 to 103.4% and by 48.8 to 70.4%, resp.). Hellenaes et al. (1995a) found in different Swedish domestic early potatoes combined α-chaconine and α-solanine contents in range from 51 to 221 mg.kg^{-1}. Sixty six per cent of the samples of the Magnum Bonum variety exceeded a temporary max. residue limit 200 mg.kg^{-1} and 8% were above 400 mg.kg^{-1} (Hellenaes et al. 1995b). Peeling did not significantly remove the glycoalkaloids in tubers with high content.

Glycoalkaloids have been found in almost all parts of the potato but the highest concentrations are usually associated with tissues that are undergoing high metabolic activity (Schreiber 1966; Jadhav et al. 1973; Jadhav and Salunkhe 1974a, b, 1975). These include potato flowers, unripe berries, young leaves, sprouts, peels, and the area around potato eyes. Typical concentrations of glycoalkaloids found in the potato plant and tuber are summarized in Table 4. Most commercially produced potatoes average 100 mg.kg^{-1} fresh weight of total glycoalkaloids, but a few commercial varieties can average close to 150 mg.kg^{-1} (Uppal 1987). Also, small, immature tubers normally are high in glycoalkaloids since they are still metabolically active (Gelder et al. 1988). In normal tubers, potato glycoalkaloids appear to be concentrated in a small 1.5 to 3.0 mm layer immediately under the skin (Schulzová et al. 1992). Kalač (1994) studied the distribution of SGAs in tubers of six varieties and found that the skin contained 83 to 96%, the cork layer 3 to 14% and the pulp 1 to 3% SGAs. With normal tubers, peeling removes from 60 to 96% of the glycoalkaloids present, while if tubers are high in glycoalkaloids, peeling will only remove up to 35% (Maga 1994). The glycoalkaloid diffusion from the outside to the inside can occur in potatoes with high glycoalkaloid levels. Easton (1998) and Friedman and Dao (1992) bring total SGAs (mg.kg^{-1}) in potato products (Table 5). The highest levels were found in potato chips (32–720), potato skins fried (55–242), and potato peel wedges, frozen (76–120). Potatoes containing 66 mg.kg^{-1} SGAs produced starch with no detectable SGAs (< 0.2 mg.kg^{-1}, Driedger and Sporns 1999). Dry protein concentrate and dry pulp produced from the same tubers contained 600 and 500 mg.kg^{-1} SGAs, resp. The pulp contained an average 220 mg.kg^{-1} SGAs, indicating that efficient washing can reduce SGAs concentration. The apparent partitioning of SGAs into protein concentrate and pulp indicates that toxicity is a legitimate concern for these by-products, given that a maximum suggested level of 200 mg.kg^{-1} SGAs is usually cited for tubers.

Percival and Dixon (1996a) found higher glycoalkaloid concentrations in aerial than in subterranean tubers (in aerial tubers of cv. Kerrs Pink 1343.0 mg.kg^{-1}). Ratios of α-chaconine:α-solanine in aerial tubers differed significantly from those in subterranean tubers indicating that exposure to light during aerial tuber growth enhanced the synthesis of one glycoalkaloid to a greater degree than the other. As Griffiths et al. (1994) have shown, there were significant differences between the genotypes in their rates of increase of glycoalkaloids and chlorophyll when they were exposed to light. There were significant differences between the cultivars in their rates of greening and increase in glycoalkaloid content (Dale et al. 1993). Percival et al. (1994) found that glycoalkaloid concentrations accumulated with time in dormant and sprouted tubers exposed to sodium and fluorescent light but fluctuated in those exposed to mercury light. Dormant tubers were more sensitive to illumination than sprouted tubers regardless of light source. Fluorescent light was associated with maximal glycoalkaloid synthesis in dormant tubers, while sodium light enhanced glycoalkaloid synthesis in sprouted tubers to the greatest extent. Exposure of dormant tubers to continuous light altered the ratio of α-chaconine to α-solanine irrespectively of illumination source. Changes in glycoalkaloid content depends on light intensity (Percival and Dixon 1996b) and temperature (Percival et al. 1993). Irrespective of genotype, light intensity $L \leq 500$ μmol.m^{-2}.s^{-1} photosynthetically active radiation significantly increased ($P < 0.01$) glycoalkaloid content of all cultivars tested, whereas light intensity $L \geq 750$ μmol.m^{-2}.s^{-1} resulted in a decrease in contents with

