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## Segregation Analysis of Wheat-Rye Chromosomes with Different Marker Systems

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**Abstract:** Segregation of different marker types (RFLP, PCR and protein marker) were successfully used to detect new IRS wheat-rye-translocation lines. The lines were derived from androgenetic wheat progenies of the  $F_1$ -hybrids (IJ61...IJ64), containing the translocated chromosomes 1AL.IRS<sub>insave</sub> and 1BL.IRS<sub>petkus</sub>. Their distinct IRS<sub>insave</sub> and IRS<sub>petkus</sub> arms originated from different rye varieties. In four lines the complete IRS<sub>petkus</sub> arm of the wheat-rye translocated chromosome 1BL.IRS<sub>petkus</sub> was exchanged for the IRS<sub>insave</sub> arm. Three lines contain new recombinant IRS arms in the 1AL.IRS or 1BL.IRS chromosomes. These recombinants are likely to be derived from crossing-over events in the interstitial segments of IRS between the centromer and the loci of polymorphic markers (major and minor secalins; PCR based STS-IAG95; subtelomer rye specific RFLP probe). Their probable chromosomal genetic map is shown. Till now, a strong linkage is detected between the loci of minor secalin and STS-IAG95. The latter is derived from Xiag95, a locus of a major gene for powdery mildew resistance. The correlation between the markers and the powdery mildew resistance gene locus *Pm17* should be investigated by performing resistance tests.

**Keywords:** *Triticum aestivum*; anther culture; wheat-rye translocation; genetic analysis; recombination; protein marker; DNA marker; restriction fragment length polymorphism (RFLP); polymerase chain reaction (PCR); Sequence tagged site (STS)

Translocations of rye chromatin into the wheat genome have widely been used in wheat breeding programmes to introduce genes of agronomic importance (GRAYBOSCH *et al.* 1999). The 1BL.IRS<sub>petkus</sub> translocation has positive effects on the yield potential of wheat (see discussion by SINGH *et al.* 1998). Its chromosome arm IRS<sub>petkus</sub> derived from Petkus rye, is connected to the long arm of wheat chromosome 1B. Besides other genes, such as *Yr 9*, *Lr 26* and *Sr 31*, this short rye chromosome arm contains the powdery mildew resistance gene *Pm 8*. However, this gene is already overcome in Europe. To join the positive yield effects of 1BL.IRS<sub>petkus</sub> with the effective powdery mildew resistance gene *Pm 17*, derived from the short arm of chromosome 1of *Insave* rye, a further translocation (1AL.IRS<sub>insave</sub>) was incorporated via the wheat-rye translocation variety Amigo (VAHL *et al.* 1993). These double translocated wheat lines contain both the wheat-rye translocation chromosomes 1AL.IRS<sub>insave</sub> and 1BL.IRS<sub>petkus</sub> simultaneously. The distinct short rye chromosome arms, replacing the homoeologous group 1 wheat chromosome arms, are normally transmitted as a block without detectable recombination

(GRAYBOSCH *et al.* 1999; JOUVE *et al.* 1997). However, recombination between IRS marker loci occur in intermatings of wheat-rye translocation lines with different IRS chromosome arms (MARAIS *et al.* 1994; GRAYBOSCH *et al.* 1999). In order to identify new recombinants, double translocation lines (1AL.IRS<sub>insave</sub>, 1BL.IRS<sub>petkus</sub>) were included in crosses. In doubled haploid (dh) lines, derived from the  $F_1$ -hybrids of these crosses using anther culture, new recombinant 1BL.IRS<sub>insave</sub> chromosomes were detected (VAHL & MÜLLER 1994; MÜLLER *et al.* 1998). In these wheat-rye translocation lines the whole IRS<sub>petkus</sub> chromosome arm is replaced by the IRS<sub>insave</sub> arm, containing the gene *Pm 17* (MÜLLER *et al.* 1991; VAHL, MÜLLER 1994; HSAM *et al.* 1995).

This publication reports the detection of wheat lines, which contain new wheat-rye translocation chromosomes derived from the homologous recombination between the different IRS<sub>petkus</sub> and IRS<sub>insave</sub> arms. These new translocation lines were selected by the segregation analysis of different marker systems (protein, RFLP, and PCR-based), associated with different chromosomal regions.

**MATERIAL AND METHODS**

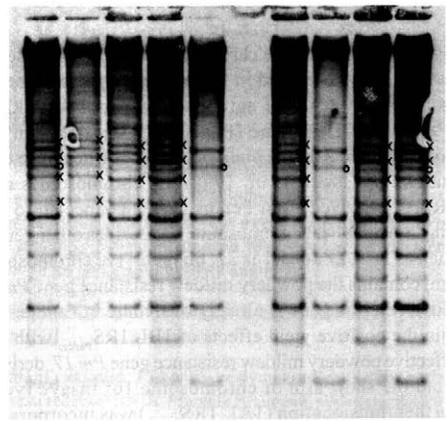
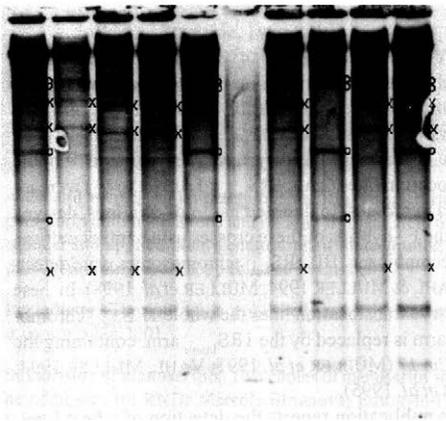
The analysed plant material consisted of doubled haploid (dh-) lines. These homozygous lines were produced using anther culture as described by MÜLLER and VAHL (1993) from the F<sub>1</sub> hybrids IJ 61... 64. The F<sub>1</sub> hybrids were derived from crosses between the variety Schwabenkorn and double 1RS translocated dh-lines. The incorporated dh-lines contain two distinct 1RS chromosomes arms (derived from the different rye origin Petkus and Insave) in their disomic 1AL-1RS<sub>Insave</sub> and 1BL.1RS<sub>Petkus</sub> wheat-rye translocation chromosomes (MÜLLER *et al.* 1998). The anther culture derived plants were characterised by patterns of prolamins (gliadins/secalins) separated by SDS-PAGE (VAHL *et al.* 1999). According BOTHE and KOEBNER (1998) or MARAIS *et al.* (1994), it was deduced from the position in their electrophoretic profile, that the

1RS<sub>Insave</sub> and 1RS<sub>Petkus</sub> specific major secalins are encoded from the gene Sec1 in the locus of the short arm of the rye chromosome one.

The following specific primer sets were used for the PCR-based identification of the 1RS-wheat-rye translocation chromosome type as described by BRINGEZU *et al.* (1999):

- PR55 (VAN CAMPENHOUT *et al.* 1998), specific for 1RS, ribosomal gene spacer sequences, locus 5S-rRNA-R1
- IAG-95 (MOHLER – pers. communication 1999), specific for 1RS<sub>Insave</sub>, sequence derived from a rye DNA clone, locus Xiag 95 (Xiag95 is a locus of a major gene for powdery mildew resistance *Pm*, WRICKE *et al.* 1996; compare with SENFT & WRICKE 1996),
- PSP2999 (DEVOS *et al.* 1995), specific for 1AS, constructed to the flanking regions of the internal microsatellite (CAG/CAA compound) in a low molecular weight

Hybridization with 7250								cultivar/ dh-line	Hybridization with 7200											
Dh <sub>2</sub> 15151a	dh <sub>2</sub> 15244a	dh <sub>2</sub> 15248a	dh <sub>2</sub> 15142a	dh 932	Greif	Iris	Amigo		Kavkaz	dh 483	Dh <sub>2</sub> 15151a	dh <sub>2</sub> 15244a	dh <sub>2</sub> 15248a	dh <sub>2</sub> 15142a	dh 932	Greif	Iris	Amigo	Kavkaz	dh 483
									DT											DT
	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	nontransl.	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS-type (RFLP)	DT	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	nontransl.	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>	IRS <sub>Insave</sub>



The RFLP patterns were detected by nonradioactive “Southern”-hybridization of electrophoretically separated Hind III-digested total DNA from green plant material after blotting to positive charged “Nylon”-membran. For comparison, different 1RS translocated wheats Amigo (1AL.1RS<sub>Insave</sub>), Iris, Kavkaz (1BL.1RS<sub>Petkus</sub>), dh932 (1BL.1RS<sub>Insave</sub>), and the double translocated (1AL.1RS<sub>Insave</sub>, 1BL.1RS<sub>Petkus</sub>) dh-line 483 as well as the nontranslocated wheat cultivar Greif were incorporated.

DT= 1RS<sub>Insave</sub>, 1RS<sub>Petkus</sub>; x = 1RS<sub>Petkus</sub>-typical fragments; o = 1RS<sub>Insave</sub>-typical fragments

Fig. 1. RFLP characterization of translocated 1RS rye chromosome arms of dh-lines from the F<sub>1</sub>-hybrids IJ 61 (Schwabenkorn [nontransl.] × dh3957c [DT]) and IJ 64 [dh3957c (DT) × Schwabenkorn (nontransl.)] on basis of the repetitive rye DNA clones Sc.BS+. 7250 (left) and 7200 (right)

Table 1. Details for the classification with the different marker systems see by material and methods or in Fig. 1

Number of classified doubled haploid wheat lines of the F <sub>1</sub> -hybrids IJ 61 and 64 with different recombinant IRS arms translocated to 1AL as well as 1BL arms												
Column-no.	1	2	3	4	5	6	7	8	9	10	11	
Classification with	RFLP	IRS Insave	IRS Petkus	IRS Petkus	IRS Petkus	IRS Petkus	IRS Insave	IRS Insave	IRS Insave	IRS Insave	+IRS Petkus	nontrans- located
	PCR	IRS Insave	IRS Insave	IRS Petkus	IRS Petkus	IRS Petkus	IRS Petkus	IRS Insave	IRS Insave	IRS Insave	+IRS Petkus	nontrans- located
	minor secalin	IRS Insave	IRS Insave	IRS Petkus	IRS Petkus	IRS Petkus	IRS Petkus	IRS Insave	IRS Insave	IRS Insave	+IRS Petkus	nontrans- located
	major secalin	IRS Insave	IRS Insave	IRS Insave	IRS Petkus	IRS Petkus	IRS Petkus	IRS Petkus	IRS Insave	IRS Insave	+IRS Petkus	nontrans- located
Translocated to	1AL	1AL	1AL	1AL	1BL	1BL	1BL	1BL	1AL	+1BL	–	
Number of lines	3	1	1	0	4	0	2	4	2	4	21	

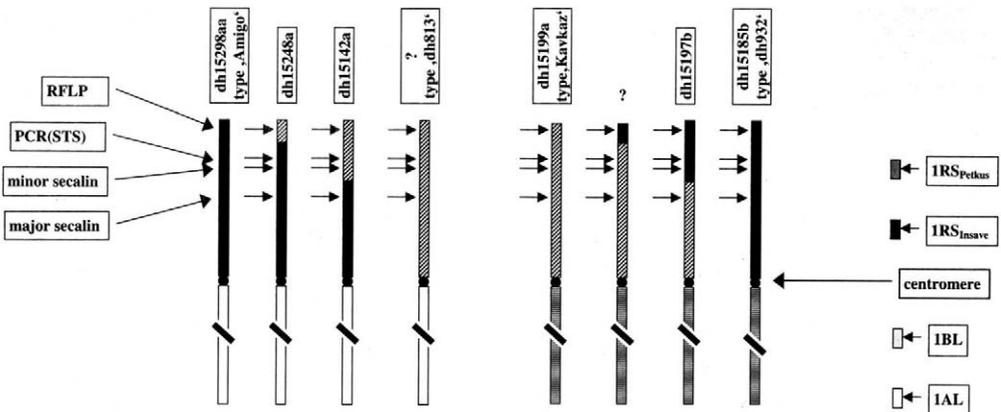
(LMW) glutenin gene, locus Xpsp2999 (Glu-3)-1A (Xpsr11)

– O11B (VAN CAMPENHOUT *et al.* 1995; DE FROIDMONT 1998), specific for 1BS, sequence from a low molecular weight (LMW) glutenin gene, locus Glu-B3

RFLP analysis (BRINGEZU *et al.* 1998) was performed using repetitive rye DNA clones, specific to the sub-olomeric region of rye chromosomes (SCHWARZACHER 1996). Furthermore, Giemsa C-banding was used for the cytological characterization (MÜLLER *et al.* 1991) of the marker selected wheat rye translocation lines.

