Table 2 Variation in the individual HMW glutenin subunit alleles in Slovak wheat varieties.

Cultivar	HMW glutenin subunits			Glu-score	SDS test
	Glu-A1	Glu-B1	Glu-D1		(cm^3)
Astella	0	7+9	5+10	7	57
Barbara	0	7+9	5+10	7	50
Blava	0	7+9	5+10	7	60
Fundulea 29*	0	7+9	5+10	7	43
Ilona	2*	7+9	5+10	9	61
Iris*	0	7+9	2+12	5	48
Košútka	0	7+9	5+10	7	64
Lívia*	0	7+9	5+10	7	35
Maris Marksman	0	7+8	2+12	6	40
Rada	0	7+9	5+10	7	47
Sana*	0	7+9	2+12	5	32
SK3756-76*	0	6+8	2+12	5	46
Solida	0	7+9	5+10	7	52
Torysa	0	7+8	2+12	6	54
Vala	0	7+9	2+12	5	56
Viginta	0	7+9	5+10	7	70
Vlada	1	7+9	5+10	9	79
Regia	1	7+9	5+10	9	70
Average				6.8	53.6
Min.				5	32
Max.				9	79
v (%)				19.2	23.3

- sekaline block GLD 1B

MULTIVARIATE ANALYSIS OF SELECTED MORPHOMETRIC TRAITS OF BARLEY CULTIVARS

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Summary

Paper is focused on searching for separating value of selected morphological traits of barley grain which are used for cultivar identification by morphometric method (epispermoscopy). The method is used for rapid screening during purchase of malting barley. Cluster Analysis and Principal Coordinates Analysis confirmed possibility for separation of selected cultivars according suggested methods.

Key words: morphometry, epispermoscopy, barley, multivariate analysis.

Introduction

Genetic influence on shape and dimension of plants, which is realized through gene activities and morphogenesis is at present the base for the most common methods of genotype description in the frame of internationally used systems. Other group belongs to biochemical description of genotypes with application of variability in storage proteins and isozymes. The third group are methods of DNA-profiling, where is analyzed extracted plant DNA (Hulman et al., 1998).
Diversity of species in plant empire is in the frame of intact plant conserved on the level of seeds (Jureková, 1990). Special method which could be used in species and cultivar identification is so-called epispermoscopic analysis (*epi*-surface, *sperma*-seed, *scopia*- observation). In spite of situation, in the history was known that seeds of individual species, variets and cultivars are different. There was not stressed possibility of application detailed microscopic evaluation of seeds for practical use as a method for identification individual cultivars (Lužný et al., 1989).
Morphometric identification of cultivars is modern and very perspective with possibility of application in breeding, seed production, seed inspection, variety testing but most of all in inspection of mercantile commodities delivery, for example food wheat, malting barley etc. (Hulman et al., 1998).

Acquired information from study of variability of individual characters and traits in plant species, first of all solid recognition of morphology individual plant parts and determination of genotypic deviations is basic assumption for managing morphometric methods of identification selected genotypes (Brindza et al., 2000).

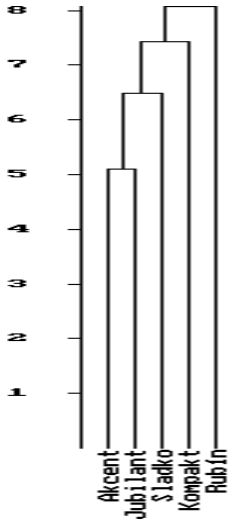
Material and methods

In 1998 according request of joint-stock company Zlatý bažant in Hurbanovo was prepared catalogue and method for identification of 5 selected cultivars of malting barley (*Hordeum vulgare* subsp. *distichon* L.) –"Akcent", "Jubilant", "Kompakt", "Rubín" and "Sladko" at the level of grain morphology. For experiments basic seeds SE1 generation were used, for macroscopic description of grains was used microscope ZEISS STEMI 2000-C and for taking of macro pictures CCD camera SONY model NO.SSC-DC 30P.
Description of traits (binary, quantitative and multistate) in grains was prepared and published as a company catalogue (Brindza et al., 1998). Degree of genotype dissimilarity was counted by Hierarchical Cluster Analysis. For evaluation of suitability selected discriminating traits was used Principal Coordinates Analysis from the reason, that data character needs to use other measure of distance than common Euclidean distance. For mixed data (binary, qualitative, multistate) we applied Gower's coefficient. In analysis statistical package SYNTAX ver.5.1 was used.

