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PLANT GENOME MAPPING

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Summary

Genome mapping is the term used to create a set of ordered fragments of a particular genome. A genome map describes the order of genes or other markers and the spacing between them on each chromosome. The genome maps are constructed on several different scales or levels of resolution. At the coarsest resolution are genetic linkage maps, which depict the relative chromosomal locations of DNA markers (genes and other identifiable DNA sequences) by their patterns of inheritance. Physical maps (chromosomal map, cDNA map, contig map, macrorestriction map, sequence map) describe the chemical characteristics of the DNA molecule itself.

Key words: genome mapping, genetic map, physical maps

Introduction

Plant cells contain genetic information within nuclei, chloroplasts and mitochondria. In all of these cell organelles the genetic information is encoded within molecules of deoxyribonucleic acid (DNA). The nuclei of plant cells contain various linear DNA molecules, the number and length of which vary between different plant species. The chloroplasts and the mitochondria also contain DNA but in the form of circular molecules. Most of the plants genetic information is contained of the nucleus. Each nucleus contains sets of chromosomes (large molecules of DNA) composed of the genes. This set of genetic information is known as the nuclear genomes. The nuclear genomes of different plant species contain different amounts of DNA.
Genome mapping is the term used to create a set of ordered fragments of a particular genome. Mapping involves dividing the chromosomes into smaller fragments that can be propagated and characterized and mapping them to correspond to their respective location on the chromosomes. The base sequence of each of the ordered DNA fragments are determined. The goals of plant genome analysis is (a) to find all the genes in the DNA sequence of particular plant species, (b) to develop tools for using this information in the study of biology and agriculture and (c) automating methods to extract the maximum useful information from maps and sequences (http://www.ort.edu.uy/REDOC/maphugen.htm, 2001).
A genome map describes the order of genes or other markers and the spacing between on each chromosome. Genome maps are divided into two general types (a) genetic linkage maps and (b) physical maps. Genetic linkage maps or phenotype maps shows the relative chromosomal location of DNA markers (genes and other identifiable sequences) by their patterns of inheritance. Physical maps describe the chemical characteristics of the DNA molecule itself (http://www.ort.edu.uy/REDOC/maphugen.htm, 2001).

Genetic mapping methods review

Genetic linkage maps

A genetic linkage map shows a relative locations of specific DNA markers along the chromosome. Any inherited physical or molecular characteristic that differs among individuals and is easily detectable in the laboratory is a potential genetic marker. Markers can be expressed DNA region (genes) or DNA segment that have no known coding function but whose inheritance pattern can be followed. DNA sequence differences are especially useful markers because they are plentiful and easy to characterize precisely (http://www.ort. edu.uy/REDOC/maphugen.htm, 2001).
On the genetic map, distances between markers are measured in the terms of centimorgans (cM), named after the American geneticist Thomas Hunt Morgan. Two markers are said to be 1 cM apart, if they are separated by recombination 1 % of time. A genetic distance of 1 cM is roughly equal to a physical distance of 1 million bp (1Mb).
Three kinds of genetic maps are available in *Arabidopsis* genetic map: (1) classical, (2) RI and (3) mi-RFLP.
The classical map was originally built using genetic distances based on recombination frequencies between visible markers. Other mutant genes were later cloned and placed on the recombinant inbred (RI) map or assigned a position relative to molecular markers on the RI and / or physical maps. The major function of the classical genetic maps is to show the relative locations of genes identified by mutation. The Recombinant Inbred (RI) lines were generated from a cross of *Arabidopsis* ecotypes Columbia and *Landsberg erecta*.
The RI map was constructed using a „framework" of markers that act as a consistent backbone for the map. 129 *Arabidopsis thaliana* RFLP (restriction fragment length polymorphisms) markers were established based upon DNA fragments cloned in the pUC119 plasmid vector and insert end sequences of P1 clones. These markers are useful for map-based gene isolation

and genome physical mapping in *Arabidopsis thaliana* as well as studies of chromosome colinearity with related species (http://www.arabidopsis.org/map Viewer/help /mapkey2a.htm, 2001).
Genetic maps may consist of actual gene coding regions, phenotypic traits, and molecular markers that do not encode genes but serve as landmarks, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers (http://bldg6.arsusda.gov/benlab/soybean_mapping.htm, 2001).
The soybean genetic linkage map contains over 200 RFLP, SSR, AFLP, RAPD and phenotypic markers. The map encompasses over 1200 cM of the soybean genome. Many RFLP markers placed on the map represent cDNAs of known function (http://bldg6.arsusda. gov/benlab/genetic_map.html, 2001).

Physical maps

Different types of physical maps vary in their degree of resolution. The lowest-resolution physical map is a **chromosomal** (cytogenetic) map. Higher resolution provides a **cDNA map**, which shows the locations of expressed DNA regions (exons) on the chromosomal map. More detailed cosmid **contig map** depicts the order of overlapping DNA fragments spanning the genome. A **macrorestriction map** describes the order and distance between enzyme cutting sites. The highest-resolution physical map is the complete elucidation of the DNA base-pair sequence (**sequence map**) of each chromosome in the genome (http://www. ort.edu.uy/REDOC/maphugen.htm, 2001).
In a **chromosomal map**, genes or other identifiable DNA fragments are assigned to their respective chromosomes. These DNA fragments can be physically associated with particular bands (identified by cytogenetic staining) primarily by *in situ* hybridization, a technique that involves tagging the DNA fragment with an observable label. The location of the labeled probe can be detect after it binds to its complementary DNA strand in an intact chromosome.
A **cDNA map** shows the position of expressed DNA regions (exons) relative to particular chromosome or bands. The cDNA molecule is synthesized in the laboratory using the mRNA molecule as a template. A cDNA map provide the chromosomal location for genes.
The result of top-down mapping is a **macrosrestriction map**. In this mapping, a single chromosome is cut (with rare-cutter restriction enzyme) into large pieces, which are ordered and subdivided. The smaller pieces are then mapped further. The resulting macrorestriction maps depict the order of a distance between sites at which rare-cutter enzyme cleave. This approach yields maps with more continuity and fewer gaps between fragments than contig maps. The map resolution is lower and may not be useful in finding particular genes. In addition, this strategy generally does not produce long stretches of mapped sites. This approach allows DNA fragments to be located in regions measuring about 100 000 bp to 1 Mb.
The result of bottom-up mapping is a **contig map**. The bottom-up approach involves cutting a chromosome into small pieces, each of which is cloned and ordered. The ordered fragments form contigous DNA block (contigs). Currently, the resulting library of clones varies in size from 10 000 bp to 1 Mb. An advantage of this approach is the accessibility of these stable clones to other researchers. Contig construction can be verified by FISH method, which localizes cosmid to specific regions within chromosomal bands (http://www.nalsuda. gov/pgdic/tutorial/lesson8.htm, 2001).
The ultimate physical map is the complete DNA sequence determination (**sequence map**) of all base pair on each chromosome. This map will be formed by assembling contigs of at least 2 Mb in length with sequence-tagged sites (STS) spaces every 100 kb. A STS is a 100 – 1000 nucleotide sequence with three characteristics: (1) unique in the genome, (2) identifies a mapped element and (3) amplifiable by the polymerase chain reaction. STS can be used to identify clones to generate overlapping inserts for a physical map. STS can also be used as a correspondence points between genetic and physical maps (http://www.npac.syr.edu/users/ houle/GeneticMaps.html, 2001).

Conclusion

Linkage maps are important for genetic analysis, breeding and population studies. By knowing the locations of a particular gene in reference to other genes and markers, the breeders can devise strategies for developing new crop varieties. Linkage analysis using polymorphic DNA markers has expanded our knowledge of genomes tremendously and has allowed quantitative traits to be linked with molecular markers (QTL – quantitative trait loci). Molecular linkages to important traits also provide footholds for the molecular biologists to clone genes by map position. For example, specific genes encoding economically important phenotypes, such as resistance to fungi, insects, bacteria etc., could be cloned by without having knowledge of the product encoded gene, but just by knowing the location of the phenotype on the map.

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THE TESTING OF EFFICACY OF CHA GENESIS® FOR PRODUCTION OF HYBRID WHEAT AND HYBRID TRITICALE

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Summary

The objectives of the joint project "Hybrid Wheat and Hybrid Triticale Research in the Czech Republic" conducted by Monsanto Company (USA) and the Agricultural Research Institute Kroměříž, Ltd. were to evaluate the efficacy of the CHA Genesis under climatic and soil conditions of the Czech Republic using potential parent components of wheat and triticale, to assess an efective Genesis dose and produce a necessary amount of experimental hybrid wheat and hybrid triticale seed for yield trials. Evaluating the yield of tested wheat parental components, significant differences were found between individual variants of treatment. If the percentages of sterility and hybrids using the technique of bagging were assessed, significant differences in all doses in relation to the untreated check and significant differences between the dose of 1.6 kg.ha^{-1} Genesis to both increased doses were calculated. Insignificant differences were found between the dose of 3.6 and 4.9 kg.ha^{-1} Genesis. If the percentage of hybrids was assessed by electrophoresis of storage proteins, significant differences were also calculated between the dose 3.6 and 4.9 kg.ha^{-1} Genesis. Based on the obtained results, it is possible to conclude the CHA Genesis dose of 4.9 kg.ha^{-1} appeared sufficient to induce nearly 100% (99.5% in wheat and 96.7% in triticale) male sterility of female plants under investigated soil and climatic conditions in the Czech Republic.

Key words: chemical hybridising agent Genesis, wheat, triticale, male sterility, hybrid purity, electrophoresis

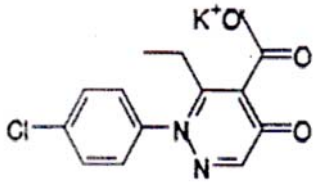
Introduction

Chemical hybridising agents (CHA) are chemical substances that are used to induce male sterility in female plants. The possible use of CHAs has been investigated in at least 40 species, including all the major cereales of the world and several other crops of great economic importance (Pickett, 1993). CHAs prevent from normal production of pollen at keeping female fertility which enables to produce hybrid seed and to overcome a lot of problems associated with exploitation of cytoplasmic male sterility (Nesvadba et al., 1998). Great experience with utilization of CHA and production of hybrid wheat has been obtained in the USA and France. On the basis of Genesis, the first wheat hybrids of the Quantum series in hard and soft red wheats were developed and released to farmers for commercial production in the USA in 1996. In France, six hybrid wheats (Cockpit, Cabestan, Domino, Mercury, Sextant and Twin) have been registered since 1995. In Germany the first hybrid wheat Hybnos 1 from Nordsaat - Saatzucht was registred in a List of Registered Varieties in 1999 (Nesvadba, Vyhnánek, 2001).
This paper presents the results of the yield evaluation, male sterility and hybrid purity of twenty varieties and lines of wheat and five genotypes of triticale produced using Genesis compound.