maximal decreases following light exposure at 1500 $\mu mol.m^{-2}.s^{-1}$. The accumulation of glycoalkaloids that normally takes place in aerobically incubated potato tuber disks is inhibited by the ethylene-releasing substance ethephon (Bergenstraahle et al. 1993). The enzyme S-adenosyl-L-methionine:sterol C 24 methyltransferase (SMT, EC 2.1.1.41), located at one presumed branching point in the sterol and glycoalkaloid pathway, was characterized and found to exhibit similar activity of SMT increased in aging tuber disks and this increase was further stimulated by ethephon, but inhibited by norborna-2,5-diene. The glycoalkaloid accumulation was also inhibited by the sterol synthesis inhibitor, tridemorph (Bergenstraahle et al. 1992). The accumulation of glycoalkaloids was not affected by addition of ascorbic acid, gibberelic acid or (2-chloroethyl)trimethylammonium chloride to the disks.

The concentration of solanine is greatest in or directly beneath the peel. Peeling is effective in removing most of the affected tissue. Cooking in steam or water reduces solanine to 60 to 70% of the value in raw material. A large level of variability in solanine content of potatoes can be attributed to differences in variety of the tuber. A certain variety may increase or decrease in solanine content with maturation, and increase or decrease or remain unaffected by fertilization. Other varieties may be affected greatly or slightly by storage conditions. Some varieties have shown increased solanine content upon storage (Concon 1988). Sprouting potatoes contain greater amounts of solanine, with higher concentrations found near the skin. Solanine amounts decrease toward the centre of the potato (Tanner 1952). Cheeke (1998) found variety Lenape with significantly high solanine levels (approximately 300 $mg.kg^{-1}$). But most commercial varieties contain less than 120 $mg.kg^{-1}$ and are normally between 20 and 130 $mg.kg^{-1}$ (Concon 1988). Greened potatoes are often higher in solanine than those not greened. The bitter taste associated with greened potatoes is caused by the solanine, not the chlorophyll. Wounding stimulated synthesis of α-solanine more than α-chaconine in cv. Home Guard and Desiree (Percival and Dixon 1996a). Regardless of cultivar, total glycoalkaloid α-solanine and α-chaconine were higher in wounded than unwounded aerial tubers. Potato peelings from French fry production are a potential source of dietary fiber, but the presence of naturally occurring glycoalkaloids could limit their use as a food additive (Zhao et al. 1994). Extrusion did not change α-chaconine/α-solanine ratios. Neither α-chaconine nor α-solanine decreased on a dry basis from non-extruded peels (390 and 8000 $mg.kg^{-1}$, resp.). Swaaij (1993) tested glycoalkaloid content with seed potatoes of 3, 6, 9 and 12 months old. Glycoalkoid content of tubers markedly decreased when plants were grown from 12 months old seeds. The glycoalkaloid content also decreased by nematode infection, especially in the cultivar Elles. Gerstner et al. (1999) investigated the effect of genetic modifications of potatoes on the concentrations of glycoalkaloids. The genetically modified lines exhibited significantly reduced concentrations of the total glycoalkaloids compared to the control. This effect could be confirmed at different dates of harvest. The concentrations of SGAs decreased during maturation. However, at each harvest, the amounts of glycoalkaloids in transgenic lines were lower than in the wild type potatoes. It is correlated with the influence of genetic modifications of the carbohydrate metabolism (invertase and sucrose synthase) on the content of glycoalkaloids. The accumulation of glycoalkaloids and chlorophylls in stored tubers investigated Edwards and Cobb (1996) and Percival et al. (1993). From their studies it is apparent that potato tubers must to be held at low temperatures and under subdued lighting.