## RESULTS AND DISCUSSION

Doubled haploid (dh) wheat lines, derived from anther culture regenerates, obtained from the F<sub>1</sub> hybrids IJ 61... IJ 64, were tested with protein (Secalin/Gliadin) as well as with RFLP (Fig. 1) and PCR technology (Sequence tagged site (STS) and microsatellite (MS) markers). Till now, twenty one doubled haploid lines were classified by consideration of their specific marker loci of 1RS (1RS<sub>Insave</sub> (*Pm17*) and 1RS<sub>Petkus</sub>), 1AS and 1BS arms. The results are summarized in Table 1. Doubled haploid



Details for the classification with the different marker systems see by material and methods or in Fig. 1.

Fig. 2. Probable chromosomal genetic linkage map of recombinant IRS arms in the wheat-rye translocation chromosomes 1AL.1RS and 1BL.1RS

lines with the initial parental constitution of two disomic chromosomes 1A and 1B (nontranslocated, column 10 of Table 1) or of two disomic wheat-rye translocation chromosomes 1AL.1RS<sub>Insave</sub>, 1BL.1RS<sub>Petkus</sub> (column 9) were identified. The expected two types with only one original translocated chromosome (1AL.1RS<sub>Insave</sub>; column 1 as well as 1BL.1RS<sub>Petkus</sub>; column 5) were also detected.

In addition, 8 dh lines were detected with recombinant wheat-rye translocated chromosomes. In four of these wheat lines a crossing-over event has probably happened near the centromere and therefore the complete 1RS<sub>Petkus</sub> arm of 1BL.1RS<sub>Petkus</sub> was exchanged for the 1RS<sub>Insave</sub> arm (Table 1 – column 8). These dh lines contain the same type of wheat-rye translocation chromosome (1BL.1RS<sub>Insave</sub>) as detected by VAHL *et al.* (1994) in the dh 932 (Fig. 2). The other possible crossing-over event, a complete exchange of 1RS<sub>Insave</sub> for 1RS<sub>Petkus</sub> was not observed (type dh 813 in Fig. 2). Further four dh lines were identified to possess translocated wheat-rye chromosomes with three different types of recombinant 1RS arms (column 2, 3, and 7 in Table 1). These recombinant 1RS arms were derived from homologous recombinations of both the 1RS<sub>Insave</sub> and 1RS<sub>Petkus</sub> chromosome arms, caused by crossing-over events in different positions distally to the centromere.

In all 8 dh lines crossing-over events have occurred in the interstitial segment between the centromere and the RFLP marker locus. Seven lines carry recombinant 1RS translocated chromosomes derived from crossing-overs between the centromere and STS (IAG95, *Pm17*) or minor secalin marker loci, whereas only four dh lines have a recombinant 1RS translocated chromosome, derived from crossing-over events between the centromere and the major secalin locus. Therefore, the likely genetical order of the marker loci on the 1RS chromosomal arm is: centromere – major secalin (Sec-1) locus – minor secalin and/or STS (IAG95/*Pm17*) locus – RFLP (subtelomeric DNA) locus. The probable chromosomal genetic maps of the identified recombinant 1RS chromosome arms are shown in Fig. 2.

With the restriction, that investigation with the RFLP marker is still needed, recently additional 11 dh lines were classified. These lines contain normal parental 1RS translocated chromosomes. Therefore altogether 32 lines are characterized. In spite of the small number of the investigated dh lines, it is possible to make a first verification of workability of the included markers for the selection of the powdery mildew resistance gene locus *Pm17*. Provided it is possible to confirm by resistance analysis of these recombinant dh lines the linkage between the powdery mildew resistance gene locus *Pm17* and the loci of markers of the PCR based STS (IAG95/*Pm17*) as well as of the minor 1RS *Insave* secalin, it can be assumed, that both markers detect the *Pm17* resistance gene in wheat with a reliability of 100%. Nevertheless, in most cases the wheat lines carrying *Pm17* was also correctly selected by RFLP and major secalin marker. (Only in 3 or 9% of all cases,

respectively, deviations were observed in the *Pm17* classification in comparison with STS or minor secalin.)

Thus, new recombinant 1RS wheat-rye translocation chromosomes are detected in doubled haploid lines from crosses of common wheat with 1RS double translocation wheat lines by segregation analysis. These recombinant 1AL.1RS and 1BL.1RS chromosomes are likely to be derived from crossing-over events in the interstitial segments between the centromere and the loci of different markers (major secalin; minor secalin and PCR based STS-IAG95; subtelomeric rye specific RFLP probe). Till now, a strong linkage is detected between the loci of minor secalin and STS-IAG95 derived from Xiang95, a locus of a major gene for powdery mildew resistance. The workability of the marker in selection of the powdery mildew resistance gene locus *Pm17* should be investigated by performing resistance tests.

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## Abstrakt

BRINGEZU T., VAHL U., MÜLLER G. (2001): **Analýza štěpení pšenično-žitných chromozomů s odlišnými systémy markerů.** *Czech J. Genet. Plant Breed.*, 37: 1–5.

Štěpení různých typů markerů (RFLP, PCR a bílkovinných markerů) bylo úspěšně využito k detekci nových linií s pšenično-žitnou translokací IRS. Linie byly odvozeny z androgenních potomstev pšenice z  $F_1$  hybridů (IJ61...J64), které obsahovaly translokované chromozomy 1AL.1RS<sub>insave</sub> a 1BL.1RS<sub>Petkus</sub>. Jejich lišící se ramena 1RS<sub>insave</sub> a 1RS<sub>Petkus</sub> pocházela z odlišných odrůd žita. U čtyř linií bylo kompletní rameno 1RS<sub>Petkus</sub> z pšenično-žitného translokovaného chromozomu 1BL.1RS<sub>Petkus</sub> vyměněno s ramenem 1RS<sub>insave</sub>. Tři linie obsahují nové rekombinace ramene IRS v chromozomech 1AL.1RS nebo v 1BL.1RS. Tyto rekombinace vznikly pravděpodobně při crossing-overech v intersticiálních segmentech IRS mezi centromerou a lokusy polymorfních markerů (major a minor secalinů; PCR based STS-1AG95; subtelomerní vzorek RFLP specifický pro žito). Je uvedena jejich pravděpodobná genetická chromozomová mapa (Fig. 2). Doposud je zjišťována silná vazba mezi lokusy minor secalinu a STS-1AG95. STS-1AG95 je odvozen z Xiag95, což je lokus pro major gen rezistence k padlí travnímu. Korelace mezi markery a lokusem genu rezistence k padlí travnímu *Pm17* musí být ověřeny v dalších testech rezistence.

**Klíčová slova:** *Triticum aestivum*; prašnikovká kultura; pšenično-žitná translokace; genetická analýza; rekombinace; proteinový marker; marker DNA; restriction fragment length polymorphism (RFLP); polymerase chain reaction (PCR); Sequence tagged site (STS)

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## Stability of 1AL.1RS, 1BL.1RS Wheat-Rye Double Translocation Lines

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**Abstract:** Chromosome counting, Giemsa C-banding, biochemical and molecular markers were used to determine the chromosomal constitution and stability of the doubled haploid line dh 17029 with 1AL.1RS<sub>insave</sub>, 1BL.1RS<sub>insave</sub> wheat-rye double translocation. As a result of this study, it can be concluded that the line dh 17029 exhibits a normal behavior in the meiosis and that all in all, such doubled haploid lines with 1AL.1RS<sub>insave</sub>, 1BL.1RS<sub>insave</sub> wheat-rye double translocation derived via anther culture are relatively genetically stable.

**Keywords:** *Triticum aestivum* L.; doubled haploid lines; wheat-rye double translocation; biochemical markers; molecular markers; C-banding; chromosome counting; powdery mildew resistance

Transferring useful genes from related species into hexaploid wheat is a valuable and proven technique for improving wheat cultivars (MILLER *et al.* 1996; VAHL *et al.* 1999). Recently (MÜLLER *et al.* 1990; BRINGEZU *et al.* 1997, 1999), we have described doubled haploid (dh) wheat lines with four doses of 1RS (two 1AL.1RS chromosomes and two 1BL.1RS chromosomes). It is known that many resistance genes are located on the short arm of 1R chromosome (MÜLLER *et al.* 1991, 1997; VILLAREAL *et al.* 1998, and references therein). Hence we report here on a further new doubled haploid winter wheat line (dh 17029) which carries a 1AL.1RS<sub>insave</sub>, 1BL.1RS<sub>insave</sub> wheat-rye double translocation and the high-molecular-weight (HMW) glutenine subunit groups 2\*, 7+9, 5+10. The short arm 1RS<sub>insave</sub> of these translocation chromosomes derived from Amigo wheat carries the powdery mildew resistance gene *Pm17* (HSAM & ZELLER 1997a, b; MÜLLER *et al.* 1991; VAHL & MÜLLER 1994; VAHL *et al.* 1996, 1998).

In the present study, the chromosomal stability and chromosome constitution of such double translocation lines are characterized by utilizing a combination of chromosome counting, C-banding, biochemical and molecular markers.

Analyses were carried out on different single individuals (at least 10) of dh<sub>1</sub>- and dh<sub>2</sub>-karyotypes or young plants of the line dh 17029 and on different single grains of three F<sub>1</sub> hybrids produced by crossing of the double translocation line dh 17029 with wheat forms without 1RS translocation (F<sub>1</sub>UE117: dh 17029 × dh 20313; F<sub>1</sub>UE118: dh 17029 × dh 19710; and F<sub>1</sub>UE119: dh 17029 × Cardos).

**Cytological analysis:** The chromosome number was determined in Feulgen-stained root tip cells (SCHMID *et al.* 1985). The Giemsa C-banding was employed to identify the wheat-rye translocation (MÜLLER *et al.* 1990).

**Biochemical and molecular marker analysis:** The following marker systems were used to estimate the presence or absence of short arms 1AS, 1BS and 1RS for characterizing the 1RS translocation type and the genetic stability:

- Gliadins and secalins according to VAHL *et al.* (1993, 1999)
- Multiple glucosephosphate isomerases (GPI) separated by isoelectric focusing (VAHL & MÜLLER 1997a, b)
- HMW-glutenins (MÜLLER & VAHL 1993; VAHL *et al.* 1993)
- PCR marker according to BRINGEZU *et al.* (1999).

### MATERIAL AND METHODS

**Plant materials:** The doubled haploid 1AL.1RS<sub>insave</sub>, 1BL.1RS<sub>insave</sub> wheat-rye double translocation line dh 17029 was developed in our group via anther culture (MÜLLER & VAHL 1993) of the F<sub>1</sub> hybrid IJ111 in accor-

### RESULTS AND DISCUSSION

Based on mitotic root tip cell analysis (a total of 40 cells was analysed), all individuals of the dh<sub>1</sub>- and dh<sub>2</sub>-descendants of the doubled haploid line dh 17029 contain 42 chromosomes with only two satellite chromosomes

caused by the presence of 6BL.6BS chromosomes but the absence of the 1BL.1BS chromosomes (Fig. 1a). The C-banding karyotypes reveal that all patterns involve a pair of 1AL.1RS and a pair of 1BL.1RS (Fig. 2).