Materials and methods

Genesis is a chemical agent developed by Monsanto (USA) for HybriTech for wheat hybridization. Genesis contains 244 g.l^{-1} active ingredient clofencet [2-(4-chlorphenyl)-3-ethyl-2,5-dihydro-5-oxopyridazine-4-karboxylate, potassium salt]; C13H10N2O3ClK (Fig. 1).
Clofencet is a systemic product which is translocated in wheat from leaves to flowers. It is in accordance with development of anthers when it sterilizes pollen grains (Fichet and Adams, 1996).
The experiments in both years were established in the fields of the Agricultural Research Institute Kroměříž, Ltd. which is located in the mild dry area of the warmer sugar beet-growing region with mild winter (a long-term annual sum of rainfall is 599 mm and average daily temperature is 8.7 °C). The altitude of experimental fields is 210 to 220 m. The soil is Luvi-haplic Chernozem with pH 6.1- 6.8.

Fig. 1: Structural chemical formula of clofencet



A) The experiment "Evaluation of wheat parents responses to Genesis application" was conducted to test selected varieties and lines of winter wheat and their reaction to three doses of the gametocide Genesis (1.6 - 3.6 - 4.9 kg.ha^{-1}) in combination with a surfactant (MON 8161, 1 % c.v.) and a humactant (MON 8165, 2 % c.v.) in comparison with the untreated check. Water carrier volume (c.v.) was 500 l.ha-1.The details of the experimental layout have been reported in a previous paper (Nesvadba, Vyhnánek, 2001).
B) The experiment "Top cross of triticale" was conducted to produce hybrid triticale seed in five varieties and line in one block with one male component for subsequent yield trials. Hexaploid winter triticale (2n=42) was used for this experiment. The varieties Chrono (POL), Kolor (CZE), Presto (POL), Tornado (POL) and line KM 779 from Agricultural Research Institut Kroměříž, Ltd. were the female components. The German variety Modus was used as male parent. The details of the experimental layout have been described in a paper (Vyhnánek, Bednář, 1999).

Results and discussion

In our experiments we tested the efficacy of the chemical hybridizing agent Genesis for induction of male sterility and subsequent crosses of female and male components on selected varieties and lines of winter wheat. Based on the obtained results, it is possible to conclude that the Genesis dose of 4.9 kg.ha-1 appeared sufficient to induce nearly 100% (99.5%) male sterility of female plants under investigated soil and climatic conditions in the Czech Republic.
Evaluating the yield of tested wheat parental components, significant differences were found between individual variants of treatment. If the percentages of sterility and hybrids using the technique of bagging were assessed, significant differences in all doses in relation to the untreated check and significant differences between the dose of 1.6 kg.ha^{-1} Genesis to both increased doses were calculated. However, insignificant differences were found between the dose of 3.6 and 4.9 kg.ha^{-1} Genesis (Tab. 1).

Tab. 1: Analysis of variance - estimation of feasibility of female components after the application of Genesis *technique - bagging*

Source of variation	Number of degrees of freedom (df)	Variance (MS)		
		yield	% sterility	% hybrid seeds
Year (A)	1	10.55**	1.31	0.41
Dose (B)	3	493.44**	147483.15**	141292.03**
Variety (C)	19	12.44**	25.31**	35.27**
A x B	3	10.13**	6.18	6.73
A x C	19	2.15**	31.77**	60.96**
B x C	57	1.71**	12.10	20.96
Residual	217	0.77	8.80	17.31

Individual wheat genotypes were differentiated by means of prolamin storage proteins and used for the assessment of the percentage of hybrid seed when their codominant inheritance was used. The doses of 1.6 and 3.6 kg.ha^{-1} displayed the efficacy on the average of two years from 41.3 to 56.3 % depending on genotypic specificity. If the percentage of hybrids was assessed by electrophoresis of storage proteins, significant differences were also calculated between the dose of 4.9 and 3.6 kg.ha^{-1} Genesis (Tab. 2).
At the heading stage, pollen grains were present in anthers of wheat plants that had been treated with the dose of 4.9 kg.ha^{-1} Genesis. The anthers were reduced in size and green-yellow in colour in comparison with the untreated check. There was only an exine in the pollen grain. Based on visual evaluation of treated spikes, it can be assumed that pollen grains are formed even after Genesis application, however, they are not shed out of anthers at the flowering stage and are not able to pollinate either.
The prolamine spectra of grain of all the analysed triticale parental genotypes are homogenous, with the exception of variety Tornado and the line KM 779 which are heeterogenous in terms of prolamine. These genotypes consist of two sister prolamine lines (identity index (ii) 0.88 (Tornado) and 0.69 (KM 779)).

Tab. 2: Effect of selected female genotypes on examined characters

Genotype	Yield (t.ha^{-1}) 1)	% sterility 2)	% hybrid seeds2)	% hybrid seeds3)
Hana	3.76 a	99.8 a	99.7 a	60.4
Astella	6.21 b	99.5 a	98.8 a	68.9

1) mean of 16 assessments – doses of 0, 1.6, 3.6 and 4.9 kg.ha^{-1} Genesis
2) mean of 12 assessments - technique of bagging – doses of 1.6, 3.6 and 4.9 kg.ha^{-1} Genesis
3) mean of 6 assessments – technique of electrophoresis – doses of 1.6, 3.6 and 4.9 kg.ha^{-1} Genesis
a, b – homogeneous groups, significance at P > 95%

Specific female and male bands of the prolamine spectrum were found in the F1 generation of triticale. Their expression shows that the dose 4,9kg.ha^{-1} Genesis, mode and date of application was 100% efficient. Only in the hybrid combination Chrono x Modus we found the lower efficacy (83.3%) (Tab. 3).

Tab. 3: Efficiency of the CHA Genesis gametocide in the top-cross of triticale
% cross-pollination

female \ male		MODUS
CHRONO		83.3
KOLOR		100
PRESTO		100
TORNADO	linie „A“ – 80% linie „B“ – 20%	100
KM 779	linie „A“ – 80% linie „B“ – 20%	100

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VARIATION IN THE HMW GLUTENIN SUBUNIT ALLELS IN WORDS WHEAT CULTIVARS AND IN SLOVAK WHEAT VARIETIES

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Summary

Method of SDS-PAGE was used for separation, detection and evaluation of the high molecular weight glutenin subunits (HMW) in the different wheat varieties. The standard reference method by ISTA organization was applied for identification and characterization of wheat varieties. ISTA method is very convenient for identification purposes of wheat varieties.
The relation has been studied between the HMW glutenin subunit alleles and the bread-making quality of 25 words wheat cultivars and 18 regional varieties common wheat varieties (*Triticum aestivum L.).* The differences in the variation of the HMW glutenin subunit alleles in words wheat cultivars and in Slovak wheat varieties especially in the presence of alleles at the Glu-A1 and Glu-B1 locus were found. The verified correlations between bread-making quality and specific HMW subunits of glutenin can be taken advantageous to wheat breeders, using SDS-PAGE of proteins as a screening test for the prediction of bread-making quality of wheat.

Key words: *genetic marker, storage proteins, HMW glutenin subunits, SDS-PAGE, Triticum aestivum L.*

Introduction

The glutenin proteins of wheat, particularly the highly aggregative glutenin fraction strongly influence the breadmaking properties of flours. There is the significant relationship between the presence or the absence of some specific high molecular weight (HMW) glutenin subunits and breadmaking quality of wheat varieties. The most important subunits are 5+10 and 2+12 which are expressed from the Glu - D1 loci. Method of SDS-PAGE is possible to apply for separation, detection and evaluation of HMW glutenin subunits in wheat (*Triticum aestivum L*).
The aim of our present contribution was to compared the variation in the HMW glutenin subunit allels in words wheat cultivars and in Slovak wheat varieties (*Triticum aestivum L*.).

Material and methods

The relation has been studied between the HMW glutenin subunit allels and the bread - making quality of different wheat varieties. 25 words wheat cultivars and 18 regional varieties common wheat varieties (*Triticum aestivum L*.) has been analysed. Samples were obtained from the Slovak Republic Genbank and from the Czech Republic Genbank. Cultivars Neepawa, Chinese spring and Marquist were used as standards.
The standard vertical discontinuous electrophoretic reference method by ISTA organization was applied for identification and characterization of wheat varieties (Wrigley, 1992). Glutenin wheat proteins were extracted from individually ground kernels and separated on 10% polyacrylamide gels in the presence of SDS. The gels were stained with Coomassie Brilliant Blue solution and destained with de-ionized destilled water. The stained bands were qualified by scanning densitometry (LD-01 Instrument). The HMW subunits of glutenin were designated according to the numbering system of Payne & Lawrence (1983). SDS sedimentation test was determined by Seditester LS 03.

Results and discussion

The storage proteins of hexaploid wheat are important nutritionally but, above all, because of the unique cohesive-elastic properties they bestow on doughs made from wheat flours. The HMW subunits of glutenin are considered to be the most important components with the respect to the baking quality. Correlations have been established between particular HMW glutenin subunits and bread-making quality of wheat [1,2,3,4,5].
From the electophoretic spectra the individual HMW glutenin subunits were determined and the so-called Glu-score was calculated. The results of 25 worlds wheat cultivars showed (Table 1) the high frequency of occurence of HMW glutenin subunits with composition of 2*(74%), 13+16 (37%) and 5+10 (70%). The highest value of Glu-score (10) was achieved by the cultivars Laurel, Super X, Utud G-12 and Pobeda. The average Glu-score was 7.6.
The most common banding patterns in 18 slovak wheat varieties (Table 2) were subunits „zero“ (83%) from the locus Glu-A1, subunits 7+9 (83%) from the locus Glu-B1 and subunits 5+10 (67%), 2+10 (33%) located on the locus Glu-D1. The highest valus of Glu-score (9) was determined in the varieties Ilona, Vlada and Regia and the lowest one (5) in the varieties Iris, Sana, Vala and Sk 3756-1-76. The average value of Glu-score was 6.8. Positive correlations between SDS – sedimentation test and Glu-score were determined.