The highest solanine content was found in the potato fruit (as high as 10 000 $mg.kg^{-1}$). Large variations in both α-chaconine and α-solanine contents were found in fresh leaves (Dao and Frieman 1996). Laurila et al. (1996) found that the leaves of *S. tuberosum* contained solanidine (396 $mg.kg^{-1}$ dry wt.) and solanthrene (49 $mg.kg^{-1}$ dry wt.), whereas the leaves of *S. brevidens* contained tomatidine (8173 $mg.kg^{-1}$ dry wt). In addition to these parental-type SGAs, all somatic hybrids contained demissidine in the leaves, and the total SGAs contents ranged from 290 to 7774 $mg.kg^{-1}$ dry wt.

SGAs are produced following the general steroid biosynthesis pathway, starting from acetyl-CoA and followed by the intermediates mevalonic acid, squalene, cycloartenol, and cholesterol (Valkonen et al. 1996). Low production of SGAs is a dominant character inherited in a relatively simple manner and can be selected for in potato-breeding programs. Love et al. (1996) used mutation breeding with 35 Gy of γ-rays to produce mutants of the potato breeding selection NDA 1725-1 with reduced levels of tuber glycoalkaloids and at the end of six clonal generations they obtained three selections with acceptable levels of tuber glycoalkaloids.

Potato glycoalkaloids in resistance of plants

Potato glycoalkaloids, mainly chaconine and solanine, are believed to provide resistance to insects and fungi (Shih and Kuc 1973). A need exists to develop new potato varieties which are resistant to bacteria, fungi, and insects. Since potatoes from the wild species, *Solanum acuale*, are known to be frost tolerant and to resist potato virus X (PVX), potato spindle tuber viroids, and cyst nematodes, Kozukue et al. (1999) carried out a collaborative study to assess the potential value of somatic hybrids derived from the wild cultivar and a standard variety, *Solanum tuberosum*. Some of the tubers from the hybrids had excellent quality characteristics such as size, shape, skin colour and depth of eyes on tuber skins. The hybrid tubers contained variable amounts of four glycoalkaloids, two from each parent. Glycoalkaloids act synergistically against pathogens (Rayburn et al. 1995). Regarding this fact, the new cultivars provide a useful resource for the development of resistant potatoes with a lower glycoalkaloid content, but broader structural

spectrum. Field experiments showed that there were significant differences in susceptibility to attack by the wireworm *Agriotes obscurus* (Jonasson and Olsson 1994). The most susceptible cultivars had the lowest total glycoalkaloid contents.

α-Chaconine is reported antifeedant (1 μM), fungicide, nematistat (EC 50 = 85 μg/ml), pesticide, α-solanine antifeedant (1 μM), fungicide and pesticide, β-chaconine as pesticide, solanidine as antifeedant (1 μM), and pesticide, solasodine as fungicide and pesticide, tomatine as bactericide, fungicide, molluscocide and pesticide, tomatidine as antifeedant (1 μM), and pesticide, demissine as antifeedant and pesticide and demissidine as antifeedant and insectifuge (Duke 1992b).

Potato breeders frequently use wild species of potatoes as sources of genes for resistance to insect pests. Glycoalkaloids in the leaves can act as insecticidal compounds. Yencho et al. (1997) located genes for glycoalkaloids on chromosomes 1, 4, 6 and 12. For estimation of glycoalkaloids an immunoassay with series of monoclonal antibodies has been developed (Stanker et al. 1994).