The uniform presence of this double translocation type in the  $dh_2$ -progeny (after selfing of  $dh_1$ -plants) is also confirmed by the biochemical and molecular marker analysis. Thus, the IEF-patterns of GPI (Fig. 3) show clearly the double translocation type for all 10 analysed individuals due to lack of bands by the absence of the short arms 1AS and 1BS. Moreover, the gliadin and secalin analysis of 30  $dh_2$  single seed samples indicates that in this double translocations type only 1RS<sub>insave</sub> chromosome arms (Fig. 4) are involved as expected in accordance with the scheme:

Cross: 1BL.1RS<sub>insave</sub> transl. × 1AL.1RS<sub>ins</sub> transl.  
(e.g. F<sub>1</sub> UJ111): (dh<sub>2</sub> 932) × (dh<sub>2</sub> 3815)

F<sub>1</sub> hybrid: 1AL.1AS / 1AL.1RS<sub>insave</sub>  
1BL.1RS<sub>insave</sub> / 1BL.1BS  
:  
:

The hybrid segregates with regard to 1RS-containing chromosomes in  $2^2 = 4$  different types of gametes (types of pollen plants):

1AL.1AS	1AL.1RS <sub>insave</sub>	1AL.1AS	1AL.1RS <sub>insave</sub>
1BL.1BS	1BL.1BS	1BL.1RS <sub>insave</sub>	1BL.1RS <sub>insave</sub>
:	:	:	:
:	:	:	:

In a classically produced F<sub>2</sub> the desired homozygous state of combination of 1AL.1RS<sub>insave</sub> translocation and 1BL.1RS<sub>insave</sub> translocation (realized in dh 17029) is found once among  $2^{2 \times 2} = 16$  plants, but in the anther culture progeny, once among  $2^2 = 4$  regenerants. This shows the advantage of haploid technique.

Homogeneity of  $dh_2$ -progeny is also observed in the HMW-glutenin patterns (cf. Fig. 5). They demonstrate for all 20 tested  $dh_2$ -seeds of line dh 17029 the subunits 2\*, 7+9, 5+10 in agreement with the analogous data of  $dh_1$ -progeny.

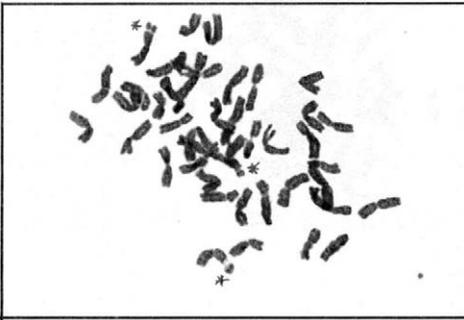
The uniform presence of the short arm 1RS<sub>insave</sub> was confirmed on the basis of PCR marker for the short arms 1AS, 1BS and 1RS (Fig. 6). As expected, all 6 examined



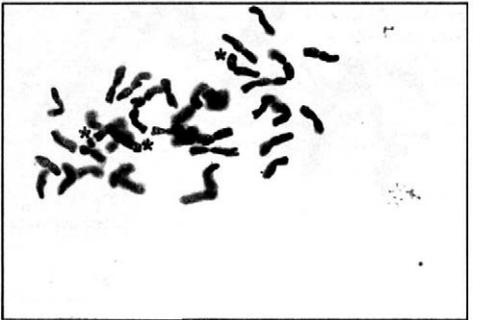
a) Example of a  $dh_2$ -individual of dh 17029 (2 SAT)



b) Example of a F<sub>1</sub>-individual of hybrid UE117 (3 SAT)

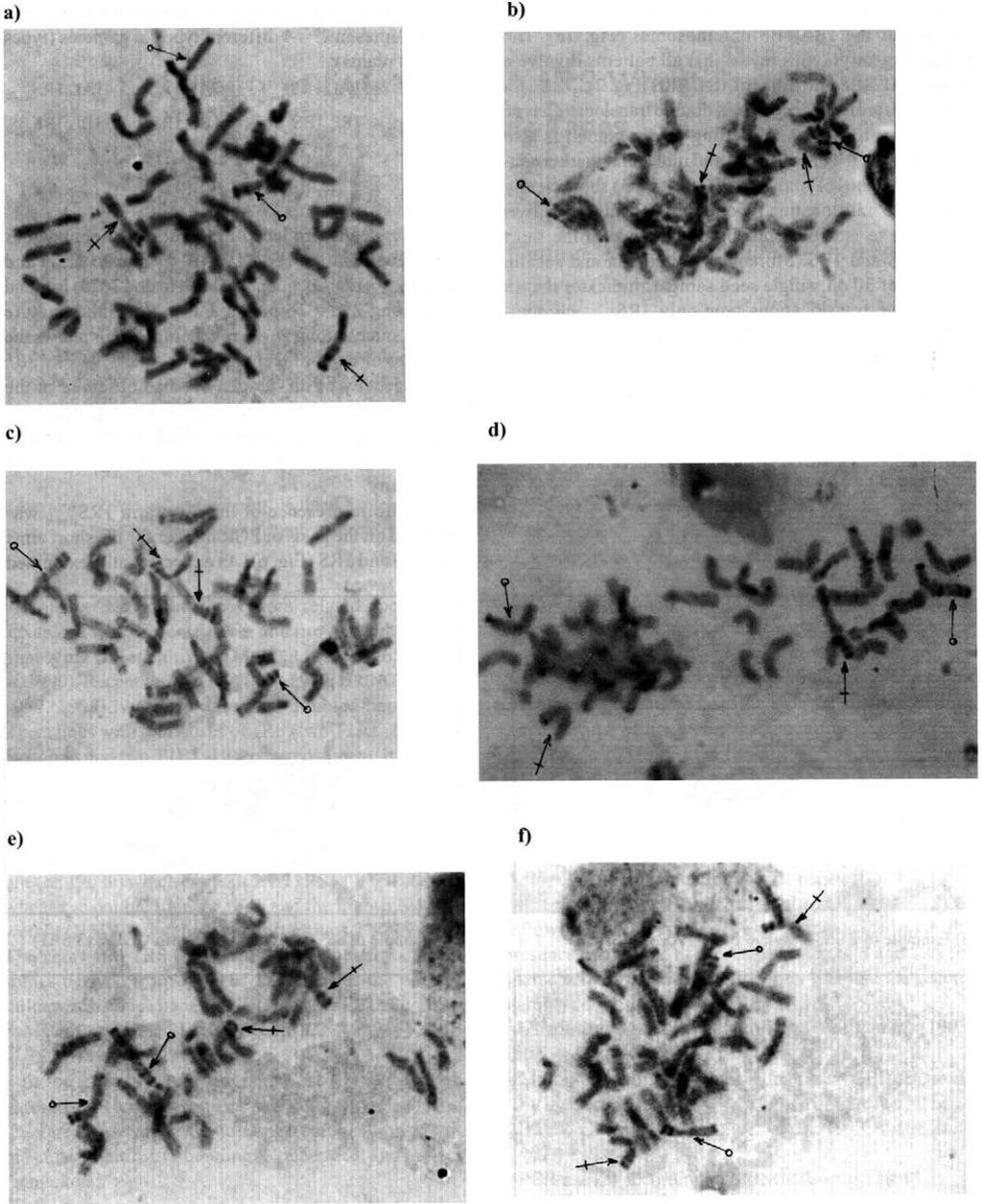


c) Example of a F<sub>1</sub>-individual of hybrid UE118 (3 SAT)



d) Example of a F<sub>1</sub>-individual of hybrid UE119 (3 SAT)

Fig. 1a–d. Chromosome numbers in mitotic root-tips after Feulgen-technique for proving euploid state (42 chromosomes) in the progeny of a wheat-rye double translocation line (\* – symbols indicate satellite chromosomes)



a = dh<sub>1</sub> plant K6; b = dh<sub>1</sub> plant K11; c = dh<sub>2</sub> plant K3; d = dh<sub>2</sub> plant K4; e = dh<sub>2</sub> plant K5; f = dh<sub>2</sub> plant K6; (1AL.1RS indicated by arrow: ←+; 1BL.1RS indicated by arrow: ←○)

Fig. 2 a–f. Giemsa-C-banding patterns of mitotic root-tip chromosomes of several individuals from dh<sub>1</sub>- and dh<sub>2</sub>-progeny of doubled haploid line dh 17029 as confirmation of disomic 1AL.1RS and 1BL.1RS wheat-rye double translocation

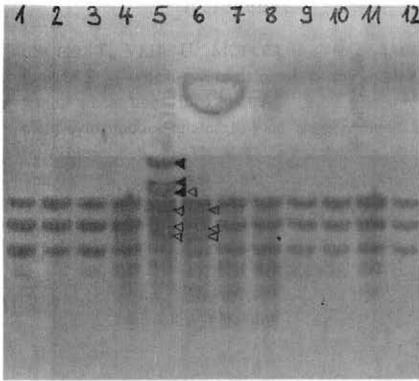
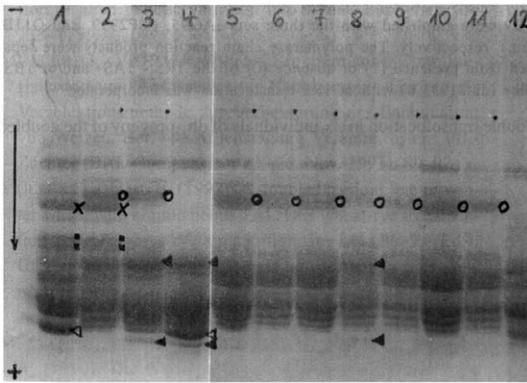


Fig. 3. Using multiple glucose phosphate isomerases as marker for the absence of wheat chromosome arms 1AS and 1BS (indirect proof of 1AL.1RS, 1BL.1RS double translocation) in several individuals of  $dh_2$ -progeny of  $dh_17029$

Separation by IEF at pH 5–8/3–5 (2:1). Lanes 1–4, 7–12: Ten single kernels (each from another ear) of  $dh_17029$ . For comparison: 5 = Rektor (cv. without a rye translocation; 6 = Iris (cv. with 1BL.1RS translocation)

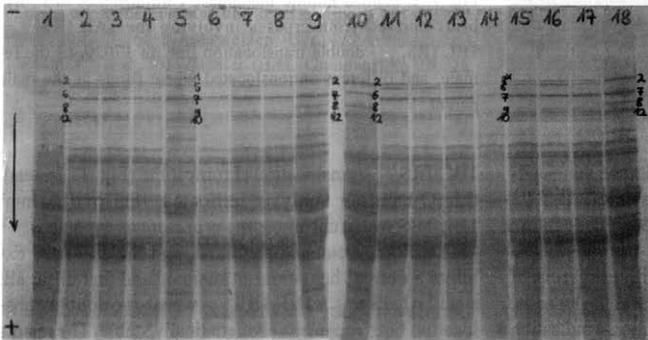
- ◁ dimers involving the *Gpi-A1* product  $\alpha$ , absent in 1AL.1RS wheat forms
- ◄ dimers involving the *Gpi-B1* product  $\beta$ , absent in 1BL.1RS wheat forms



Separation of ethanol-soluble extracts by SDS-PAGE (10% acryl-amide, 0.3% BIS). Lanes 5–7 and 9–11: Six single kernels (each from another ear) of  $dh_17029$ . For comparison wheats with different translocation types: 1 = 1BL.1RS<sub>Petkus</sub>; 2 = 1AL.1RS<sub>Insave</sub>; 1BL.1RS<sub>Petkus</sub>; 3 and 8 = 1AL.1RS<sub>Insave</sub>; 4 and 12 = no translocated wheats