Secaline block GLD 1B3 (*), marker of bad baking quality, but at the same time a marker of resistence to stem rust of grain, was identified from the gliadin spectra of the five varieties – Fundulea 29, Iris, Lívia, Sana and Sk 3756-1-76. HMW glutenin subunits is possible to use as molecular marker of bread – making quality of wheat. The verified correlations between bread-making quality and specific HMW subunits of glutenin can be taken as advantageous to wheat breeders, using SDS-PAGE of proteins as a screening test for the prediction of bread-making quality of wheat.

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Table 1 Variation in the individual HMW glutenin subunit allelesin worlds wheat cultivars.

Cultivar	Country of origin	HMW subunit of loci			Glu-score	HMW: LMW	SDS-sedimentation test (cm^3)	Nitrogen of proteins (%)
		Glu-A1	Glu-B1	Glu-D1				
Kosava	Jugoslavia	0	7+8	2+12	6	0,1	50	1,98
Turda	Rumania	2*	7+8	5+10	9	0,109	33	1,83
Fundulea	Rumania	0	7+8	2+12	6	0,110	43	1,86
Bezostaja	Russia	2*	7+9	5+10	9	0,226	52	1,88
F-122	China	2*	13+16	5+10	8	0,137	44	1,85
MV-14	Hungary	2*	13+16	4+12	5	0,123	39	1,95
MV-15	Hungary	2*	7+9	5+10	9	0,104	32	1,89
Recitál	France	2*	7+9	5+10	9	0,124	53	1,73
Delta	Poland	0	13+16	2+12	4	0,138	32	1,66
TAM-200	USA	2*	7+9	5+10	9	0,154	55	1,82
Madsen	USA	2*	13+16	5+10	8	0,113	36	2,00
Laurel	China	2*	7+8	5+10	10	0,110	56	1,82
SK-26	Jugoslavia	0	13+16	2+12	4	0,151	44	1,84
Jasen	Bulgaria	2*	7+9	5+10	9	0,139	50	1,82
Super X	Mexico	2*	17+18	5+10	10	0,148	58	1,81
MV-16-85	Hungary	2*	13+16	5+10	8	0,091	38	1,71
MV-08-85	Hungary	2*	7	5+10	8	0,102	30	1,82
NS-62-38	Jugoslavia	2*	13+16	5+10	8	0,166	69	1,90
NS-65-84	Jugoslavia	0	13+16	2+12	4	0,226	52	1,69
Utud G-12	Turkey	2*	17+18	5+10	10	0,134	69	1,84
Utud G-21	Turkey	2*	7+9	5+10	9	0,073	48	1,77
BAU-402	China	2*	13+16	5+10	8	0,120	45	1,80
Pobeda	Bulgaria	2*	7+8	5+10	10	0,208	69	1,99
Zlatostrul	Bulgaria	2*	13+16	5+10	8	0,116	53	1,65
Alba	Poland	2*	7+9	5+10	9	0,072	52	1,81
x					7,6	0,130	47,7	1,83
max.					10	0,226	69,0	2,00
min.					4	0,073	30,0	1,65
ν %					27	30,480	23,1	5,11

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).

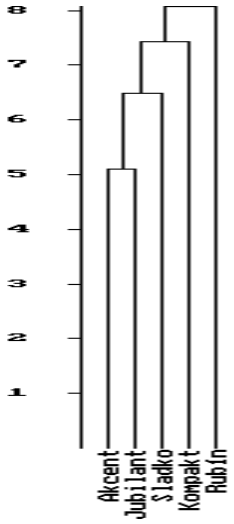
Material and methods

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

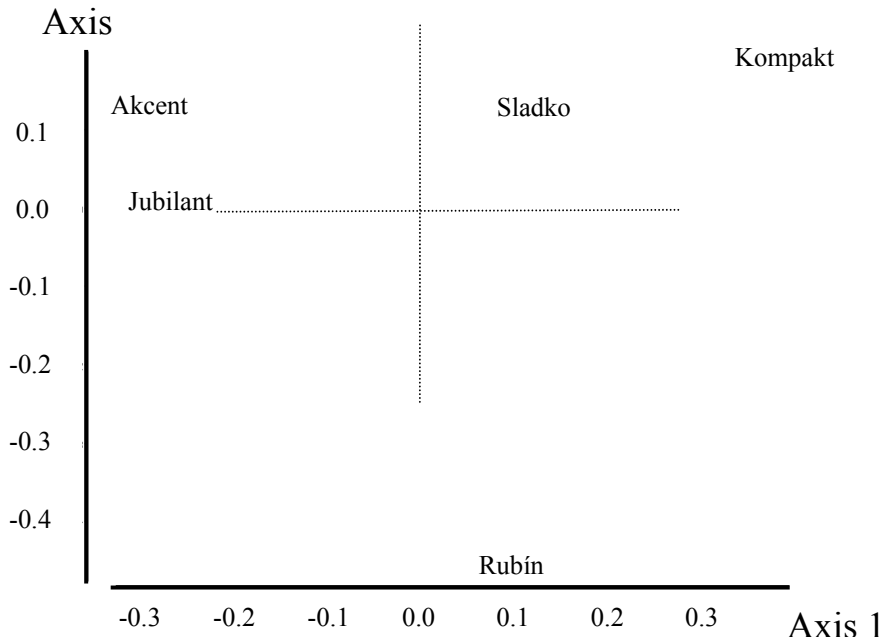


Figure 2 Biplot of evaluated cultivars accounted to component axis 1 and 2

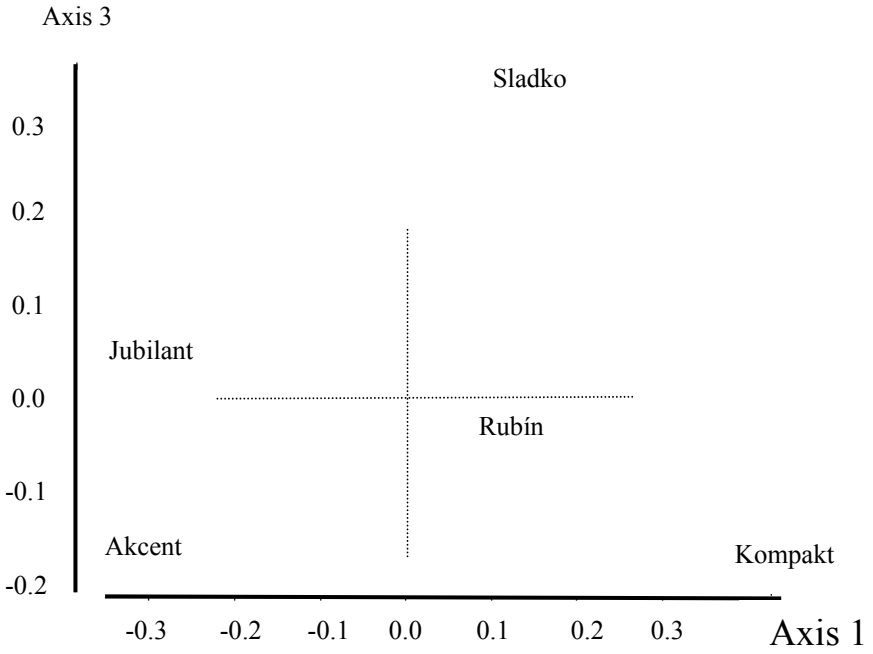


Figure 3 Biplot of evaluated cultivars accounted to component axis 1 and 3

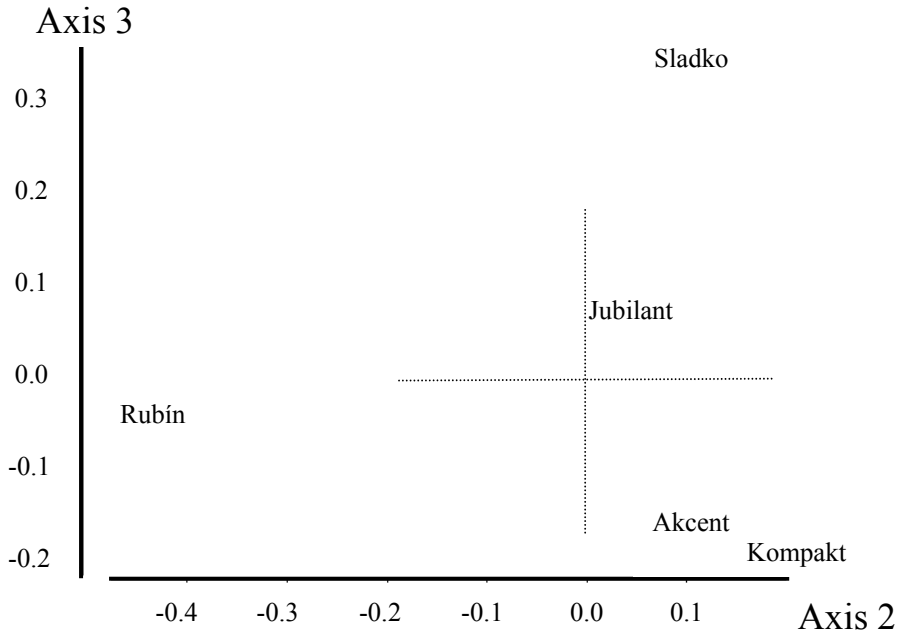


Figure 4 Biplot of evaluated cultivars accounted to component axis 2 and 3

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

of the coat protein and primer S2 (virus sense) 5'-CTG GAT CCA TGC CGC CTA AAC CAG ATC C-3' - coded for amino acids MPPKPDP on 5' end of 42K ORF of PVS RNA.
RNA extraction. RNA was purified from plant material by a modification of the method of Pawlikowski et al. (1994).
Standard RT-PCR was performed according to Barker et al. (1993) using thermocycler MJ Research PTC-150-16. The samples were subjected to 30 cycles of amplification, each consisting of 45 sec. denaturation at 94 °C, 1 min. of primer annealing at 55 °C and 2 min. of primer extension at 72 °C. Aliquots (10 μl) of each reaction were analysed by electrophoresis in 1,5 % agarose gel stained with ethidium bromide.
ELISA was carried out according to Clark, Adams (1977) with using of antibodies purchased from Bioreba.
Sensitivity of standard RT-PCR and ELISA for PVS detection. To determine sensitivity of RT-PCR purified RNA solutions were serially diluted in sterile distilled water. To determine sensitivity of ELISA for detecting PVS in potato plants, infected tissue was tested in tenfold serial dilutions.