Results and discussion

Preparation of practical cultivar identification used during malting barley purchase needs to submit economic and time unpretending method with appropriate level of reliability. Selected method of morphometric identification based on analysis and identification of morphological properties on level of grain represents rapid expedite method. Method requires solid study of morphologic and metric characteristics which are identified by common tools (magnifying glass, binocular) and then compared with standard parameters identified on homogenous breeding materials.
Selected cultivars were characterized by suitable descriptor list (Brindza et al., 1998), were we selected 14 discriminating traits, 8 of them were multistate (9 point scale), 3 quantitative (characterized by variability characteristics) and 3 binary (alternative). By 11 qualitative characters describing morphological properties of cultivars is possible to identify any of selected cultivars in anonymous sample. For mentioned characters was prepared method for rapid identification of cultivars easy managed after short training in special course.
The method was prepared for selected group of cultivars and therefore there arise question for its application as universal method for genus Hordeum. Our aim was with the help of multivariate methods to find how variable material we worked with and how were we successful in selection of characters which could be applied in genotype detection.
By Hierarchical Cluster Analysis (Method single linkage, Euclidean distance fig.1) we found that cultivars "Akcent" and "Jubilant" are the most similar in evaluated characters and cultivar "Rubín" represents genotype with the lowest similarity to searching sample of cultivars. Cultivars "Jubilant" and "Akcent" were bred by hybridization and registered in 1991 and 1992. Cultivar "Rubín" was registered in 1982 and represents genotype created by complicated high level crossing.

Figure 1 Dendrogram describing similarity level of evaluated cultivars counted by single linkage



Another question was to estimate level of influence of selected characters on determination of cultivars. For this purpose was used Principal Coordinates Analysis. We identified 10 positive eigenvalues, from which 3 covers 95% of total variability

investigated traits. Percentage of cultivar variability accounted on each component is described on table 1. Percentage of character variability accounted on each component is described on table 2. PCoA analysis helps to divide characters to three groups, on morphological characters of grain including quantitative characters accounted for the first component.

Table 1 Percentage of variance of objects accounted for by each component

Cultivar(1)	1st Component(2)	2nd Component(3)	3rd Component(4)
Akcent	68.53	3.64	21.29
Jubilant	77.67	2.51	0.78
Rubín	20.18	78.75	0.13
Sladko	0.002	30.94	65.28
Kompakt	77.23	15.52	6.13

Table 2 Percentage of variance of variables accounted for by each component

	1st Component(1)	2nd Component(2)	3rd Component(3)
Grain shape (4)	88.763	0.258	10.440
Shape of dorsal side (5)	78.055	9.627	10.294
Base profile (6)	77.687	13.937	8.183
Base hump (7)	2.296	26.258	66.839
Foot of base (8)	62.201	5.644	28.178
Lemma base (9)	31.964	47.072	16.949
Edge of lemma (10)	50.235	46.745	2.213
Firmness of lemma (11)	21.670	72.057	0.454
Lemma and palea overlapping(12)	4.711	89.978	4.356
Rachilla length (13)	36.914	49.814	13.102
Rachilla position (14)	78.071	0.140	16.411
Grain length (15)	32.000	28.498	12.787
Grain width (16)	89.624	3.868	6.485
Grain thickness (17)	81.062	1.879	0.587