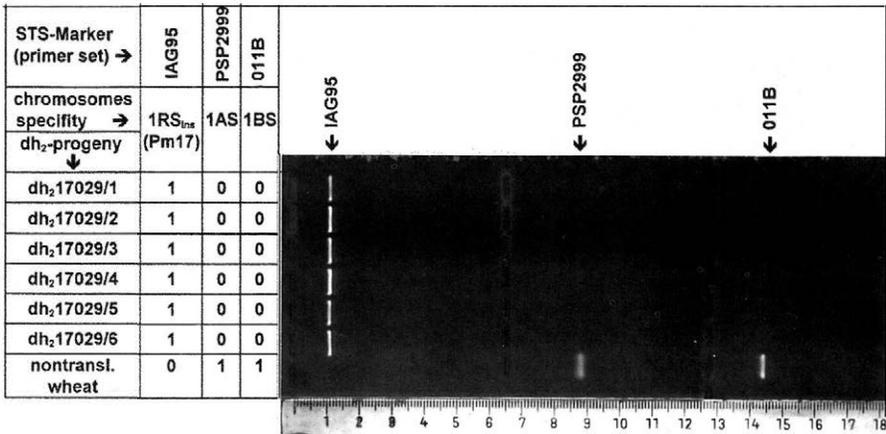
- = 1RS<sub>Inx</sub> encoded major secaline; ○ = 1RS<sub>Inx</sub> encoded minor secaline; x = 1RS<sub>Pet</sub> encoded major secaline; ■ = 1RS<sub>Pet</sub> encoded minor secaline; ◁ = 1AS encoded gliadine; ◄ = 1BS encoded gliadine

Fig. 4. Proof of the 1AL.1RS<sub>Insave</sub>, 1BL.1RS<sub>Insave</sub> wheat-rye double translocation in several individuals of  $dh_2$ -progeny of the doubled haploid line  $dh_17029$  by means of gliadin and secalin patterns



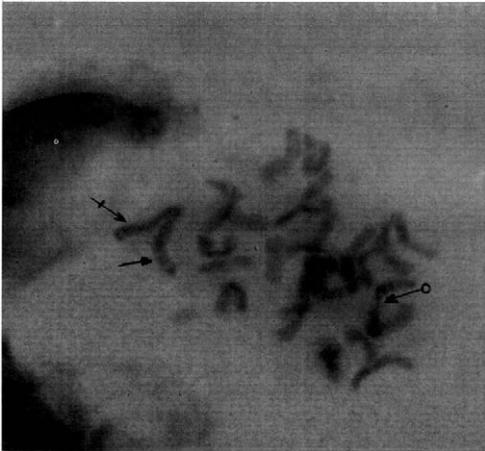
Separation by SDS-PAGE (12% PAGE-Plus from AMRESCO). Lanes 2–4, 6–8, 11–13, 15–17: twelve single kernels of  $dh_17029$ ; for comparison wheat cultivars are applied: 1 and 10 = Apollo; 5 = Bussard; 14 = Amigo; 9 and 18 = Kanzler

Fig. 5. Demonstration of uniform HMW glutenin patterns (2\*, 7+9, 5+10) for several individuals of  $dh_2$ -progeny of doubled haploid line  $dh_17029$



DNA from single plants (each from another ear) of dh<sub>2</sub> 17029 were amplified with the three sets IAG95, PSP2999, and 011B, specific for 1RS<sub>Insave</sub>-(Pm17), 1AS-, and 1BS-chromosomes arms, respectively. The polymerase chain reaction products were separated in 1.5% agarose. The translocation type was determined from presence (1) or absence (0) of the 1RS-, 1AS- and/or 1BS-specific PCR amplification product. For comparison a wheat line (dh<sub>2</sub>21933 b) without 1RS translocation was incorporated

Fig. 6. Proof of the 1AL.1RS<sub>Insave</sub>, 1BL.1RS<sub>Insave</sub> wheat-rye double translocation in six individuals of dh<sub>2</sub>-progeny of the doubled haploid line dh 17029 by means of PCR amplification



Giemsa pattern is showed for seedling K3 of F<sub>1</sub> hybrid UE118 1AL.1RS indicated by arrow (↖); 1BL.1RS indicated by arrow (←); 1BL.1BS indicated by arrow (↙)

Fig. 7. C-banded mitotic metaphase chromosomes of F<sub>1</sub> hybrids derived from crosses involving the 1AL.1RS<sub>Insave</sub>, 1BL.1RS<sub>Insave</sub> double translocation line dh 17029, as the female, and different untranslocated wheat forms as the male parent

dh<sub>2</sub>-plants had uniformly the Pm17 resistance gene coded by the short arm 1RS<sub>Insave</sub> on the basis of the STS marker-primer sequences (MOHLER 1999 – pers. comm.; BRINGEZU *et al.* 1999).

The cytological data obtained by the investigation of mitotic root tip cells of single seedlings of F<sub>1</sub> hybrids: UE117, UE118 and UE119 provide an additional indication for the relative genetic stability of such double translocation lines. Thus, all F<sub>1</sub> plants contain 42 chromosomes which include three satellite chromosomes (2 × 6B, 1 × 1B; cf. Fig. 1b–d) and only one 1AL.1RS<sub>Insave</sub> and one

1BL.1RS<sub>Insave</sub> translocation chromosome (Fig. 7). Instead, this C-banding analysis indicates, that one normal 1BL.1BS wheat chromosome is present as expected.

Therefore, it can be concluded, that the dh 17029 exhibits a normal behaviour at meiosis and that all in all, such 1AL.1RS<sub>Insave</sub>, 1BL.1RS<sub>Insave</sub> wheat-rye double translocation lines are relatively genetically stable. The results support consequently earlier statements (VAHL *et al.* 1993). Further investigations are in progress in order to analyse the chromosomal stability in meiosis.

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## Abstrakt

MÜLLER G., VAHL U., BRINGEZU T. (2001): **Stabilita linií s dvěma pšenično-žitnými translokacemi 1AL.1RS a 1BL.1RS.** Czech J. Genet. Plant Breed., 37: 6–11.

K určení struktury chromozomů a stability dihaploidní linie dh17029 s dvěma pšenično-žitnými translokacemi 1AL.1RS<sub>Inavc</sub> a 1BL.1RS<sub>Inavc</sub> bylo využito stanovení počtu chromozomů, Giemsa C-banding, biochemických a molekulárních markerů. Získané

výsledky umožňují závěr, že u linie dh17029 probíhá meiosa normálně a že obecně takové dihaploidní linie s dvojitou pšenično-žitnou translokací 1AL.1RS<sub>1msvc</sub>, 1BL.1RS<sub>1msvc</sub> získané z prašnickových kultur jsou geneticky relativně stabilní.

**Klíčová slova:** *Triticum aestivum*; dihaploidní linie; pšenično-žitná dvojitá translokace; biochemické markery; molekulární markery; C-banding; počítání chromozomů; odolnost k padlí travnímu

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## First Results on QTL-Analysis for *Barley Yellow Dwarf Virus* (BYDV) Tolerance in Barley (*Hordeum vulgare* L.)

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**Abstract:** Two DH-populations derived from crosses of the *Barley yellow dwarf virus* (BYDV) tolerant cultivar Post to cultivars Vixen and Nixe were tested for variation in yield, plant height, and yield components. Measurements were gathered on both non-infected plants and plants of the same DH-line artificially inoculated with BYDV-PAV bearing aphids in pot and field experiments for three years at two locations. For all traits a continuous variation was observed suggesting a quantitative mode of inheritance for tolerance against BYDV-PAV. Two QTL for relative grain yield/plant after BYDV-infection have been identified in the population Post × Vixen which explain nearly 50% of the phenotypic variance.

**Keywords:** barley (*Hordeum vulgare* L.), *Barley yellow dwarf virus* (BYDV-PAV), tolerance, QTL analysis

*Barley yellow dwarf virus* causes one of the economically most important diseases of cereals world-wide. In barley, yield losses up to 40% have been reported (LISTER & RANIERI 1995). The virus is transmitted by aphids, of which *Rhopalosiphum padi* and *Macrosiphum (Sitobion) avenae* are the prevalent vectors in Northern Europe (PLUMB & JOHNSTONE 1995). Symptoms caused by the virus are dwarfing of shoots and roots and leaf yellowing. Furthermore, the number of ears per plant and kernel weight are reduced, the heading-date is delayed and the plants are more susceptible to abiotic stress and fungal diseases compared to healthy plants (HUTH 1995). BYDV-infections can be reduced by sowing cereals when aphid-abundance is low and by application of insecticides. Since monitoring of aphid-populations in the field is very laborious, most sprays are prophylactic. For economical and ecological reasons cultivation of highly BYDV-tolerant cultivars with satisfactory grain yield would be advantageous. For effective breeding of BYDV-tolerant cultivars, knowledge about the genetics of tolerance against this virus is a prerequisite. Therefore, the aim of this study was to analyse the inheritance of tolerance against a German strain of BYDV-PAV by QTL mapping in two segregating doubled haploid (DH) populations.

The mode of inheritance of tolerance against BYDV-PAV was examined on DH-lines of crosses between cultivar Post to cultivars Vixen and Nixe, respectively. Post displayed a high level of tolerance against a German isolate of BYDV-PAV in previous experiments, while Vixen and Nixe showed lower levels of tolerance (HUTH 1995), even though Vixen carries the *Yd2* gene which is known to confer tolerance against BYDV (RASMUSON & SCHALLER 1959). For this study, 80 DH-lines of the cross Post × Vixen and 70 lines of the cross Post × Nixe have been used for map construction and phenotyping in pot- and field experiments. Briefly, plantlets were artificially inoculated with a German isolate of BYDV-PAV by viruliferous *R. padi* in the greenhouse at the one-leaf stage. It was assured that a minimum of 5 aphids fed on each plant resulting in 100% infection rate. Aphids were killed after four days by insecticides. Field and pot experiments were conducted in the growing seasons 1996/1997 to 1998/1999 at Braunschweig, Lower Saxony and Giessen, Hesse, respectively. Due to the fact that the virus content estimated by ELISA is only weakly correlated with yield losses caused by the virus isolate used, the level of tolerance of parents and DH-lines was assessed by measuring kernel yield/plant, thousand kernel weight (TkW),

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ears/plant, kernels/ear, and plant height on infected and non infected controls of the same lines. Additionally, the heading date was recorded in pot experiments. Molecular analysis using random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs, *EcoRI/MseI*) and simple sequence repeats (SSRs) was performed basically as described by ORDON *et al.* (1995), VOS *et al.* (1995) and LIU *et al.* (1996). AFLP and SSR detection was carried out on a DNA-sequencer (LiCor L-4200S-2). RAPDs and AFLPs were named according to the respective primer or primer combination and fragment size or numbered from the smallest to biggest fragment. Linkage maps using Haldane's mapping function (HALDANE 1919) were constructed by Mapmaker 3.0 (LANDER *et al.* 1987) according to SCHÄFER-PREGL *et al.* (1999) using SSRs as anchor markers. Additionally, a CAPS marker, YLpPCRM (FORD *et al.* 1998), was used to screen for *Yd2* in the Post × Vixen population. QTL-analyses was performed using the software PLABQTL Version 1.0 by UTZ and MELCHINGER (1996) and the composite interval mapping procedure (CIM). QTL were calculated across 6 environments (2 locations, 3 years) applying a threshold LOD value of 3.0. Statistical analyses were carried out using the SPSS Version 8.0 (SPSS Inc. 1998).