Results

Detection of different PVS isolates. Samples were taken from leaves of 14 PVS infected potato genotypes grown in glasshouse and under *in vitro* conditions. PVS was readily detected in all potato genotypes both RT - PCR and ELISA. Using the S1 and S2 primers, the 1061 bp of the PVS RNA was expected to be amplified by PCR. In agarose-gel electrophoresis, a band was detected at about 1000 bp from all PVS isolates.
Sensitivity. The end point of detection with the polyclonal antibodies in ELISA was between $1:10^{-3}$ and $1:10^{-4}$ of leaf sap of all PVS isolates. RT - PCR allowed to detect PVS RNA in dilution $1:10^{-6}$ (Figure 1).

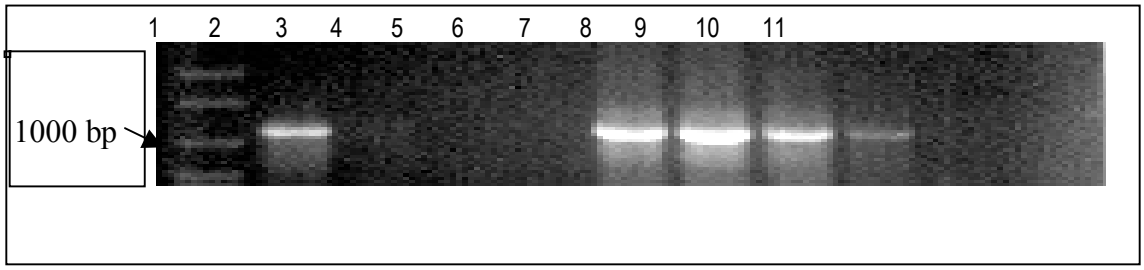

Figure 1. The effect of dilution on RT - PCR of PVS RNA. Lane 1, DNA marker; lane 2, PCR positive control of PVS; lanes 3, 4, 5, virus free *in vitro* plantlets; lane 6 - PVS from single cutting diluted 1:100; lane 7 - PVS from single cutting diluted 1:1000; lane 8 - PVS from single cutting diluted 1:10000, 9 - PVS from single cutting diluted 1:100000; 10 - PVS from single cutting diluted 1:1000000; 11 - water.

Fifty eight meristems were excised from seven genotypes and placed on R medium. The majority of the meristems were differentiated to entire plantlets in four months. Significant differences were noticed related to the genotype response to the heat treatment (VL-11/86 - 25%; VL 60/70 - 100%). Tests for the presence of PVS in regenerants were carried out at two stages: firstly from a single cutting of each regenerated plantlet derived from a meristem tip and secondly from a single cutting of each plantlet grown from a single cutting. Thirty two meristem regenerants were tested by ELISA and RT - PCR. Negative ELISA values were obtained from 34,4% of regenerants whilst virus free status determined by RT - PCR was recorded only in 28,1% of regenerants. The single cuttings of all regenerants were further subcultured and tested again by both methods. ELISA revealed the presence of PVS in regenerants which were in the first tests negative.

Discussion

The procedure described in this paper permits the direct detection of PVS in regenerants derived from meristem tips. Until now direct detection of PVS in regenerants grown under *in vitro* conditions was not adequate reliable therefore PVS is usually detected in plants grown in glasshouse (Rosenberg, 1985; Kotkas, Rosenberg, 1999). Detection procedure of regenerants grown in glasshouse is time demanding and unsuitable for early introduction of advanced clones and new varieties in multiplication under *in vitro* conditions.
An assay for the detection of PVS by use of reverse transcription of RNA followed by amplification of the synthetised cDNA has been evaluated. The sensitivity of the assay for the detection of PVS in the single cutting has been determined to be $1:10^{-6}$, well below the detection limits of ELISA. The PCR based technique described here simplify the methods currently used for detection of PVS in meristem derived plants.

The efficiency of PVS eradication depends on genotype, conditions of thermotherapy and size of excised meristem tip. Failure of ELISA to detect PVS in the first regenerants was probably related to inoculum size which was represented by amount of infected tissue on excised meristem tip. The concentration of PVS in the first regenerants was likely lower than the detection limit of ELISA. However, the size of the meristem played an important role on the differentiation rate and the virus eradication effect.

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INDIRECT REGENERATION OF *HYPERICUM PERFORATUM* L. UNDER *IN VITRO* CONDITIONS

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Summary

Hypericum perforatum L. is a traditional medical plant. Naphtodiantrones are considered to be one of the most important secondary metabolites. There are possibilities to expand genetic variability using modern biotechnology methods. The callus induction was observed on the MS medium supplemented with 2,4- dichlorophenoxyacetic acid (2,4-D). The callus growth is possible to induce with supplementation of cytokinins (kinetin – KIN, 6-benzylaminopurine – BAP) and auxins (2,4-D) to the medium. The best callus growth, from the point of size, was observed on the medium supplemented with 1 mg.l^{-1} KIN + 1 mg.l^{-1} 2,4-D (10 %) and 1 mg.l^{-1} BAP + 1 mg.l^{-1} 2,4-D (12,7 %). The most intensive regeneration (> 20 regenerants from callus) were observed on the medium supplemented with 0,1 mg.l^{-1} indole-3-butyric acid (IBA) + 5 mg.l^{-1} BAP (7,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP + 80 mg.l^{-1} adenin (6,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN (5,6 %) + 80 mg.l^{-1} adenin, 3 (5,1 %) and 5 mg.l^{-1} BAP (4,1 %).

***Key words*:** *Hypericum perforatum*, *in vitro* cultivation, callus formation, regeneration

Introduction

Hypericum perforatum L. is a traditional medicinal plant, which received great attention in recent years (http://www.admin.ch/bbw/abstracts/abstr-99/abstracts/cost/c97.0068.html, 2001). *Hypericum perforatum* L. as well as its extracts are used in the treatment of psychovegetative disorders and minor depressions. These activities are attributed among others to hypericin and compounds similar to hypericin (Biza et al., 1999). Despite of these activities, naphtodiantrones – hypericin and similar compounds – are responsible for anti-viral and anti-retroviral activity (Büter et al., 1998).
It is important to cultivate the genotypes with higher content of desired compounds. The genotype of cultivated plants of *Hypericum perforatum* present a key factor for an economically successful cultivation (Büter et al., 1998).
There is a possibility to utilize the modern biotechnology methods for potential creation of new genotypes, which may have higher content of desired compounds.
The aim of our study was to ulitilze modern biotechnology methods for expanding genetic variability using:

- callogenesis,

- indirect regeneration of *Hypericum perforatum* L. plants via callus cultures.

Material and methods

The seeds of *Hypericum perforatum* L. were obtained from the Regional Research Institute of Agroecology in Michalovce in Slovak Republic.
The plants, grown under *in vitro* conditions on a basal MS medium (Murashige, Skoog, 1962) were the primary explantats for callogenesis. The leaf and stem segments were placed on the MS medium supplemented with 2,4-D (1, 3 and 5 mg.l^{-1}). The calluses were transferred on the medium supplemented with other plant growth regulators (Table 1) after one month of cultivation. The phenotypic variability was evaluated using classification according to Bežo (1995) after one month of cultivation.
The calluses were primary explantats for indirect regeneration. Calluses were transferred on the medium supplemented with plant growth regulators (Table 2) after one month of cultivation on the medium for callus growth. The regeneration was evaluated after one month of cultivation.
Obtained data were processed using statistical program STATGRAPHICS. The tissue cultures were cultivated at 22 – 24 °C, with a 16 hours light photoperiod under 3000 lx irradiance.

Table 1 The content of plant growth regulators supplemented in the medium for callus growth

Medium	Plant growth regulators
1	1 mg.l^{-1} KIN + 1 mg.l^{-1} 2,4-D
2	5 mg.l^{-1} KIN + 5 mg.l^{-1} 2,4-D
3	1 mg.l^{-1} BAP + 1 mg.l^{-1} 2,4-D
4	5 mg.l^{-1} BAP + 5 mg.l^{-1} 2,4-D
5	5 mg.l^{-1} 2,4-D

Table 2 The content of plant growth regulators in the medium for indirect regeneration

Medium	Plant growth regulators	Medium	Plant growth regulators
1	1 mg.l^{-1} KIN	8	1 mg.l^{-1} IBA
2	5 mg.l^{-1} KIN	9	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN
3	1 mg.l^{-1} BAP	10	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP
4	5 mg.l^{-1} BAP	11	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP + 80 mg.l^{-1} adenin
5	0,1 mg.l^{-1} NAA	12	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN + 80 mg.l^{-1} adenin
6	1 mg.l^{-1} NAA	13	Control
7	0,1 mg.l^{-1} IBA		

Abbreviations; 2,4-D – 2,4-dichlorophenoxyacetic acid, BAP – 6-benzylaminopurine, KIN – kinetin, IBA – indole-3-butyric acid, NAA – α-naphtalene acetic acid, MS – Murashige, Skoog (1962) medium

Results

The phenotypic variability of calluses was evaluated. According to the descriptor (Bežo, 1995), the form, consistency, structure, colour, shape, exterior and size of calluses were assessed. The statistical agreement was confirmed between the variants of medium for callogenesis induction and form, consistency, structure and surface of calluses. The statistical agreement was confirmed also between the variants of medium for growth of calluses and form, consistency, structure and surface of calluses. The statistical agreement was not confirmed between colour (χ^2 – 69,76; f – 12; P – 0,00 %), shape (χ^2 – 22,53; f – 6; P – 0,00 %) and size (χ^2 – 43,04; f – 6; P – 0,00 %) of calluses and medium for callogenesis induction. The statistical agreement was not confirmed likewise between colour (χ^2 – 194,85; f – 12; P – 0,00 %), shape (χ^2 – 62,38; f – 8; P – 0,00 %) and size (χ^2 – 160,46; f – 8; P – 0,00 %) of calluses and medium for growth of calluses. The best callus growth, from the point of size, was observed on the medium supplemented with 1 mg.l^{-1} KIN + 1 mg.l^{-1} 2,4-D (10 %) and 1 mg.l^{-1} BAP + 1 mg.l^{-1} 2,4-D (12,7 %).
The indirect regeneration was assessed as a number of regenerants from callus cultures according to scale for classification (Štefúnová, 2000). The most intensive regeneration (> 20 regenerants from callus) were observed on the medium

supplemented with 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP (7,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP + 80 mg.l^{-1} adenin (6,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN (5,6 %) + 80 mg.l^{-1} adenin, 3 (5,1 %) and 5 mg.l^{-1} BAP (4,1 %). The statistical agreement between medium for indirect regeneration and degree of regeneration was not confirmed (χ^2 – 224,04; f – 36; P – 0,00 %).