Potatoes will also produce high levels of glycoalkaloids in response to rotting caused by fungi or bacteria. Also glycoalkaloids derived from tomatidine, such as α-tomatine, were reported to have toxic properties against *Fusarium* wilt (Durbin and Uchytil 1969).

Role of potato glycoalkaloids in human and animal nutrition

Solanum alkaloids are cholinesterase inhibitors which result in neural function impairment (Hopkins 1995). A cholinesterase inhibitor is a chemical compound that inhibits acetylcholinesterase by removing acetylcholine from neuromuscular junctions. When an inhibitor of acetylcholinesterase, such as solanine is present, acetylcholine accumulates in the cleft. The presence of acetylcholine in nerve tissue or organs is responsible for the neurological signs associated with solanine poisoning. Contents as high as 1000 mg.kg^{-1} have been measured in the skin along with lethal amounts in the sprouts (Concon 1988). The ability to have such high levels has led to several cases of potato poisoning. Since they contain not just solanine but also other glycoalkaloids, it is likely that the symptoms of potato poisoning are due to a combination of alkaloids. In humans the oral dose of 28 mg.kg^{-1} body mass may cause both neurological impairment in the form of hyperesthesia, dyspnea, itchy neck, and drowsiness. These symptoms can be accompanied by gastrointestinal effects such as diarrhea and vomiting (Chaube and Swinyard 1976). Therefore new varieties are screened for solanine content and glycoalkaloid levels must be less than 200 mg.kg^{-1} (Cheeke 1998). In the human α-solanine and α-chaconine toxicity begins with gastrointestinal disturbances, vomiting, diarrhea, abdominal pain, headache, then followed by neurological disorders, at higher doses low blood pressure, fever, rapid weak pulse, rapid breathing, hallucinations, delirium and finally a coma (Groen 1993; Friedman and Mc Donald 1997). Regarding the chronic toxicity of glycoalkaloids, there are indications that α-solanine and other potato glycoalkaloids can accumulate in tissues. But these glycoalkaloids have a low oral toxicity due low solanine levels after ingestion due to poor absorption by the gastro-instestinal tract. It is removed from the body fairly rapidly in both the urine and the faeces, usually within 12 hours, preventing accumulation in the tissues. Intestinal bacteria also aid in the detoxification by hydrolyzing the glycoside into aglycone solanidine, which is less toxic than solanine and poorly absorbed. Comparative evaluations were carried out on the triglycosides α-chaconine and α-solanine, the diglycosides β_1- and β_2-chaconine and β_2-solanine, and the monoglycosides γ-chaconine and γ-solanine (Rayburn et al. 1994). The results show that biological activity is influenced by the chemical structure of the carbohydrate, i.e. galactose, glucose, or rhamnose; the number of carbohydrate groups making up the side chain attached to the 3-OH position of the aglycone solanidine, and the stereochemical orientation of the chaconine saccharides. Solanine is the most active cholinesterase inhibitor found in food due to its location in edible portions of plants. It has the ability to prevent the breakdown of acetylcholine – a very active neurotransmitter – in the human body. The accumulation of acetylcholine in neuromuscular junctions impairs the function of the nervous system and its effect organs. Solanine levels above 140 mg.kg^{-1} are bitter in taste. Cultivar with greater content than 200 mg.kg^{-1} causes a burning, sensation in the throat and mouth. A fatal oral dose for an adult would be about 420 mg. In ruminant animals (Kotowski 1967), potato glycoalkaloids are hydrolyzed to solanidine. Solanidine is then further metabolized into 5,6-dihydroxyanalog (Cheeke 1998). Some combinations of α-chaconine and α-solanine mixtures ranging from approximately 3:1 to 1:20 exhibited strong synergism (Rayburn et al. 1995).