For Post × Vixen, a genetic map of all chromosomes consisting of 117 markers spanning 1328 c.l. with an average distance between markers of 12.1 c.l. was constructed. The linkage map for Post × Nixe consists of 122 markers with an average spacing of 8.4 c.l. and a total length of 958.5 c.l.. To align maps, markers which showed segregating polymorphic RAPD or AFLP fragments of the same size in both populations were used. Within both populations, the investigated parameters showed a significant variation regarding the reaction to a BYDV-PAV infection in pot- and field experiments (Table 1). Virus infection resulted in reduced grain yield, plant height, number of ears/plant, kernels/ear and kernel weight compared to healthy plants. Besides this the heading date of

infected plants was delayed (data not shown). The level of BYDV-tolerance, estimated as the relative grain yield/plant after infection was lower in the Post × Nixe population than in the Post × Vixen population. The frequency-distribution of the respective traits shows a continuous variation and therefore suggests a quantitative mode of inheritance of tolerance to BYDV-PAV. Based on these data, QTL-analyses on all the above mentioned traits were carried out in the population Post × Vixen. From the breeder's and farmers point of view the relative grain yield is the most important character and two major QTL were detected for this trait (Fig. 1). The positive allele for one was derived from cv. Vixen on chromosome 3HL in the *Yd2* region (LOD 7.7). The second QTL with the positive allele derived from Post was detected on the long arm of chromosome 2H (LOD 3.3). These two QTL together explain 46.8% of the phenotypic and 73.7% of the genotypic variance. For the relative number of ears/plant three QTL with the positive alleles derived from Vixen were detected at LOD 3.6, 3.0 and 4.7, respectively on chromosomes 3H, 4H and 5H. The QTL on chromosome 3 maps to the same chromosomal region as the QTL for relative grain yield. Together these three QTL explain 34% of the phenotypic and 97.7% of the genotypic variance. For relative plant height, one QTL was detected in an interval on the long arm of chromosome 3H adjacent to the *Yd2* region. It was detected at LOD 3.5 and it explains 19% of the phenotypic and 46% of the genotypic variance.

At present, QTL-analyses in the population Post × Nixe are in progress in order to obtain information on the transferability of the respective QTL derived from cv. Post. If this will be the case e.g. for the QTL for relative grain yield/plant on chromosome 2HL respective markers together with those for *Yd2* on chromosome 3HL may facilitate marker based pre-selection for BYDV tolerance in barley reducing the number of plants to be tested in a laborious and time consuming manner by artificial virus transmission using aphids.

Table 1. Means, standard deviation and range of kernel yield/plant, number of ears/plant, thousand kernel weight, number of kernels/ear and plant height relative to non-infected controls averaged across three years and two locations of cvs. Post, Vixen and Nixe and the respective DH-populations

Trait (%)	Post	Vixen	Nixe	Post × Vixen	Min–Max	P*	Post × Nixe	Min–Max	P*
Kernel yield	77.5 ± 17.0	84.5 ± 20.3	58.1 ± 10.8	75.5 ± 11.9	52.2–102.6	0.32	65.2 ± 11.6	41.7–89.1	0.78
Ears per plant	95.0 ± 32.6	99.1 ± 26.3	81.7 ± 23.8	88.5 ± 10.4	67.6–117.2	0.91	81.9 ± 12.4	52.4–110.4	0.85
TKW	96.8 ± 9.4	89.5 ± 4.1	90.5 ± 5.7	93.5 ± 3.9	84.5–103.0	0.65	89.8 ± 4.8	78.1–103.7	0.82
Kernels per ear	97.4 ± 22.4	96.6 ± 3.6	71.5 ± 0.9	91.7 ± 8.9	69.4–119.2	0.51	79.3 ± 8.6	62.7–102.5	0.82
Plant height	98.1 ± 3.3	96.5 ± 4.2	86.8 ± 11.3	94.2 ± 4.2	84.1–102.7	0.93	91.8 ± 4.8	80.3–100.0	0.25

\*P < 0.05 would indicate a significant deviation from normal distribution according to Kolmogorov-Smirnov statistics

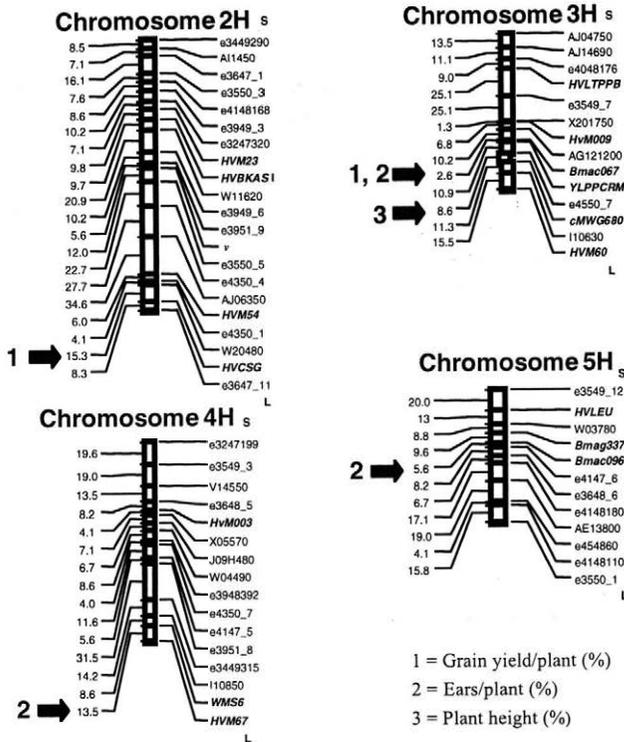


Fig. 1. QTL for relative grain yield/plant, relative number of ears/plant and relative plant height after BYDV-PAV infection detected in a DH-population of the cross Post × Vixen. Markers used as anchors for map construction are written in *italics*

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**Abstrakt**

SCHEURER K. S., HUTH W., WAUGH R., FRIEDT W., ORDON F. (2001): První výsledky QTL-analýzy tolerance ječmene (*Hordeum vulgare* L.) k viru žluté zakrslosti ječmene (BYDV). Czech J. Genet. Plant Breed., 37: 13–16.

U dvou dihaploidních populací, pocházejících z křížení odrůdy Post tolerantní k viru žluté zakrslosti ječmene (BYDV) s odrůdami Vixen a Nixe byla zjišťována variabilita ve výnosu, ve výšce rostlin a v komponentách výnosu. U každé DH-linie byla v nádobových a v polních pokusech (3 roky na 2 lokalitách) prováděna měření na rostlinách jak neinfikovaných, tak na rostlinách uměle infikovaných mšicemi nesoucími BYDV-PAV. Ve všech znacích byla zjištěna kontinuální variace, což poukazuje na kvantitativní dědičnost tolerance k BYDV-PAV. Dva QTL pro relativní výnos zrna z rostliny po infekci BYDV byly identifikovány v populaci Post × Vixen podílející se zhruba na 50 % fenotypické variance.

**Klíčová slova:** *Hordeum vulgare* L.; virus žluté zakrslosti ječmene (BYDV-PAV); tolerance; analýza QTL

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## Genetic Diversity of BYDV Resistant Barley Cultivars Determined by Molecular Markers

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**Abstract:** Genetic diversity was studied in 24 selected spring and winter barley cultivars, that showed a high or intermediate level of resistance in field infection tests in 1994–1997. The majority of lines carrying the *Yd2* resistance gene originated from BYDV nurseries of CIMMYT and ICARDA. Microsatellite (SSR) and AFLP analyses were used to study the diversity of the collection. Resulting dendrograms and principal component analysis (PCA) divided the accessions into several groups, but no clear-cut separation between *Yd2* and non-*Yd2* germplasm could be observed. Most *Yd2* genotypes representing American cultivars were grouped together, whereas other *Yd2* lines of CIMMYT and ICARDA were intermingled with non-*Yd2* genotypes and susceptible checks. Czech spring malting barley cultivars were clearly separated by PCA. The obtained results indicate that the choice of parental lines for *Yd2* gene introgression should be based on DNA fingerprints complemented by pedigree studies and field evaluations.

**Keywords:** BYDV resistance; genetic diversity; barley (*Hordeum vulgare* L.)

Barley yellow dwarf virus (BYDV) causes a serious disease of barley in many parts of the world. The virus is transmitted by aphids and several virus pathotypes have been identified (RASOCHOVÁ & MILLER 1996). The PAV pathotype is the most prevalent in Central Europe. The most widely used sources of resistance to BYDV in barley breeding contain the semi-dominant resistance gene *Yd2* (RASMUSSEN & SCHALLER 1959). The gene was found in landraces of Ethiopian origin and provides the so far most efficient protection against BYDV (BURNETT *et al.* 1995). MAKKOUK and GHULAM (1994) reported that the genetic background of different *Yd2* genotypes may highly influence the rate of virus spread within a plant.

The majority of both spring and winter barley cultivars grown in Central Europe have been found susceptible or very susceptible to the PAV pathotype of BYDV. Among the barley cultivars registered in the Czech Republic, only the spring barley cultivars Malvaz and Atribut and the winter barley cultivar Sigra showed moderate resistance to BYDV (VACKE *et al.* 1997, 1998). This stresses the necessity to improve the level of resistance in European cultivars using resistant exotic germplasm. In spite of this obstacle, DELOGU *et al.* (1995) has developed new winter barley genotypes that combin both *Yd2* resistance and superior agronomic traits. Also other *Yd2* based resistant cultivars have been released (LARKIN *et al.* 1991).

There are, however, several reasons for the search for new resistance genes and genetic resources: (1) *Yd2* does not protect against all virus pathotypes and pyramiding of resistance genes is therefore highly required, (2) the effectiveness of *Yd2* depends much on the genetic background and (3) introgression of the *Yd2* gene from exotic germplasm is a rather time consuming.

The assessment of genetic distances of resistance sources from elite germplasm can help to find suitable donors of resistance genes. The impact of diversity studies on practical breeding has been demonstrated (ORDON *et al.* 1997a; DÁVILA *et al.* 1999). Techniques based on DNA analysis are considered a suitable tool for such investigations (ORDON *et al.* 1997b; ORDON *et al.* 1998; HAN *et al.* 1997). The characterisation and the selection of potential genetic sources of BYDV resistance for barley breeding were the main aims of this study. Their possible importance for practical breeding is discussed in the article.

### MATERIAL AND METHODS

Twenty-four breeding lines and cultivars previously tested for their resistance to BYDV (OVESNÁ *et al.* 2000) by the use of the *Y1p* marker and in field tests were used

Table 1. Characteristics of the examined barley cultivars and lines, their average response to BYDV infection and evidence of *Yd2* gene presence obtained with the use of Y1p (FORD *et al.* 1998) PCR diagnostic marker

Cultivar/line	Pedigree	Origin	Y1p	Response*
81BQCB- 10	F3 Bulk Hiproly	CANADA	+	3.5
Atlas 68	Atlas*3/Ciho 3920/1//Atlas 46/3/4*Atlas/Ciho 1179//2*Atlas57	USA	+	3.4 <sup>+</sup>
Atribut	KMV3-83/EP 79	CZ	–	5.2
CIM 152	UC 76252/NK 71	CIMMYT	+	2.9
CIM 156	WB 163 (Wong in the pedigree)	CIMMYT	+	3.4
CIM 164	Giza 121/Pue	CIMMYT	+	3.0
CIM 173	Br. 6705-15-1/Feebar/4/SP(6h)/Apro//C al. Mr/3/Clla“S”	CIMMYT	+	3.9
CIM 174	F3 Bulk Hiproly	CIMMYT	+	3.8
CIM- CYDBa 88 79	MZ/M 59.247//MI/DS/3/BEN/4/Bahtim-10/5/AP1-CM 67//Alaska	CIMMYT	+	2.4
CIM-CYD Ba 92 16	HASSAN/UNA 1614//Lignee 640	CIMMYT	+	3.3
CIM-CYD Ba 92 71	NOHA“S”/3/ROBUR/42//Astrix/Sutter332.3/4/Gloria“S”/Copal“S”	CIMMYT	+	3.1
Corris	Ciho 3906/1/ABED DEBA (Denmark – DEU based origin)	CIMMYT	+	2.5 <sup>+</sup>
IC-BKL 85 237	PI 002325	ICARDA	+	2.8
IC-BKL 86 35	Gaines/Ore“S”	ICARDA	+	3.1
IC-BKL 92 65	Lignee 527/NK 1272	ICARDA	+	4.1
IC-BKL 93 78	Lignee 527/NK 1272	ICARDA	+	4.5
IC-BYDV 86 141	Br. 6705-15-1/Feebar/4/SP(6h)/Apro//Cal. Mr/3/Clla“S”	ICARDA	+	2.3
Jaspis	ST 6984/72/Opál	CZ	–	7.9
Luxor	LU 27/LU 16	CZ	–	8.9
Madras	R. 62761/4.2606 //Alexis	DEU	–	5.1
Malvaz	28-75/293-77//P1447-77	CZ	–	5.1
Okal	W 77/ HVW – 860	CZ	–	8.7
Perry	Mo. B-475/ Ludwig//Carstens(DEU)	USA	–	5.2
Sigra	Dunja/Ogra (Wong in the pedigree)	DEU	–	4.9
Sutter	Ciho 1237/2(Godollo, Hun)/Winter Tennessee	USA	+	3.5 <sup>+</sup>
Wysor	BYDV resistant Atlas/Hanover (Wong in the pedigree)	CIMMYT	+	2.5 <sup>+</sup>