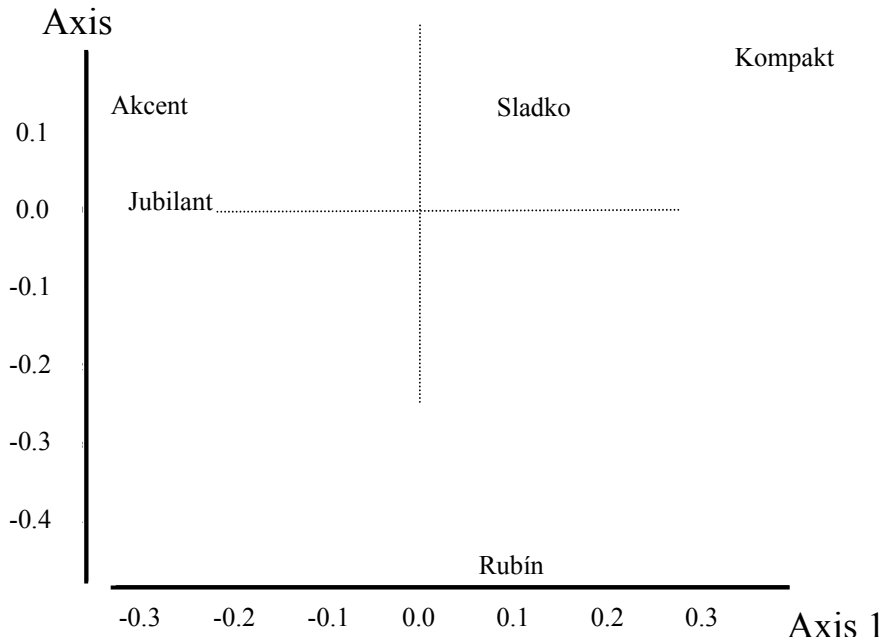


Figure 2 Biplot of evaluated cultivars accounted to component axis 1 and 2

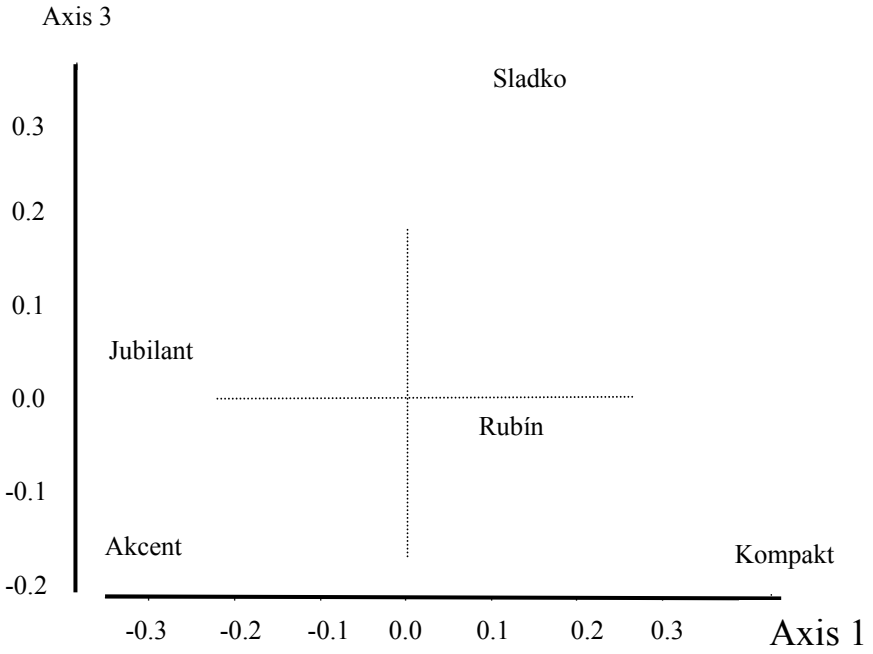


Figure 3 Biplot of evaluated cultivars accounted to component axis 1 and 3

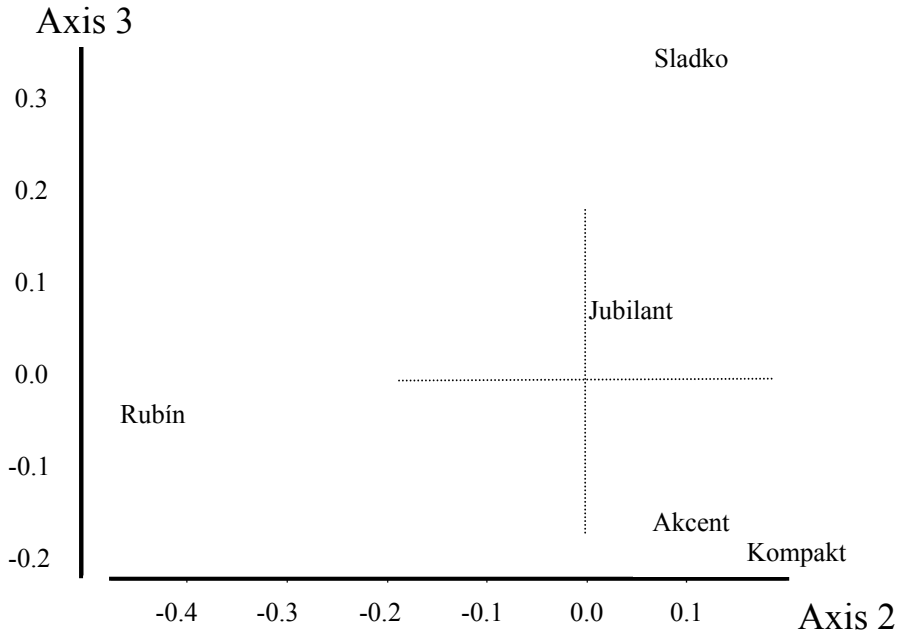


Figure 4 Biplot of evaluated cultivars accounted to component axis 2 and 3

Characters describing properties of lemma are accounted for the second component. Character basal hump represents the highest portion on the third component. Between investigated characters are three which are accounted at the same time for the first and the second component (edge of lemma, rachilla length, grain length). Graphic description of cultivar position in three dimensional space to component axes is described on figures 2-4.