Potato vines also contain solanine but they are a valuable feed for livestock. When the vines are harvested, it is done before the vines dry out and die. At the point of harvest the vines are non-toxic and serve as a nutritious food source for ruminants. Green potatoes may cause food poisoning and since some of the symptoms are similar to gastroenteritis, it is possible that some undiagnosed cases of gastroenteritis have been caused by eating green potatoes. Human and livestock deaths have been recorded as a result of the consumption of greened or damaged potatoes with very high glycoalkaloid levels (Bolin 1962; Gelder 1991; Buczek and Majeran 1969). It is advisable that green or damaged potatoes are avoided by pregnant or women who are likely to become pregnant, as there is some evidence of possible foetal damage or loss of the foetus from glycoalkaloid poisoning in animals. The peels of dry or moist cooked potatoes retain up to twenty more sprout inhibitor residue than the pith of the potato. About 30 to 80% of the glycoalkaloid content of a potato is in its peel with the remainder in the flesh of the tuber. The concentration of these glycoalka-

loids is highest in potato sprouts and green potato skins (Kline et al. 1961).

New potato varieties cannot be introduced unless they contain less than 200 mg glycoalkaloids.kg^{-1}. The glycoalkaloids are more poisonous than the steroid alkaloid aglycones. An appreciable amount of *Solanum* – type glycoalkaloids is hydrolyzed in the gut of mammals to the less toxic aglycones. Sanford et al. (1995) by backcrossing the F_2 genotypes (*S. chacoense* × *S. tuberosum*) to *S. tuberosum* reduced mean SGAs content to 150 mg.kg^{-1}. A second backcross reduced further the content to 90 mg.kg^{-1}. The gene, or antisense DNA derived from it, in particular an antisense DNA, can be used to limit expression of the gene and thereby limit glycoalkaloid synthesis in potato (Moehs et al. 1998). For SGAs quantification an enzyme immunoassay with bovine serum albumin coating conjugates was developed (Plhak and Sporns 1992).

Conclusions

Potatoes as well as eggplants, tomatoes and other plants contain glycoalkaloids that are important in their defence system against viruses, bacteria and insect attack. In the potatoes the most dominant structures are α-solanine and α-chaconine, but there are contained also other minor forms as β- and γ-solanine, β- and γ-chaconine, α- and β-solamarine, demissidine, 5-β-solanidan-3-α-ol, in wild potatoes leptines, commersonine, demissine, tomatine, etc. α-Solanine and α-chaconine represent 95% of total glycoalkaloid content of potatoes. *Solanum* glycoalkaloids are cholinesterase inhibitors which result in neural function impairment, so the presence of acetylcholine in nerve tissue or organs is responsible for the neurological signs associated with the glycoalkaloid poisoning. This is dominant reason why new varieties are screened for the glycoalkaloid levels less than 200 mg.kg^{-1}. Solanine levels above 140 mg.kg^{-1} are bitter in taste. Fortunately, most commercial varieties contain less than 120 mg.kg^{-1} and are normally between 20 and 130 mg.kg^{-1}. Of the many factors affecting glycoalkaloid content in potatoes the variety, greening, maturity and tuber damage seem to cause the largest variation. On the other hand, a need exists to develop new potato varieties which are resistant to bacteria, fungi, and insects. The new approach is to obtain somatic hybrids derived from the wild cultivars (such as *Solanum acuale*) and a standard variety (*Solanum tuberosum*). The new cultivars provide a useful resource for the development of resistant potatoes with a low glycoalkaloid content, but broader spectrum of glycoalkaloids that act synergistically against pathogens.