Discussion

The importance of *Hypericum perforatum* L. has an increased tendency in recent years, especially because of the anti-viral and anti-retroviral activity (Čellárová, 1995).

Yazaki et al. (1990) present the callus initiation of the *Hypericum erectum* on the cultivation medium supplemented with combination of auxin and cytokinin plant growth regulators – IAA (10^{-5} mol.dm^{-3}) + BAP (10^{-5} mol.dm^{-3}). According to Brutovská et al. (1994) it is possible to initiate callus culture of *Hypericum perforatum* L. from the leaf segments on the medium with growth regulators – NAA (0,2 mg.l^{-1}) + KIN (0,2 mg.l^{-1}) + 2,4-D (0,2 mg.l^{-1}). Karting et al. (1996) found callus formation of seven species of the genus *Hypericum* on the medium supplemented with BAP (10^{-6} mol.dm^{-3}) + NAA (10^{-7} mol.dm^{-3}) from the sterile seedlings. Dias et al. (1998) present callus cultures creation from the stem segments.

According to our results it is evident, that the initiation of the callus formation is possible on the medium with 2,4-dichlorophenoxyacetic acid from the leaf or stem segments. The growth of callus cultures was observed on the medium supplemented with auxin and cytokinin (1 : 1); KIN (1 mg.l^{-1}) + 2,4-D (1 mg.l^{-1}), KIN (5 mg.l^{-1}) + 2,4-D (5 mg.l^{-1}), BAP (1 mg.l^{-1}) + 2,4-D (1 mg.l^{-1}), BAP (5 mg.l^{-1}) + 2,4-D (5 mg.l^{-1}). The necrosis of callus cultures were observed on the medium with 2,4-D (5 mg.l^{-1}).

The most intensive regeneration was observed on the medium supplemented with BAP (1 and 5 mg.l^{-1}), IBA (0,1 mg.l^{-1}) + BAP (5 mg.l^{-1}), IBA (0,1 mg.l^{-1}) + BAP (5 mg.l^{-1}) + adenin (80 mg.l^{-1}) and IBA (0,1 mg.l^{-1}) + KIN (5 mg.l^{-1}) + adenin (80 mg.l^{-1}). Čellárová (1997) found indirect regeneration from the green compact calluses on the medium with variable concentration of BAP.

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EVALUATION OF COMMON FLAX (*LINUM USITATISSIMUM)* GENEPOOL VARIABILITY FOR ANOTHER UTILIZATION AND CONSERVATION IN SLOVAKIA.

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Summary

A research program GENOFOND on SAU in Nitra, besides other things is provided collection, evaluation and long-term preservation of linen genetic resources. At the first stage realized experiments are provided basic evaluation, classification and cataloguing of 144 collected genotypes of linen. Also the descriptor list is innovated for complete classification. We are provided, besides descriptive and numeral evaluated characters also image documentation of specific characters of genotypes on the level of leaves, flowers, bolls and seeds. Large-scale of descriptive, numeral and picture data are gradually processing in created an information system for evaluation and evidence of genetic resources – GENOTYPEDATA – LINUM.

Key words: flax, evaluation, conservation of genetic resources, list of descriptors

Introduction

The conservation and protection of plant biodiverzity has the historical and current importance in all parts of human life values. The man is realizing an irredeemable and unique reality of a genetic resource, which was created during the nature and life generation process. Living space for natural diverzity evaluation of species is strongly limited when only small number of plant species (mostly monoculture) is planted and spread on expanded fields. Genepool of cultivated plants represented important natural and cultural heritage each country. It has high economic value as an initial genetic material on current and future needs of plant breeding. Therefore, in the process of conservation the evaluation and classification of particular cultivated plants on the level of all available genetic forms have their irredeemable place. First of all, the evaluation is provided description and determination basic economic values of collected material. Also, acquired passport and descriptor information expand and increase possibilities of evaluated genetic resources utilization for the needs of plant breeder and grower.
Submitted contribution is oriented on evaluation of flax genepool. Flax from the historical aspect is one of the oldest cultivated plants of oil and fiber origin. It is one of the first crop which a man started to utilized and cultivated for his needs at the normal life. Current utilization of flax is in these areas: textile industry, producing of body car, building industry, paper industry, also producing of varnish, linoleum, lacquer, paint. Flax is also important for medicine, nutrition and food industry. Signs and characters known variability of wild and semi-cultural forms, and breed cultivars will provide new knowledge. This new information will possible to use on better flax utilization for technical, food and other use.

Materials and methods

Solving of the evaluation and classification problem of selected flax genotypes has taken a part in project "Rescue and protection of endangered plant genepool in Slovakia" in year 2000 coordinated by Slovak Agriculture University in Nitra.
The main aim solving this problem is oriented in:

1. Basic valuation of economic values of flax genotypes in collected collection.
2. Descriptor list innovation on evaluation of signs and characters, and basic characterization of flax genetic resources.
3. Development and creation of specialized database on evaluation and evidence of flax genetic resources in GENOTYPDATA system.

We got the collection of evaluated flax genotypes from Slovak National Gene Bank which is located in Piešťany. It consists of X11 common flax – lines, X12 common flax – land races, X13 common flax. Biological material is cultivated and tested at the Breeding Station in Vígľaš – Pstruša. Chosen region has suitable climate, soil composition for cultivating flax plants.
Plant material of 144 flax genotypes we evaluated in the first year of our experiment at 28 selected descriptors of morphological signs and characters. List of descriptors and passport data selected for evaluation of biological material have come out from international lists of descriptors: UPOV (1980, 1991, 1995), VIR (1989), from list of descriptors made in Šumperk (Pavelek, Faberová, 2000), from list of descriptors for flax (Rosenberg, Trnka, Procházdka, 1978), and from suggested list of descriptors for international flax database – IFDB (1993, 1994). List of descriptor innovation is parallel with the process of selected flax genetic resources evaluation.

Results

The first collection of flax genotypes was completely evaluated in the first term of solving the problem in year 2000. Collection was evaluated during the vegetation period and also on the level of individual analysis.

During the vegetation period individual genetic resources were evaluated at these characters: size, shape and color of leaf, size of corolla, color of petals, length, termination and shape of petal leaves, length, termination, shape and color sepal leaves.
After harvesting these morphological and crop yield signs and characters were evaluated: total length, technical length, branching, number of branches, branching at the base, thickness of stem, size, shape of boll and shape at the base of boll, total number of bolls per plant, color, shape of seed, weight per 1.000 seeds and number of seeds per boll.
Acquired experimental data, which are gradually processed by suitable mathematical-statistical methods, mentioned on high variability of evaluated signs. This fact is also presented in the table 1.

Table 1: Flax genepool variability of selected evaluated signs

	Total length of plant (mm)	Weight per 1.000 seeds (g)	Weight of seeds per plant (g)	Number of seeds per plant	Number of bolls per plant	Number of seeds per boll
AVERAGE	40,8	5,83	0,21	37,63	9,11	4,19
MINIMUM	22	4,57	0,001	1	2	0,12
MAXIMUM	71	7,94	1,54	203	46	9,88
VARIATIONAL INTERVAL	49	3,37	1,53	202	44	9,76

Experimental data received during the process of classification and cataloguing we have utilized for innovation of descriptor list. First of all we have concentrated to the quantification of individual signs, and it will help us with better characterization of signs. We have compared known descriptors lists in the process of creation innovated descriptor list. Summary design is presented at the table 2.

Table 2: Evaluation of variability signs and characters of species *Linum spp.* according to selected list of descriptors.

	LISTS OF DESCRIPTORS			
SIGN	UPOV	ŠUMPERK 2000	ROSENBERG	INNOVATED
MORPHOLOGICAL SIGNS				
Stem:				
Total length	x	X	x	xp
Technical length	x	X	x	xp
Branching	o	X	x	xi
Number of branches	o	X	x	xi
Branching on the base	o	x	x	xp
State of top	o	x	x	xp
Leafage	o	o	x	xn
Thickness	o	x	x	xp
Thickness of hypocotyl	o	o	x	xn
State of bracts according to branching	o	o	x	xn
State of bracts in the space	o	o	x	xn
Cotyledons:				
Shape	o	x	x	xp
Size	o	x	x	xp
Leaf:				
Shape	o	x	x	xp
Size	o	x	x	xi
Color	o	x	x	xi
Anthocyan coloring	o	x	x	xp
Surface of leaf	o	x	x	xn

table continues at the next page

Flower:				
Size of corolla	x	x	x	xi
Shape of corolla in vertical section	o	x	x	xn
Shape of corolla in horizontal section	o	x	x	xn
Flower petals:				
Color – before blooming	x	o	o	xn
Color in time of full blooming	x	x	x	xi
Length	o	x	x	xi
Shape (length : width)	o	x	x	xp
Termination of petals	o	x	x	xp
Shape of petal edge	o	o	x	xn
Form of petals	o	o	x	xn
Color of petal nerves	o	x	x	xi
Longitudinal folding	x	o	o	on
Sepal:				
Size of sepal leaves	o	x	x	xi
Termination of sepal leaves	o	x	x	xp
Shape of sepal leaves	o	x	x	xp
Surface of sepal leaves	o	o	x	xn
Color	o	x	x	xi
Dotting of sepal	x	x	x	xn
Composition of population according to dotting	o	o	x	xn
Sexual organs:				
Position	o	x	x	xn
Color of style	x	o	x	xn
Shape of stigma	o	o	x	xn
Color of stigma	o	x	x	xn
Color of stamen	x	x	x	xn
Color of anther	x	o	o	on
Color of pollen grain	o	o	x	on
Boll:				
Shaper	o	x	x	xp
Shape of base	o	x	x	xp
Size of boll	x	x	x	xp
Surface	o	o	x	xn
Dehiscence	o	x	x	xp
Anthocyan coloring of boll	o	x	x	xn
Intensity of ciliation	o	o	x	xn
Ciliation of septa	x	x	x	xn
Length of ciliation	o	o	x	xn
Width of septa	o	o	x	xn
Width of boll wall	o	o	x	xn
Number of bolls per plant	o	x	o	xp
Seed:				
Color of seed	x	x	x	xi
Shape of seed from side	o	o	x	xn
Shape (width : length)	o	x	x	xp
Weight of 1.000 seeds	x	x	x	xp
Number of seeds per boll	o	x	x	xp
Edge of seed	o	o	x	xn

Abbreviations:
Sign is presented in the list **(x)**
Signs is not presented in the list **(o)**
Innovation of sign **(i)**
New included sign **(n)**
Original, evaluated sign **(p)**

Development and processing of complex system on evaluation and evidence of genetic resources was supplied by modern information and communication technology – video camera set for system SVHS. At the first phase was provided video

documentation on the level of leaf, corolla, petal, boll and seed. Completion and processing of these video records is still in processing. In the second year we are planed to sow and evaluate another 121 new genotypes of flax. These genotypes were provided by AGRITEC Šumperk – Temenice Ltd.