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DETECTION OF POTATO VIRUS S BY RT - PCR IN POTATO REGENERANTS DERIVED FROM *IN VITRO* HEAT - TREATED SHOOT TIPS

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Summary

An assay, based on amplification of cDNA synthesised from genomic viral RNA, has been evaluated to detect potato virus S (PVS) in infected *in vitro* plantlets and regenerants derived from *in vitro* heat - treated shoot tips. Two primers were selected to prime the synthesis of 1061 bp fragment for specific detection of PVS. One single cutting of *in vitro* plantlet was sufficient for the detection of PVS and the presence of virus was detected in a $1:10^{-6}$ dilution. The use of RT - PCR allowed to detect the presence of PVS about one multiplication step earlier than ELISA.
Key words: potato virus S, PVS, polymerase chain reaction, PCR, tissue culture, thermotherapy, virus elimination

Introduction

New varieties for rapid multiplication under *in vitro* conditions and slow-grow conservation are required to be free from any pathogens including potato virus S (PVS). Thermotherapy *in vitro* combined with meristem tip culture enables to produce pathogen-free *in vitro* plantlets and tuber nuclear stocks. Meristem clones derived from buds of heat-treated *in vitro* plantlets are tested for presence of PVS in *in vitro* plantlets by ELISA method and planted into the greenhouse to be finally tested for presence of PVS (Kotkas, Rosenberg, 1999). This testing procedure is labour and time consuming.
PVS is a member of the carlavirus group and it is considered to be the commonest virus of potato crop. Although losses as high as 10 - 20 % have been reported, it is generally accepted that PVS alone does not usually cause significant economic loss (Bantari et al., 1993). Potato virus S (PVS) occurs widely in Slovakian potato crops at levels of 100 %.
A variety of techniques are used to detect potato viruses in infected plants and tubers. Methods depending on viral proteins includes enzyme - linked immunosorbent assay (ELISA) and techniques involving properties of the viral nucleic acid are based on hybridisation procedure or cDNA synthesis of template RNA which is subsequently used as a template for polymerase chain reaction (PCR) (Barker et al., 1993). It is of a great importance to have a reliable and rapid method for direct detection of PVS in the plantlets regenerated directly from meristem tips. The aim of this work was to evaluate an assay for detection of PVS in the first regenerants using a procedure based on PCR.

Materials and methods

Virus isolates and potato genotypes. Isolates used in the study included PVS in potato plants collected from potato fields in Slovakia and isolates in potato *in vitro* plantlets of 10 genotypes. All infected and healthy plants (advanced clones VL-25/87, VL-120/87, VL-95/87, VL-11/87, VL-23/87, VL-116/87 and varieties Lipa, Rema, Juliver, Freika, Bettina, Amazone, Maradona a Diamant) were maintained in greenhouse and under *in vitro* conditions.
Thermotherapy of *in vitro* plantlets. Stem cuttings were cultivated *in vitro* for 2 weeks on MS medium (Murashige, Skoog, 1962) and subjected to heat treatment. Plantlets were cultivated at 37 °C under 16 h photoperiod and 33 °C in dark during four weeks. Meristem tips were excised from heat treated plantlets and transferred on MS medium supplemented with 0,25 $mg.l^{-1}$ GA_3. Regenerants were cut and two cutting were used for further multiplication and two cuttings were tested for the presence of PVS by both methods.
Oligonucleotide primers. Oligonucleotide primers for PVS were designed on nucleotide sequence of PVS RNA (McKenzie at al., 1989). The primers S1 and S2 were selected from region 42K protein and capsid protein. Size of final product - 1061 bp - was designed to not interfere with known products of other potato viruses. Primer S1 (virus antisense) - 5'-CTG GAT CCT TAC TCC AAC CCC GAA TAA A-3' was complementary to the viral sequence encoding amino acids FIRGWSN near 3´ end