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ABSTRAKT

Glykoalkaloidy brambor a jejich význam v ochraně rostlin a v lidské výživě – studie

Brambory (*Solanum tuberosum* L.) obsahují mezi sekundárními metabolity glykoalkaloidy, které jsou důležité v jejich obranném mechanismu proti různým pathogenům, jako jsou viry, bakterie, houby a hmyz. Na druhé straně jsou tyto glykoalkaloidy významnými toxiny v lidské výživě i při krmení zvířat. V bramborách jsou nejvíce zastoupeny α-solanin a α-chakonin, které tvoří až 95 % celkového obsahu glykoalkaloidů. Další identifikované glykoalkaloidy představují β- a γ-solaniny a chakoniny, α- a β-solamariny a aglykony demisidin a 5-β-solanidan-3-α-ol, v divokých bramborách leptiny, komersonin,

demisin a tomatin. Glykoalkaloidy brambor jsou inhibitory cholinesterázy a způsobují otravu vedoucí k akumulaci acetylcholinu v nervové tkáni. Nové odrůdy musí obsahovat méně než 200 mg glykoalkaloidů v 1 kg hlíz a ceněné jsou odrůdy obsahující 20 až 130 mg glykoalkaloidů v 1 kg hlíz. Hlavními faktory ovlivňujícími obsah glykoalkaloidů v bramborách jsou odrůda, zelenání brambor, zralost a poškození hlíz. Vzhledem k významu těchto látek v ochraně rostlin proti patogenům poskytuje křížení mezi divokými bramborami (*S. acuale*) a standardními odrůdami (*S. tuberosum*) nové hybridy obsahující nižší množství glykoalkaloidů, avšak s širším spektrem jejich strukturálního zastoupení, které umožňuje synergický efekt vůči patogenům.

Klíčová slova: brambory; glykoalkaloidy; solaniny; chakoniny; minoritní glykoalkaloidy; obsah glykoalkaloidů; obrana rostlin; toxický účinek; hybridizace

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REVIEW

Mischanbau von Getreide- und andere Körnerfruchtarten
Ein Beitrag zur Nützung von Biodiversität im Pflanzenbau

The cultivation of cereal mixtures and other grain crops
The contribution to the utilisation of biodiversity in plant cultivation

W. Aufhammer

Verlag Eugen Ulmer, Stuttgart (Hohenheim) 1999. 305 pp., 200 tables, 8 figures and 8 colour panels

The successful book Cereals and other grain crops (Getreide- und andere Körnerfruchtarten, Bedeutung, Nutzung und Anbau) Ulmer, Stuttgart (1988) is soon succeeded by the author written by the same author, remarkable by its theme. It brings knowledge on cultivation of species mixtures and varieties. It is based on the fundamental natural situation, when the plant cover is composed of the stands of mixtures of species and cultivars. These stands are formed by different plant species in the community of plants and other organisms. The first part of the book specially studies the bases of the development of mixed stands, while the second part pays an attention to the issues of competition between components of mixtures and vegetation factors and the resistance to deleterious elements. The methods of description of properties and behaviour of components of mixtures derived from these studies are very valuable.

The second half of the book is composed of the methods of cultivation of species mixtures and varieties. There are summarised the results of the trials with cultivation of different species and cultivars in the agrarian field of the Central Europe. The stands of mixtures are concentrated as on grain production, as on forage as well as on mulching biomass. The cultivar mixtures are specialised above all on the grain production, particularly on cereals. The results of known combinations, such as the mixture of wheat and rye, or barley and oats, and also some untraditional combinations are presented. For example, beside cereals, legumes and oil crops should be suitable to be introduced into practical cultivation with respect to extension of biodiversity. Many of them may be certainly appropriate for ecological (organic) agriculture, or into zones of hygienic protection of water resources. There is valuable knowledge on the combination of crops, when one of the components (e.g. the legume) leaves excessive amount of nitrogen and the second component makes use of this nitrogen. The stands of cultural crops as a section of conditions of natural biodiversity are described in the final part.

The book of W. Auhammer represents a remarkable publishing action of the author and the publisher Ulmer, which has no equivalent in the agricultural literature in view of the theme as well as the content. It is a remarkable contribution to put through the sustainable development of agriculture by its acquisition to the extension of biodiversity of cultivated crops.

Prof. Ing. Jiří Petr, DrSc.

POKYNY PRO AUTORY

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ROSTLINNÁ VÝROBA PLANT PRODUCTION

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