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UPOV: Guadlines for the conduct of tests for distinctness, uniformity and stability, TWA/20/5 flax, Geneve, International Union for the Protection of New Varieties of Plnats, 1991.
UPOV: Guadlines for the conduct of tests for distinctness, uniformity and stability, TG/57/6 flax, Geneve, International Union for the Protection of New Varieties of Plnats, 1995.

EVALUATION OF SOME QUANTITATIVE TRAITS OF INTERGENERIC HYBRIDS TRITICUM AESTIVUM L. WITH TRITICUM SPELTA L. IN F1 GENERATION

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Summary

With aim to induce genetic variability of *Triticum aestivum* L. by intergeneric hybridization with *Triticum spelta* L. was realized complete dialel crossing of two cultivars of *T. aestivum* "Zdar", "Estica" with *T. spelta* cultivar "Renval". Biological material of parents and hybrids of F1 generation was cultivated in field conditions and analyzed for following quantitative traits: number of productive spikes per plant, length of main stem (mm), number of spikelets per spike, number of grains in main spike and weight of thousand grains (g). Hybrids were evaluated from aspect of real heterosis and hypothetical heterosis. Real effect of heterosis was find out in weight of grain in main spike (18,5 %) and hypothetical effect of heterosis (22,08%) in hybrid combination "Zdar" x "Renval". In weight of thousand grains was find out real heterosis in hybrid combination "Estica" x "Renval" (10,9%) and "Zdar" x "Renval" (6,98%). In both combinations was manifested hypothetical heterosis at the level of 16,76% and 18,88%.

Keywords: *Triticum aestivum* L., *Triticum spelta* L., intergeneric hybridization, quantitative traits, real heterosis, hypothetical heterosis

Introduction

Triticum spelta belongs to the oldest hexaploid forms of wheat. At present is considered relict species. In the history was very common its presence in stands of *Triticum aestivum*. Both species are hexaploid. Diversity of *Triticum spelta* in morphological traits is confirmed by 54 up to now known varieties. There are winter and spring forms with awned and awn-free thin spike. Spike spindle is brittle.

At present is *T. spelta* cultivated mostly in extensive conditions in higher, more rough areas of Austria, Switzerland, Germany, Belgium and in the north of Spain (Astoria region).
Archeological research in the Slovak territory revealed (Hajnalova 1999) at least 18 localities in western, eastern but mostly northern Slovakia from period since neolit till middle age (15 - 16 century). Discoveries of carbonated grains and other spike parts confirmed presence of *T. spelta* in Slovak territory for thousands years. Importance of *T. spelta* as cultivated cereal was not always the same. According archeological research it is supposed, that in the north of Slovakia, where it was the main crop its cultivation was estimated not longer than 700 years and less in other areas.
T. spelta due to its modesty, flexibility and good nutritional values is starting to be object of increased interest for direct economical utilization. It has many favorable medicinal properties, which are used in alternative medicine, treatment against allergy, high content of cholesterol, prevention to depression, cancer and rheumatic diseases.
T. spelta could be a usable genetic resource in breeding of *T. aestivum*. According other authors is known that some forms mostly wild and landraces contain in the grain 26% of rough protein. In breeding cultivars of *T. spelta* is content lower but significantly higher then in majority cultivars of *T. aestivum*.
Since the last decade were carried out field experiments at the Department of Agricultural systems at the Slovak Agricultural University in Nitra. Their aim was to evaluate possibilities for cultivation of *T. spelta* in specific field and climatic conditions in ecological system of production and to test group of cultivars according qualitative traits and mineral composition of grain (Lacko-Bartošová et al. 1997, Lacko-Bartošová et al. 1999). At the Department of Genetics and Plant breeding in Faculty of Agronomy at the Slovak Agricultural University in Nitra is with minimal financial support solved problem of genetic variability induction of *T. aestivum* by distant hybridization with *T. spelta* for present agroecological cultivation systems.

Material and methods

There was carried out complete dialel crossing of two *T. aestivum* cultivars ("Zdar" and "Estica") and *T. spelta* ("Renval") with aim to induce genetic variability. The cross was made by traditional method in field conditions. Biological material of F1 generation and parental components were cultivated in field at experimental base in Center of biology and plant ecology Faculty of Agronomy in the Slovak Agricultural University in Nitra locality Dolna Malanta. Acquired biological material was analyzed for following traits: number of productive spikes per plant, length of main stem (mm), number of spikelets per spike, number of grains in main spike and weight of thousand grains (g). Analyzed biological material of parental components and their hybrids in F1 generation was evaluated from aspect of calculation:

a) Real heterosis expressed by overcame of F1 generation in percentage for evaluated traits in comparison to better parent.
b) Hypothetical heterosis expressed by overcame of value of F1 generation in percentage to average values of both parents (Petrovic, Bezo 1989).

Results and discussion

Number of productive spikes per plant: In parental components is number of productive spikes per plant from 3 to 4 (“Zdar” and “Renval”) to 5 (“Estica”). The lowest number (2,5) of productive spikes were observed in hybrid combination “Zdar” x “Renval”. In this trait was not discovered real or hypothetical heterosis.
Length of main stem: The values varied in the range from 600 mm (“Estica”) to 1022 mm for *T. spelta* cultivar “Renval”. There was not observed hybrid combination with higher manifestation of trait then higher parent. The lowest was combination (“Estica” x “Estica”) 490 mm. The average values of this trait in hybrid combination were in most cases between values of parental components. There was not discovered real or hypothetical heterosis.
Length of main spike: Average length of main spike varied in studied material in range from 915 mm (“Estica”) to 153,5 mm (*T. spelta* “Renval”). There was not discovered real or hypothetical heterosis.
Number of spikelets per spike: In observed genotypes of parents and their hybrid combinations the highest number of spikelets per spike was find out in cultivar “Estica” (22,8) and the lowest in hybrid combination “Zdar” x “Renval” (18). There was not discovered real or hypothetical heterosis.
Number of grains in main spike: The highest number of grains per spike had cultivar “Estica” (71), the lowest *T. spelta* cultivar “Renval” (49,6). We did not find any combination with higher number of grains than had parents. On the other hand hybrid combination “Estica” x “Zdar” had the lowest number of grains (31). There was not found demonstration of heterosis.
Weight of grains in main spike: The highest weight of grains in main spike of parental components was observed in cultivar “Estica” (3g). The lowest in *T. spelta* “Renval” (2,32 g). In combination “Zdar” x “Renval” was discovered effect of real heterosis at level of 18,5 % and hypothetical heterosis at level 22,08 %.
Weight of thousand grains: In observed genotypes are values of this trait at the range from 39,29 g “Zdar” to 46,42 g *T. spelta* “Renval”. The real heterosis was find out in hybrid combination “Estica” x “Renval” at level of 10,9 % and “Zdar” x “Renval” 6,98 %. Counted hypothetical heterosis in this two hybrid combinations was 16,76 % and 15,88 %.
Extreme climatic conditions in experimental year 1999/2000, mostly spring 2000, significantly influenced values of investigated quantitative traits. In spite of extreme drought the highest weight of thousand grains was find out in *T. spelta*

"Renval". This finding corresponds with data of Vlasak (1995), Lacko-Bartošová, Antala (?...), Lacko-Bartošová et al. (1999). Cultivar "Renval" had in majority of hybrid combinations in F1 generation positive influence on values of experimental traits.

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The paper was created in the frame of institutional scientific research project: „Possibilities of application intergeneric hybridization for creation of genotypes in agroecological cultivation systems" solved at the Faculty of Agronomy in the Slovak Agricultural University in Nitra.

POSSIBILITIES OF SYNTHETIC AMPHIPLOID USE FOR ENLARGEMENT OF GENETIC DIVERSITY IN WHEAT

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Summary

The world-wide gene pool of common wheat is descended from a very small number of spontaneous interspecific hybrids, which originated as a result of two natural amphiploidisation events. In the more recent event, plant(s) of emmer wheat (*Triticum dicoccoides*), which were cultivated at the time by early Neolithic farmers, were fertilised by weedy diploid goatgrass (*Aegilops tauschii*), producing primitive common wheat. Because of the rarity of this event, today's common wheat (*T. aestivum*) has extremely low levels of polymorphism at enzyme, storage protein, and DNA marker loci, compared with its parent species, especially *Ae. tauschii*. In fact, the bulk of evolutionary evidence suggests that common wheat began its existence as a highly monomorphic species and that its genetic variation was reduced further by domestication. Despite its narrow genetic base, human-guided evolution has produced a profusion of distinct landraces over a period of 5 or more millennia, and modern breeding has maintained steady genetic improvement throughout the current century. Interspecific hybridisation has shown to be a useful tool in the breeding of cultivated species of *Triticeae* tribe. This technique has been mainly used for transferring some interesting characters, such as resistance to biotic and abiotic stresses and other traits of agronomic interest. We present an overall survey of amphiploids created with the utilisation of wheat genomes. Potential uses of synthetic amphiploids to introgress genetic material into *Triticeae* species and amphiploidisation to expand the wheat gene pool are discussed.