\* Average response (1994–1997) is based on 0–9 scale, in which 0 – no disease symptoms

+ Reference of *Yd2* gene presence available

in the study (Table 1). DNA was isolated from bulked samples as described by SAGHAI-MAROOF *et al.* (1984). To develop AFLP patterns, the Perkin-Elmer Protocol (Part number 402083, Rev. A, 1995) using EcoRI and MseI restriction enzymes was followed. Altogether 15 primer combinations with 3/3 selective nucleotides were used (AFLP-MseI CCT, MseICAT, MseICTT, MseICTA, MseI CGA combined with each of EcoRI ATG, EcoRI AGG and EcoRIACG). Twelve microsatellite primer pairs were used in our experiments (BECKER & HENZ 1995; LIU *et al.* 1996). PCR was carried out under conditions described by these authors. Amplification products (AFLP and microsatellites) were separated by capillary electrophoresis using a Perkin Elmer Generic Analyzer ABI PRISM 310. The Results were evaluated using GeneScan and Genotyper software. For each genotype, a binary matrix reflecting specific AFLP or microsatellite band presence (1) or absence (0) was produced. Pairwise distances between the

DNA samples ( $n1/n-n00$  – Jaccard's metric) were calculated using the RAPDALG program (The RAPDistance Package.ftp: life.anu.edu.au/pub/software/RAPDistance or http://life.anu.edu.au/molecular/software/rapd.html). UPGMA-clustering and the analysis of principal components were conducted using the statistical software package STATISTICA (StatSoft, Inc.).

## RESULTS AND DISCUSSION

Twelve SSR and thirty-three AFLP primer combinations were initially screened for their ability to produce polymorphic products using five accessions originating from different countries. All the microsatellite primers produced polymorphic bands. Fifteen AFLP primer combinations listed above, out of 68 initially tested, were found suitable for diversity studies in the examined set of genotypes. The

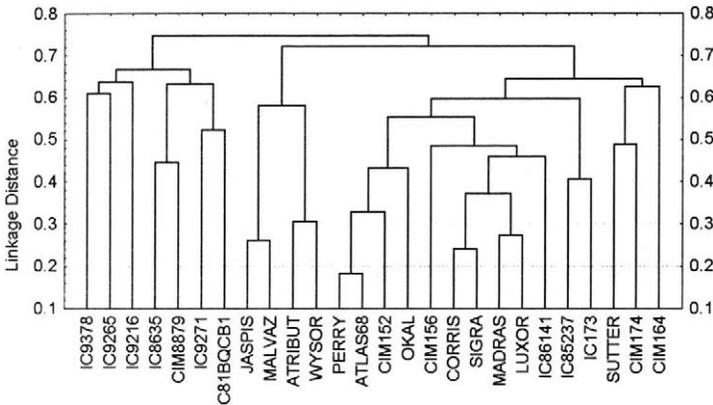
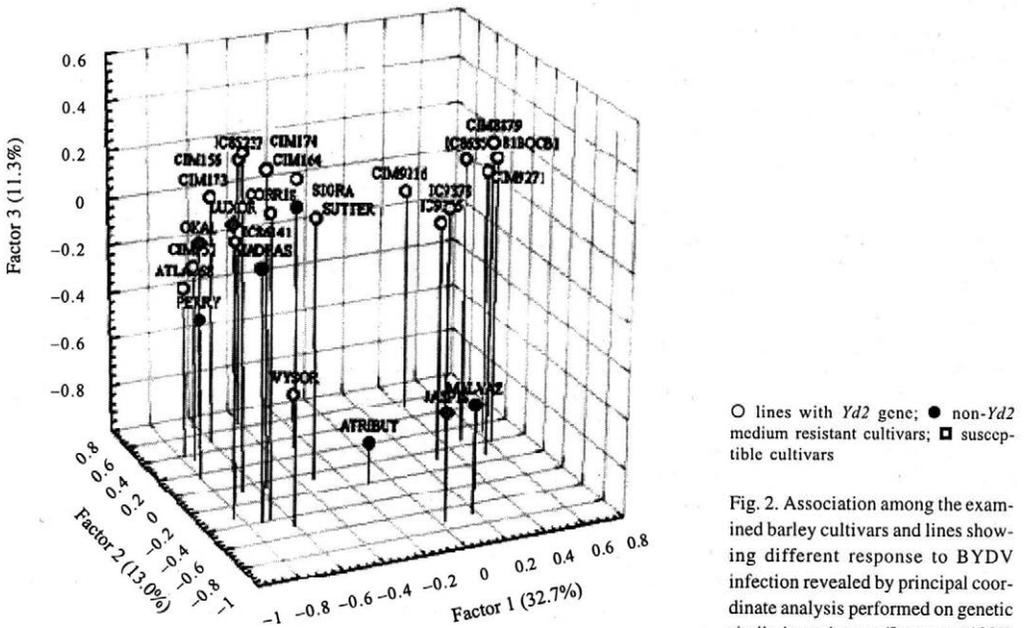


Fig. 1. Dendrogram generated using WPGMA analysis demonstrating the relationships among 26 barley (*Hordeum vulgare* L.) accessions listed in Table 1

observed level of polymorphism, produced by selected markers, was generally sufficient. The markers produced 155 different polymorphic bands across 26 studied accessions. The analysis permitted to distinguish all the genotypes, even though the sister lines IC BKL 92 65 and IC BKL 9378, 81 BQCB-10 and CIM 174, and IC BYDV 86 141 and CIM 173 originated from the same crosses (Table 1). This indicates that the set of analysed accessions does not contain genetic duplications and that the accessions with the same pedigree can be considered as sister lines. Relative genetic similarity (JACCARD 1907) within the investigated set, based on evaluation of DNA markers, was

estimated between 0.18 (Perry vs. Atlas 68) and 0.87 (Okál vs. IC92 71), which is a higher value in comparison with studies of ORDON *et al.* (1997a, b), MANNINEN and NISILÄ (1998) or OVESNÁ *et al.* (2000, 2001). Unlike ORDON *et al.* (1997a, b), who compared European and Asiatic germplasm, other authors studied local gene-pools. Barley lines and cultivars described in this paper are derived from wide crosses of genetic resources of worldwide origin. Therefore the degree of dissimilarity is rather high.

The dendrogram (Fig. 1) resulting from these data divided the accessions into four main groups. The first cluster contained only *Yd2* genotypes. Pedigree analy-



○ lines with *Yd2* gene; ● non-*Yd2* medium resistant cultivars; □ susceptible cultivars

Fig. 2. Association among the examined barley cultivars and lines showing different response to BYDV infection revealed by principal coordinate analysis performed on genetic similarity estimates (JACCARD 1907)

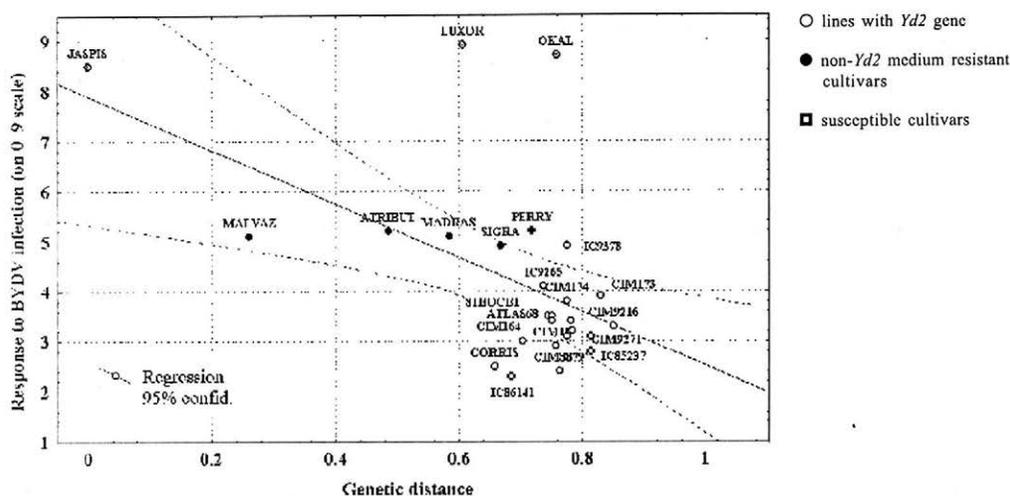


Fig. 3. Relationship between genetic similarity and average response to BYDV infection of selected barley cultivars and lines in comparison to the adapted malting barley cultivar Jaspis

sis did not indicate the presence of germplasm often used in Germany. However, it is impossible to trace back all the pedigree data. The second group included three malting spring barley cultivars and the winter cv. Wysor, pedigree of which is not clear enough, but it contains spring cv. Atlas (*Yd2*) and elite German cultivars. The third group appears to be a particular mixture of spring and winter types developed in Europe and America, but many cultivars belonging to this group involve in their pedigree a source of quality (e.g. Corris is a cross between an African landrace and high grain quality Danish barley based on Weihenstephaner Trauernresistenz II originating in Germany). Therefore, a relatively high degree of similarity between e.g. cv. Corris and the German cv. Sigra may be comprehensible. Cv. Sigra likely contains a portion of exotic germplasm, because it has in its pedigree cv. Wong – from a cross between Orel, a landrace from Russia, and a line of a Chinese cultivar from the Sechuan province (both are potential donors of resistance). Cv. Wong can be found also in the pedigree of other donors of resistance in the collection. The dispersion of CIMMYT genotypes across European germplasm was also found by HAYES *et al.* (1997). A concept of crossing different gene-pools is widely used in CIMMYT programs. This might explain the dispersion of these materials into different clusters. Three American *Yd2* genotypes, bred predominantly with the use of US genetic resources, were found in the fourth cluster.

The principal component analysis (PCA, Fig. 2) also revealed variability of the investigated set in a similar pattern as did the cluster analysis. From the total varia-

tion 57% were explained by the three components PC1, PC2 and PC3. The PC1 and PC2 components divided the *Yd2* genotypes into two groups – one of them corresponds to the cluster one in the dendrogram, the second one includes both *Yd2* and non-*Yd2* genotypes. PC3 separated clearly Czech spring malting barley cultivars Atribut, Jaspis and Malvaz.

The relationship between genetic similarity and average response to BYDV infection in comparison to the adapted malting barley cv. Jaspis (Fig. 3) demonstrated a higher degree of variability in non-*Yd2* genotypes possessing a medium resistance level as compared to *Yd2* genotypes. The relative genetic distances of *Yd2* genotypes from Jaspis were similar. However, cluster analysis and PCA grouped the *Yd2* genotypes into different clusters. This grouping corresponds better with the results obtained from pedigree studies (mentioned above) and with the observed large differences of these materials in the morphotype, heading date, plant type and other agronomically important characters (OVESNÁ *et al.* 2000). COMEAU and JEDLINSKI (1990) noted that the use of *Yd2* resources may be associated with introgression of some non-desirable traits. *Yd2* genotypes may be suitable for resistance gene transfer to adapted germplasm if broadening of genetic diversity is required. The members of the different clusters may differ in their genetic background. They may contribute to the new genotypes in a different way, even when the genetic distances from an adopted genotype are similar. The obtained results indicate, that detailed pedigree analysis and assessment of other important breeding traits are important prerequisites of a

successful selection of parental lines carrying the *Yd2* gene. Non-*Yd2* medium resistant cultivars could be suitable for faster introduction of resistance genes into well-balanced genetic background. Some of these cultivars, like Atribut and Malvaz are likely to be appropriate candidates for the incorporation of *Yd2* into malting genotypes and for pyramiding BYDV resistance genes.

The obtained DNA fingerprints can be used for monitoring saturation of the genetic background by the recurrent parent genome. DNA fingerprints complemented by pedigree analyses and field evaluation, together with the use of PCR markers like Y1p (FORD *et al.* 1998), can be considered as effective tools in breeding for resistance to BYDV in barley.

**Abbreviations:** SSR – single sequence repeats, AFLP – amplified fragment length polymorphism, PCA – principal component analysis

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#### Abstrakt

OVESNÁ J., ŠÍP V., KUČERA L., CHRPOVÁ J., NOVÁKOVÁ I., JAHOR A., VACKE J. (2001): **Genetická diverzita odrůd ječmene rezistentních k BYDV stanovená molekulárními markery.** Czech J. Genet. Plant Breed., 37: 17–22.

Studovali jsme genetickou diverzitu vybraného souboru 24 odrůd jarního a ozimého ječmene, které v polních infekčních testech v letech 1994–1997 vykazovaly vysokou nebo střední míru odolnosti vůči BYDV. Většina linií, které nesly gen *Yd2*, pocházela z programu ICARDA/CIMMYT (BYDV školky). Pro studium diverzity byla využita analýza délkové variability mikrosatelitů a AFLP. Na jejich základě byla provedena klastrová analýza. Podle dendrogramu i analýzy hlavních komponent byly genotypy rozděleny do několika skupin, avšak jasná separace skupiny genotypů s genem *Yd2* a genotypů bez tohoto genu nebyla zjištěna. Genotypy s genem *Yd2* založené na amerických odrůdách byly zahrnuty ve stejném klastru, zatímco další materiály s genem *Yd2* z CIMMYT a ICARDA byly zahrnuty do klastrů s liniemi a odrůdami bez genu *Yd2*. České odrůdy jarního sladovnického ječmene byly jasně separovány na základě analýzy hlavních komponent. Získané výsledky prokazují, že při výběru donorů genu *Yd2* do šlechtitelských programů by měla být zvažována i genetická vzdálenost rodičovských materiálů, odvozená na základě DNA fingerprintingů, současně s výsledkem analýz rodokmenů a polních hodnocení.

**Klíčová slova:** odolnost vůči BYDV; genetická diverzita; *Hordeum vulgare* L.

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## Identification of Barley (*Hordeum vulgare* L.) Cultivars using Microsatellite Analyses

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**Abstract:** Barley microsatellite length polymorphism was tested in this study for its ability to produce cultivar specific DNA profiles. Fourteen microsatellite loci were examined across 22 cultivars registered in the Czech Republic. The diversity index of individual markers varied from 0.368 to 2.413. The microsatellite marker HVM40 amplified most of the polymorphic products. Totally, 11 different alleles were identified by the marker. The marker was able to distinguish all the studied cultivars, even though they were mostly two row spring malting barley genotypes, which were known to have similar well-balanced genetic background. Microsatellite polymorphism was found to be suitable for cultivar differentiation as a possible complementary system to morphological markers and protein spectra analyses. The microsatellite analysis separated clearly the spring barley cultivars from the winter barley cultivars. The oldest spring malting cultivar Proskovcův Hanácký, developed more than 100 years ago at the end of the 19<sup>th</sup> century, was identified completely separated from the group of modern genotypes.

**Keywords:** *Hordeum vulgare* L.; microsatellite; DNA profile; cultivar identification

Barley was one of the first domesticated crops (RUSSELL *et al.* 1997) and belongs to the major cereals in the world. Barley is used for feeding and in the food and brewing industry. The knowledge of genotype differences is essential for cultivar identification and also for the selection of crossing parents in breeding for resistance to diseases or pests, for high quality and yield.

Traditionally, morphological characters have been used to evaluate officially distinctness, uniformity and stability of genotypes and to describe them (JARMAN & PICKETT 1992). An examination of morphological characters is laborious (COOKE 1984) and influenced by environment. Identification of plant cultivars is important from several aspects. It allows to protect breeders rights, to verify seed lots and to differentiate between germplasms. Electrophoresis of seed storage proteins has been used extensively for the identification of cereal cultivars (JARMAN & PICKETT 1992). Isozyme markers have also been used (COOKE 1989). Biochemical markers, however, have shown a limited ability to distinguish barley cultivars (HENRY *et al.* 1997). DNA analyses are the most effective for these purposes. RFLP (Restriction fragment length polymorphism – BOTSTEIN *et al.* 1980), RAPD (Random Amplified Polymorph DNA – WELSH & MCCLELLAND 1990; WHITKUS *et al.* 1994), SSR (Simple Sequence Repeat) or STR (Short Tandem Repeat –

TAUTZ 1989; WEBER & MAY 1989) and AFLP (Amplified Fragment Length Polymorphism – ZABEAU & VOSS 1993) are the most common methods used for DNA analysis. It was found that RAPD is not fully reproducible between laboratories (JONES *et al.* 1997). The RFLP method is very laborious (MELCHINGER *et al.* 1994; POULSEN *et al.* 1996). SSR markers are the most polymorphic, but AFLP has a higher diversity index because of a higher amount of bands. Microsatellites are particularly attractive for the differentiation of cultivars, since they exhibit the highest level of variation of all the molecular methods (SAGHAI-MAROOF *et al.* 1994; BECKER & HEUN 1995; POWELL *et al.* 1996; COOKE & REEVES 1998).

### MATERIALS AND METHODS

Twenty-two barley cultivars, registered in the Czech Republic and mostly of Czech origin, were tested in this study. The old malting quality spring barley variety Proskovcův Hanácký (also known as Proskowetz Haná Pedigrée) was included for comparison (Table 1). Genomic DNAs were extracted from young leaves (14 days old) using the protocol of SAGHAI-MAROOF *et al.* (1984) from bulked leaves of minimally 30 individual plants. Fourteen microsatellite primer pairs (BECKER & HEUN 1995; LIU *et*

Table 1. List of barley (*Hordeum vulgare* L.) cultivars used in the study, indicating their names, pedigrees and origins

No.	Cultivar	Pedigree	Origin country
1	Akcent	HVS 827(Salome)/EP 79	Czech Republic
2	Amulet	HE 2591/Salome	Czech Republic
3	Atribut	KM V 3-83/BR 2174	Czech Republic
4	Forum	(H 387/75×(Horpatsi Ketscoros×H1712(H 387-75//Horpatsi Ketscoros/H 1712-78//044-78	Czech Republic
5	Jarek	Km 1192/Sladar//Opal	Czech Republic
6	Jubilant	SK 1952-7/Dera	Czech Republic
7	Kompakt	Galan/KM-A10	Slovak Republic
8	Krona	Nebi/Trumpf/1192/Hor. 1873/Union/Gimpel	Germany
9	Krystal	Koral/Rapid	Czech Republic
10	Luxor	LU 27/LU 16	Czech Republic
11	Novum	Sk 1429/Krystal	Czech Republic
12	Okál	Rubina/HVW 860	Czech Republic
13	Olbram	HVS 1703-82/BR 2174	Czech Republic
14	Primus	Jaspis/E 1197-85	Czech Republic
15	Profit	Koral/3/H 357//Juliana/Jantar	Czech Republic
16	Proskovcův Hanácký	LV-Hana/Kargyn//Schwarzenberg barley	Czech Republic
17	Rubin	Valticky//Algerian/Valticky/Union//Diamant/H. St. 13603/64	Czech Republic
18	Scarlett	Amazone/Br. St. 2730e/Kym	Germany
19	Sladko	SK 2043-38-78/HVS 827-77	Czech Republic
20	Stabil	Orbit/HE 2592	Czech Republic
21	Terno	S 170-74/Opal	Czech Republic
22	Tolar	HE 4710/HVS 78267-83	Czechoslovakia

*al.* 1996; EU project SCRI database) were used in this investigation. The forward primers were fluorescently labelled. PCR was carried out as described by the authors. The products of amplification were separated by capillary electrophoresis using the Perkin-Elmer Genetic Analyser ABI PRISM 310. A binary matrix was prepared, representing presence (1) or absence (0) of allele specific signal. Data were evaluated using GeneScan, Genotyper (PE Biosystem) and UNISTAT software.

## RESULTS AND DISCUSSION

The application of DNA profiling for the protection of breeders rights to plant cultivars and for official registration is still a matter of discussion. Morphological traits are used along with protein analysis for cultivar description and identification according to UPOV rules. However, molecular markers represent a fast and efficient tool for cultivar authenticity and purity evaluation (LAW *et al.* 1998).

In the present work we describe the possible use of SSR analysis for the identification and distinction of 22 mainly two row spring barley cultivars registered in the Czech Republic. Fourteen SSR primer pairs were tested cover-

ing all the linkage groups – 2 microsatellites per each chromosome. At these 14 loci 72 alleles were identified, ranging from 2 to 11 alleles per locus. The distribution of allele frequencies across some of the evaluated cultivars is demonstrated in Fig. 1. A similar set of barley cultivars was evaluated using protein markers by BRADOVÁ *et al.* (1998). They have found 10 different esterase loci, of which only four are currently used for genotype identification of barley cultivars. The number of hordein alleles is also definite (ČERNÝ & ŠAŠEK 1998). Generally, protein analysis can provide information about a limited number of loci. In contrast, tens of microsatellite loci have been mapped on each barley chromosome (SCRI database, Grain genes database). Since considerably more details of cultivar genomes can be obtained with microsatellite markers, this method is more powerful. However, protein analysis provides still some advantages over microsatellite analysis. Up to now, instrumental equipment for microsatellite analysis is rather expensive and also the running costs (consumables: 4 USD per sample) seems to be rather high for the current use. Therefore, bulked samples are usually evaluated by DNA analysis. In consequence, the total variability is detected regardless of the representation of individual lines in the cultivar, which can vary in some degree during the seed

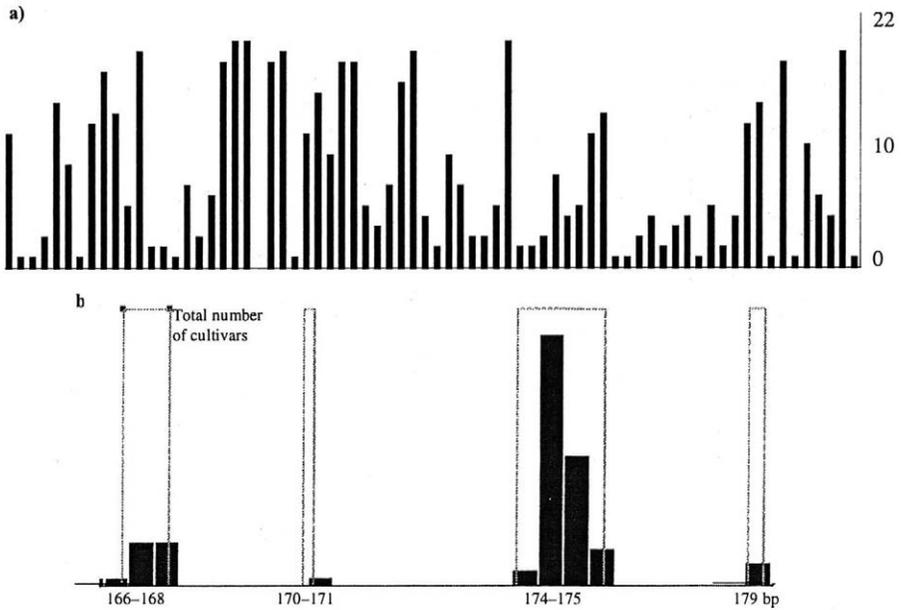


Fig. 1. The frequency of occurrence of individual alleles across evaluated accessions: a) A total overview – each column corresponds to an allele (category) sorted in the same order as in electronic profiles, the height of the column indicate the number of accessions in which the allele was identified; b) The presence of individual alleles (categories) detected by microsatellite HvPAF across all the accessions, the sizes are indicated in bp

propagation. Usually 100 individual plants/grains per cultivar are evaluated when protein analysis is considered. In case of heterogenous cultivars, protein analysis