Key words: amphiploid, synthetic, wheat, triticale, tritordeum, tritinaldia, agrotana, agrotricum, diversity, gene pool

Wild forms from regions of their natural occurrence are still an undervalued source of the genetic diversity, which will have to be incorporated into the present breeding programmes and used in agriculture. The increasing requirements for the performance of wheat varieties make the breeders use a limited range of approved parental forms (varieties) for hybridisation. The result is an increasing proportion of genetic similarities of the newly formed varieties, which has a negative impact on their longevity. In pure cultures, where the diversity of resistance genes is limited, we see a rapid selection of new virulent pathotypes of fungal diseases, which could soon overcome the resistance of their hosts. This is the reason why the search for new donors of resistance against biotic and abiotic stress factors, which would be potentially effective for a long time, is so urgent. The use of the so-called "non-host resistance", which occurs in some genetically distant wild species, has lately been frequently mentioned.
During the evolution of the *Triticeae* tribe, which includes *Triticum, Hordeum* and *Secale*, i.e. genera most frequently used in agriculture, spontaneous amphiploidy played an important role. Induced amphiploidy allows building up completely different genomes into the common organism. Table 1 presents a survey of amphiploidies.

Synthetic wheat:
Ae. tauschii (2n = 2x = 14,DD) is a generally acknowledged donor of the D genome of hexaploid wheat with a high degree of homology, even though the present study of genetic diversity within the framework of *Ae. tauschii* showed that the D genome of wheat could be of polyphyletic origin. Some authors also maintain that amphiploid hexaploid wheat with the AABBDD genome is not a product of unique hybridisation, but that its origin could occur more times through recurrent hybridisation of various tetraploid and diploid parents. Within the *Ae. tauschii* species a high degree of variability in many characters and properties can be assumed, which could also be the source of desirable genes of resistance to fungal diseases of wheat. Successful translocation of resistance to diseases and pests and resistance to stress have been recorded and *Ae. tauschii* is also considered to be the source of new alleles for storage proteins of genes localised on the 1D chromosomes. There is a sufficient degree of recombinant interchanges between the D genomes of *Ae. tauschii* and *T. aestivum*, made possible by pairing the chromosomes in meiosis, which facilitate the translocation of required properties from the diploid donor species into the genome of cultivated wheat. Therefore, there are no problems, such as can be seen in products of wide hybridisation of species with unrelated and also related, but non-homologous genomes.

Table 1 – Survey of some amphiploidy forms produced using wheat

Name		Type of hybridisation	Genomes (2n)	Reference
synthetic wheat		(*Triticum turgidum* conv. *durum*, *T. carthlicum*) x *Aegilops tauchii*	AABBDD	Mujeeb et al. (1996, 1997)
		(*T. boeoticum*, *T. monococcum*, *T. uraru*) x *T. turgidum* conv. *durum*	AAAABB	Ma et al. (1997)
other synthetic forms		*T. durum* x *Ae. caudata*	AABBCC	Aghaee-Saebarzeh et al. (2000)
		T. durum x *Ae. umbellulata*	AABBUU	
		(*T. turgidum*, *T. aestivum*) x *Ae. variabilis*	AABBUUSS, AABBDDUUSS	William, Mujeeb (1996)
		Ae. tauchii x *Agropyron cristatum*	DDPP	Martin et al. (1998)
triticale	*xTriticosecale* Wittmack	*Triticum* spp. x *Secale cereale* L.	AARR, AABBRR, AABBDDRR	extensive literature
tritordeum	*xTritordeum* Ascherson et Graebner	*Triticum* spp. x *Hordeum chilense* Roemer et Schultese	AABBH^{ch}H^{ch}, AABBDDH^{ch}H^{ch}	Martin, Cubero (1981)
tritinaldia		(*T. turgidum*, *T. aestivum*) x *Haynaldia villosa* Schur (*Dasypyrum villosum* L.) (synthetic amphiploid)	AABBVV, AABBDDVV	Pace. et al. (1985); Yao-JingXia et al. (1995)
	xHaynaldoticum sardoum Meletti et Onnis	*T.* spp x *Haynaldia villosa* Schur (*Dasypyrum villosum* L.) (spontaneous amphiploid)	AABBVV	Meletti et al. (1996)
agrotana		*T. aestivum* x (*T. bessarabicum* /*Elymus farctus* subsp. *bessarabicus* or *Thinopyrum ponticum* /*E. elongatus*/)	AABBDDJJ	Chen-Quin et al. (1995)
agrotricum (wheat x wheatgrass)		*T. aestivum* x *Thinopyrum intermedium* (*Agropyrum intermedium*, *Elymus hyspidus*)	AABBDDEE$^{+)}$	Chen-Quin et al. (1999); Liu-Bao et al. (1999)
		T. aestivum x *Thinopyrum ponticum* (*Lolopyrum elongatum* /Host/ A. Love, *Elymus elongatus*)	AABBDDEE$^{+)}$	Colmer et al. (1995); Kasai et al. (1998); Chen-Quin et al. (1998)
		T. aestivum x *Elymus sibiricus*	AABBDDEE$^{+)}$	Motsnyi et al. (2000)
		Triticum aestivum x *Thinopyrum intermediatum* (*Elymus hyspidus*)	AABBDDE^{1}E^{1} or AABBDDE^{2}E^{2}	Gao-Zhi et al. (1998); Limin et al. (1995)
(wheat x lymegrass)		(*T. aestivum*, *T. carthlicum*) x (*Leymus arenarius*, *L. mollis*)	hexaploid, octoploid	Anamthawat-Jonsson (1996)
		T. spp. x *Leymus mollis*	hexaploid	Anamthawat-Jonsson (1999)

$^{+)}$ Amphiploid forms differ in their genomes due to different species of the *Elymus* genus.

Under conditions of Central Europe it is necessary to consider its use particularly for creating sources of resistance to *Puccinia recondita* f.sp. *tritici* and to powdery mildew *Blumeria graminis* (DC) Speer f.sp.tritici (= *Erysiphe graminis* (DC) f.sp. *tritici* Marchal).
Translocation of resistance to diseases and pests and resistance to stress have been successful and *Ae. tauschii* is considered to be the source of new alleles for storage proteins of genes localised on the ID chromosomes.

Triticale

In 1875, Wilson described the first spontaneously formed sterile form. Rimpau obtained the first fertile triticale amphiploid in 1888. After the production of the first triticale variety in 1968, it was the object of keen breeding attention leading to the application of many varieties in practice. The presence of the rye genome is responsible for the specific properties of the crop, particularly its potential use in poorer conditions of growing and worse feeding quality of the grain. Great attention has recently been devoted to the possible improvement of some triticale properties by means of purposefully created amphiploids and induction of translocations. A good example is e.g. to transfer a segment of chromosome 1D from bread wheat possessing the Glu D1 (5+10) allele to hexaploid triticale through a centric break-fusion using 5D/5B translocation conditioned by the absence of the *Ph* gene (Lukaszewski, Curtis, 1992, 1994). That enabled to obtain the recombinant chromosome 1R having a small segment of 1D with the glutenin allele Glu D1 (5+10). Two types of translocation of 1R chromosome, 1R.1D5+10 differing in a size of transferred segment of chromosome from 1D were obtained. They were designated by symbols 1R.1D5+10-1 and 1R.1D5+10-2 (1 and 2 indicate these translocations differing in the length of the 1D chromosome segment). Both described translocation chromosomes in hexaploid triticale with Glu D1 (5+10) lead to a considerable increase in bread volume and breadmaking quality of triticale (personal communication, Lukaszewski, 2000).

Tritordeum

The production of tritordeum, created in Cordoba in Spain where it is intensively studied and bred, is considered to be a significant success in the area of genetics. According to literary data increasing the yield potential has been progressing in the present forms. Tritordeum is also a potential source for the translocation of some important properties of barley to wheat and, in some cases, also into other cereal species. *Hordeum chilense* is resistant to rust, especially to leaf rust (*P. recondita* f.sp*. tritici, Blumeria graminis /= Erysiphe graminis/),* septoria leaf blotch *(Septoria tritici /Mycosphaerella graminicola/),* common bunt (*Tilletia caries*), loose smut of barley (*Ustilago nuda*), loose smut of wheat (*U. tritici*), net blotch (*Pyrenophora teres)* and scald (*Rhynchosporium secalis*). It is also considered to be a source of resistance to some insect pests, i.e. *Diuraphis noxia, Rhopalosiphum padi* and *Schizaphis graminum*, and also to *Meloidogyne* spp.
The existence of a wide genetic variability of tritordeum resistance to diseases of the ear (*F. culmorum, F. graminearum [Gibberella zeae], Stagonospora nodorum*) is the prerequisite for the finding of resistance donors, which could be applied in wheat breeding. Hexaploid tritordeum has a very high content of proteins. We can therefore assume that it could be used for feeds.

Tritinaldia, *xHaynaldoticum sardoum*

Several stable hexaploid lines (2n = 42), with the genome AABBVV, were obtained from the cross T*. durum* cv. Modoc x *D. villosum* (*Haynaldia villosa*) and these synthetic amphiploids are potential donors of resistance to powdery mildew (Pace et al., 1985; Yao-Jing Xia et al., 1995). In addition to these synthetic forms there are also spontaneous amphiploid forms, which are known as „Denti de cani" (dog's teeth) and are considered to originate from crosses between durum wheat and *D. villosum*, common in durum wheat fields in Italy. Two pure lines, one with a hollow stem CV (winter) and the other with a solid stem CP (spring), were selected from populations growing in Sardinia. Since 1987, these 2 lines have been grown and evaluated in Pisa and other areas of Italy. Both lines are tall and give a grain yield of only 0.8-1 $t.ha^{-1}$, they have good resistance to *Fusarium* wilt and produce flour of excellent baking quality and high protein content. The CP line has rheological properties equal to or superior to bread wheat. The lines are considered useful in their own right as low-input crops and as sources of useful genes for wheat improvement.They did not differ greatly in accumulation of the various protein classes, but they differed in the banding pattern of the prolamins and glutelins (Meletti et al., 1996).

Use of perennial Triticeae species for wide hybridisation

Perennial *Triticeae* species comprise some of the most valuable grasses in the temperate regions of the world. In addition, they have been a source of genes for pest resistance and other traits in wide hybridisation programmes to improve cultivated cereal crops. Intense breeding efforts have been restricted primarily to species of *Agropyron*, *Psathyrostachys*, *Thinopyrum*, *Leymus*, *Elymus*, and *Pseudoroegneria* [*Elymus*]. Crested wheatgrass (*Agropyron* spp.) has received the most attention in breeding programmes as evidenced by the release of several improved cultivars. Stand establishment vigour of *Psathyrostachys juncea* has been substantially increased through breeding, and selection for improved forage quality and

disease resistance has been effective in intermediate wheatgrass, *Thinopyrum intermedium* [*Elymus hispidus*]. Interspecific hybridisation is a promising breeding procedure in the perennial Triticeae, or with cultivated wheat species.
The wheat streak mosaic rymovirus (WSMV), vectored by the wheat curl mite (WCM; *Aceria tosichella*), is one of the most important viral diseases of wheat (*T. aestivum*) in the world. Genetic resistance to WSMV and the WCM does not exist in wheat. Resistance to WSMV and the WCM was evaluated in five different partial amphiploids, namely Agrotana, OK7211542, ORRPX, Zhong 5 and TAF 46, which were derived from hybrids of wheat with decaploid *Thinopyrum ponticum* [*Elymus elongatus*] (2n = 10x = 70) or with hexaploid *Th. intermedium* [*E. hispidus*] (2n = 6x = 42). Although the amphiploids signed as Agrotricum [*Agropyron* x *Triticum*] - lines CI15321, CI15322, OK9387A are resistant to streak mosaic virus (WSMV).
Wide-hybrids between wheat (*Triticum aestivum* and *T. carthlicum*) and lymegrass (*Leymus* arenarius and *L. mollis*) can serve as a bridge to increase genetic diversity both of wheat (with respect to biotic and abiotic stress tolerance) and of lymegrass (with respect to end-use quality). The hybrids have been developed further by: (1) direct backcrossing with the parental species; and (2) chromosome doubling to produce fertile amphiploids (Anamthawat-Jonsson 1996, 1999). Simultaneously, lymegrass characters such as stress tolerance and disease resistance may be introgressed into wheat, making the cultivation more reliable in many different production regions.

Discussion

As a rule, the direct use of synthetic amphiploids is limited, due to its negative properties, which inhere to the wild forms from which they are created. The use of triticale in agricultural practice and the relative chance of tritordeum being used in practice in the future are more or less exceptions. It is interesting that a more marked breeding progress was reached in cereals with a high degree of ploidy. In this association we can assume that rapid breeding progress of hexaploid wheat and triticale could also be based on the homoeologous chromosome, which could fix the heterosis effect in the amphiploid condition. From this point of view the amphiploid forms could have a good chance to be successful in breeding.
One of the limiting factors when translocating genes from wild forms is the incidence of genetical systems determining the crossability, and/or the incidence of hybrid necrosis. While the use of *Ae. tauschii* by means of synthetic hexaploid wheat with the AABBCC genome is relatively easy, the transfer of genes from other unrelated genomes using synthetic amphiploids with wheat could be considerably difficult due to problems of meiotic pairing of non-homologous chromosomes. It is desirable to build the required genes in wheat using the induction of translocation. The homoeologous pairing suppressor gene Ph1 is localisated on the long arm of the 5B chromosome in wheat. The forms with a lack of the Ph1 gene should cause homoeologous chromosome pairing in meiosis. This makes the translocation line very useful for the induction of homoeologous chromosome pairing between alien chromosomes and wheat chromosomes in interspecific and intergeneric hybrids.

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DETECTION OF LOCUS GLU 1D5+10 IN WHEAT GENE RESOURCES WITH MRS AND LG MORFOTYPES OF SPIKE

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Summary

In selected gene resources of Triticum aestivum L. with MRS (multirow spike) morphotype of spike and long glumes, using technics of molecular biology the locus Glu 1D5+10 was detected. The presence of this allelic variant, as a genetic marker, predicts high bread-making quality. The genomic DNA from the young wheat leaves by DNasy Plant Mini Kit (Qiagen) was isolated. The presence of glutenin subunits 1D5+10 by SPLAT (D´Ovidio, Anderson, 1994) method was detected. The used primers were JEDL11 and JEDL12, the resulting product had 450 bp. The allelic variant Glu 1D5+10 was verified in all MRS and LG genotypes .

Key words: gene resources, wheat, Glu 1D5+10, SPLAT

Introduction

Progress in field of biochemistry and molecular biology allows application of genetic markers based on DNA polymorphism (Williams et al., 1990). Markers on DNA level – RAPD, SPLAT, RFLP, AFLP – are representing the groups of molecular markers used in breeding programmes. Bread-making quality of gluten is determined with proteins gliadin and glutenin. Glutenin markers of improved bread-making quality of wheat are allelic variants Glu 1A1, Glu 1B7+9 (Chloupek, 2000) and locus Glu 1D5+10. These allelic variants during determination of „Glu score" shows the highest value (4). The value of „Glu score" correlates with SDS test, the indicator of gluten swelling. In contrary, the markers of poor bread-making quality are glutenin locuses Glu 1B6+8 and Glu 1D2+12. The prediction of bread-making quality cannot be realise without qualitative analysis of the other protein fraction – of gliadins. In case of gliadins the main manifesting influence is of rye translocation

T1BL.1RS. Proteosynthesis of secalin proteins, determinate by locus in translocated segment of chromosome 1RS, is the reason of poor bread-making quality. The translocated segment of rye chromosome 1RS has localised gene controlling elements of prolamins in genetic linkage with gene Sr 31, the resistance to stem rust (*Puccinia graminis* Pers.*)* (Černý, Šašek, 1996).

Material and methods

The molecular genetic analysis in selected gene resources of *Triticum aestivum* L. with MRS (Multirow Spike) morphotype of spike and significantly enhanced glumes – LG (Long Glumes), improved by Agricultural Research Institute Kroměříž, Ltd., was proceeded. For the newly selected morphotype of spike, marked as MRS, is characteristic that higher number of spikelets (2-10) grow out together from each nodes of spike-screw abreast and above them. This is not a branchiness of spike. Morphotype MRS is probably subjected to a recessive gene. The donor of this gene is heterogeneous form of Ra1 from VIR Sankt Peterburg (Martinek, Bednář, 1998). The bigger size of glumes into *T. aestivum* L. proceeding outbreeding with tetraploid *T. polonicum* L. (variety Buitre Cometa obtained from CIMMYT, Mexico) was transferred. Production of long glumes is under the control of recessive gene *P*, localised on chromosome 7AL and having influence on improvement of characteristics of starch in tetraploid and hexaploid wheat. Intraspecific hybridisation many interesting lines of wheat originated that may be used for breeding. In molecular genetic analyses gene resources were used as is shown in tab. 1 were used.

Tab.1. Analysed genotypes

Species	**Ploidy**	**Genome**	**Field number**	**Origin**
Wheat with MRS (Multirow Spike)				
T. aestivum	(2n=6x=42)	AABBDD	683+684-99	Ra1 from VIR
T. aestivum	(2n=6x=42)	AABBDD	687-99	Ra1/ZG K 242-82//Ra1
T. aestivum	(2n=6x=42)	AABBDD	643-99	Alana/3/Ra1/ZG K 242-82//Ra1
T. aestivum	(2n=6x=42)	AABBDD	670-99	Astella/3/Ra1/ZG K 242-82//Ra1
T. aestivum	(2n=6x=42)	AABBDD	705-99	Ra1/ZG K 242-82//Ra1/3/Contra
Wheat with significantly enhanced glumes – LG (Long Glumes)				
T. aestivum	(2n=6x=42)	AABBDD	783-99	ZG K 171-1-82/Biutre Cometa//HYB 92.104
T. aestivum	(2n=6x=42)	AABBDD	789-99	ZG K 171-1-82/Biutre Cometa//unkn.genotyp
T. aestivum	(2n=6x=42)	AABBDD	815-99	ZG K 171-1-82/Biutre Cometa
T. aestivum	(2n=6x=42)	AABBDD	V1-220-99	Brea//ZG K 171-1-82/Biutre Cometa
T. aestivum	(2n=6x=42)	AABBDD	V1-485-99	Hereward//ZG K 171-1-82/ Biutre Cometa
T. aestivum	(2n=6x=42)	AABBDD	V1-214-99	Mona//ZG K 171-1-82/ Biutre Cometa
T. aestivum	(2n=6x=42)	AABBDD	V1-218-99	Brea//ZG K 171-1-82/ Biutre Cometa

The genetic variability of selected lines of wheat by SPLAT markers was characterised in Laboratory of Molecular Genetics at Department of Genetics at MUAF in Brno. SPLAT (Specific Polymorphic Locus Amplification Test) is an older type of PCR, where amplification of two-primer-defined locus occurs. The advantage of this method is its quickness, no radioisotopes and small amount of starting genomic DNA. The disadvantage is hard obtaining the primers, due to high specifity of the reaction. There were used young green leaves for analysis. Plant material in special absorbent PUR material in Knopp's nutrient solution was grown at 20 °C and under photoperiod 12 h light and 12 h darkness. Samples were collected in the stage of first real leaf. The used amount of plant material for DNA isolation was 100 mg of fresh weight (miniprep). The genomic DNA by DNasy Plant Mini Kit (Qiagen) was isolated. The presence of allelic variation of locus Glu 1D5+10 by SPLAT method (D´Ovidio, Anderson, 1994), using primers JEDL11 and JEDL12 with sequence 5´GCCTAGCAACCTTCACAATC3´ a 5´GAAACCTG CTGCGCACAAG 3´ (D´Ovidio, Anderson, 1994) was detected. The electrophoretic separation on agarose gel in TAE buffer, the visualisation by ethidium bromide, and the documentation by Polaroid DS-34 camera was proceeded.

Results and discussion

Isolation of genomic DNA using DNasy Plant Mini Kit is reliable and prompt. The disadvantage of this procedure are its financial expenses.

The genotype and related content of gluten-producing proteins (prolamines and glutenins) in grains are the most significant factors having influence on bread-making quality of grain. The marker of good bread-making quality is glutenin allelic pair 1D5+10. This locus, which can be detected by method SPLAT (D´Ovidio, Anderson, 1994), represented 450 bp product on agarose gel. In this manner verified higher bread-making quality is subjected by absence of rye translocation T1BL.1RS, which is the marker of poor bread-making quality in wheat. This marker could be detected by method RAPD (Iqbal,Rayburn,

1995), the resulting product had size 1.2 kb, or by PAGE ISTA of prolamine proteins of grain and by detection of allelic variation of gliadins Gld 1B3 (Bednář et al., 1999). The presence of secaline proteins expressed by locus in translocated segment of chromosome 1R is the reason of poor bread-making quality. In studied wheat resources allele Glu 1D5+10, as a genetic marker of good bread-making quality, in all MRS and LG genotypes was detected. Jedličková et. al. (2001) by DNA markers demonstrated absence of rye translocation T1BL.1RS in genotypes MRS (field no. 643-99, 670-99) and in genotypes LG (field no. V1-220-99, V1-485-99, V1-218-99). In these genetic resources higher poor bread-making quality can be presumed.

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