Table 2. Diversity indices (DI) calculated according DAHLEEN (1997) ( $DI = -\sum p_{ij} \ln p_{ij}$ ) from banding patterns identified by microsatellite length polymorphism of 22 barley cultivars

SSRs	DI	<i>n</i>
HVM40	2.413	11
HvLTPPB	2.259	8
HVM03	2.197	6
HVM54	2.003	7
Bmac0181	1.997	6
HvPAF	1.684	4
HvABA	1.602	3
HVM04	1.403	4
Bmag0211	1.401	3
Bmag0222	1.362	6
EBmac0541	0.923	3
Bmac0163	0.837	2
HvWaxy	0.823	3
HvJas	0.368	3

*n* – number of alleles, DI – diversity index, SSRs – microsatellites

run on individual plants/grains meets better the requirements of UPOV and official national authorities. Not all the cultivars are homogenous. The cvs. Jubilant, Olbram, Primus, Scarlet, Sladko, Stabil and consist of 2–3 lines, Terno of four lines, Akcent and Profit are heterogenous. However, different bulks prepared from the same variety did not show differences in the total variability. This permitted us to evaluate the potential of individual microsatellites for cultivar evaluation, e.g. for diversity studies.

Diversity indexes (DI) were calculated for all the markers (Table 2). The microsatellite HVM40 (LIU *et al.* 1996) detected the highest number of alleles (11) and had also the highest diversity index. Only two alleles were detected using the Bmac0163 microsatellite. The lowest diversity index was calculated for HvJas, since for it the smallest number of deviations from the main allele(s) was recorded. None of the microsatellites detected zero alleles. HVM40 is able to discriminate uniquely among all studied cultivars.

Amplification products were sized using the Genotyper program and divided into individual categories. The profiles were converted into binary data and further used for (1) the generation of cultivar-specific electronic profiles (Fig. 2) and (2) for cultivar differentiation. The electronic profiles can be given either as a table, where presence of the product belonging to certain category is marked as 1 (absence as 0) or in a graph, where the pres-

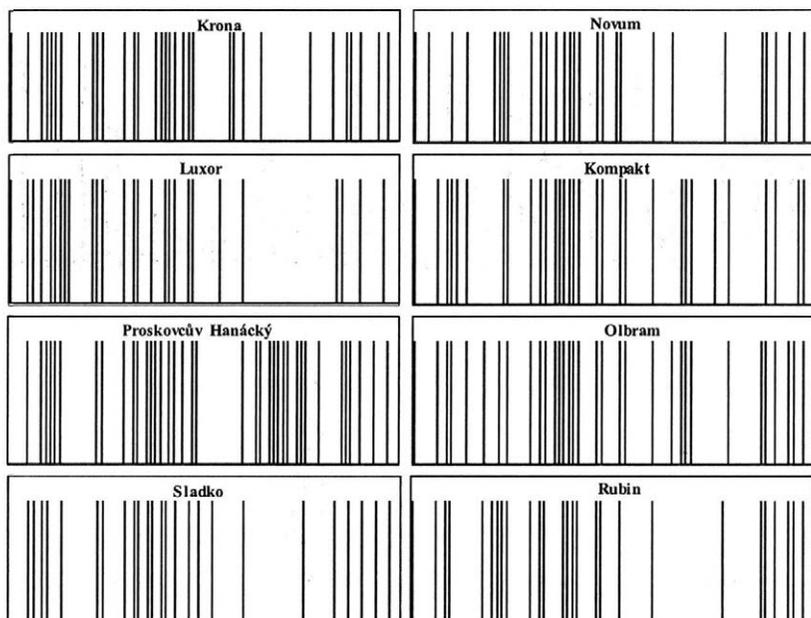


Fig. 2. Electronic DNA profiles of several barley cultivars registered in the Czech Republic based on polymorphism of 14 microsatellite loci and 72 alleles (categories). The presence of individual allele is marked as a black line

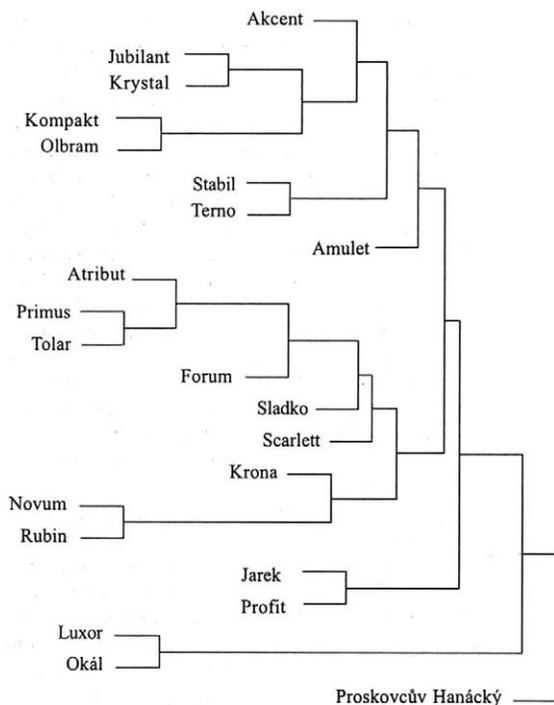


Fig. 3. Dendrogram plot based on city block analysis of allele numbers and frequencies depicting patterns of genetic diversity of a set of barley cultivars registered in the Czech Republic estimated by SSR markers

ence of the amplification product is depicted as a line. The categories are sorted in the same order for every sample. All the electronic profiles are maintained in a catalogue which is available at the RICP and will also be seen at the web page [www.vurv.cz](http://www.vurv.cz) after January 31, 2001. The profiles can be used as etalons of cultivars in future.

The level of variability, revealed by the 14 microsatellite markers, in the set of studied cultivars was sufficient to perform a cluster analysis. The dendrogram plot (Fig. 3), based on the City block analysis of the allele numbers, shows three clusters of spring cultivars. The two winter six row cultivars were separated from the spring cultivars. The over hundred years old spring malting cultivar Proskovčův Hanácký, included in the study, was identified clearly outside the pool of recent varieties by this approach. The Figure 3 graphically illustrates the utility of the marker set for cultivar identification.

Microsatellites could be useful for the identification of related malting barley cultivars (listed by LEKES 1961, and in the GRIN and EVIGEZ databases) used in the Czech Republic. Several techniques are available, that enable to use just a small piece of leaf tissue as a direct template (MARTYNKOVÁ *et al.* 1997) or to use primary products like malt as a source of DNA for SSR analysis (PECCHIONI *et al.* 1997). This is another advantage of DNA markers over protein analysis, which is regularly done only from non-processed grains. It is possible to expect, that DNA markers might become soon included into the official documentation for the registration of new cultivars.

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## Abstrakt

POLÁKOVÁ K., OVESNÁ J., LEIŠOVÁ L. (2001): **Identifikace odrůd ječmene (*H. vulgare* L.) na základě analýzy mikrosatelitů.** Czech J. Genet. Plant Breed., **37**: 23–28.

Testovali jsme možnost využití délkového polymorfismu mikrosatelitních lokusů ječmene pro odvození odrůdově specifických DNA markerů. Využili jsme 14 mikrosatelitních lokusů, které jsme použili pro rozlišení 22 odrůd ječmene registrovaných v ČR. Index diverzity se u jednotlivých markerů pohyboval v rozmezí od 0,386 do 2,413. Nejvíce polymorfních produktů bylo amplifikováno pomocí markeru HVM40. U analyzovaných odrůd bylo nalezeno 11 odlišných alel. Tento marker byl schopen rozlišit všechny testované odrůdy, i když se jednalo především o české jarní sladovnické ječmeny s dobře vyváženým genotypem. Prokázali jsme, že analýza polymorfismu mikrosatelitů je vhodnou metodou pro rozlišení genotypů ječmene a může být vhodným doplňkem morfologických hodnocení a analýzy proteinových spekter. Na základě analýz mikrosatelitů byly testované odrůdy ječmene rozděleny na jarní a ozimé. Zcela jako outlayer byla identifikována nejstarší registrovaná odrůda sladovnického ječmene Proskovcův Hanácký (syn. Proskowetz Haná Pedigrée), vyšlechtěná před více než 100 lety.

**Klíčová slova:** ječmen; mikrosatelitní DNA; DNA profil; identifikace odrůd

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## The Use of Microsatellite Analysis for the Identification of Wheat Varieties

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**Abstract:** The use of microsatellite length variability for the determination of distinctness between wheat varieties is briefly described. Within 21 microsatellite loci of 15 varieties registered in the Czech Republic we have in sum revealed 132 different alleles. The diversity index (DI) of the tested primer pairs ranged from 0.126 to 0.934 with an average of 0.719, which is generally considered sufficient for this purpose. We proved, that microsatellites are polymorphic enough to distinguish wheat varieties grown in the Czech Republic.

**Keywords:** bread wheat; microsatellite; DNA fingerprinting; variety differentiation

Hexaploid bread wheat (*Triticum aestivum* L.) is one of the most important crop species. It has been widely used for human nutrition and animal feeding, depending on the quality of different varieties. Therefore the identification of varietal authenticity is important for producers and consumers. Up to now, the identification is based on the analysis of storage proteins. This method is fast, reliable and internationally recognised by UPOV. Etalons have

been developed and published in special catalogues (METAKOVSKY 1991; ČERNÝ & ŠAŠEK 1996a, b). Nevertheless, the analysis of storage proteins does not cover the whole genome variability of wheat and requires at least a part of the kernel endosperm.

DNA analysis is at the moment more expensive in comparison with protein analysis, but it is possible to run it at any developmental stage of the plant (MARTYNKOVÁ

Table 1. List and brief description of wheat (*Triticum aestivum* L. var. *lutescens* [ALEF.] MANSF.) varieties used in this study

Name	Pedigree	Registration	Baking quality
Alka	Hana/Mercia	1995	A (7)
Asta	Astyrčanka/Maris Marksman	1994	B (6)
Astella	Viginta/SO-80-2208	1995	B (5)
Blava	Viginta/Fundulea 29	1992	A (6–7)
Brea	BR-918/Hana	1996	E
Danubia	Aurora//Nebojska/Pruduttore/3/Purdue 5517	1984	
Hana	NS-984-1//Mironovskaja 808/Moisson	1985	A (9)
Ilona	Amika//Siete Cerros/Kavkaz	1989	E (7)
Nela	HE 2394/3/Selekt//Slavia/Weihestephan 378-57	1998	A
Sofia	Mironovskaja 808/Artois Desprez//Weihestephan 378-57/M. Huntsman	1990	4
Sparta	Mironovskaja 808/Artois Desprez//A. Desprez/3/M. Huntsman/Weihestephan 378-57	1988	C (4)
Šárka	UH677/Mironovskaja nizkoroslaja//Avalon/Mironovskaja nizkoroslaja	1997	B (4–5)
Vála	Moisson/Mironovskaja 808	1980–1989	
Vlada	Mironovskaja 808/4/Kaštická osin./T. timopheevi//Harrachw./3/Harrachw./S. Pastore/Kavkaz	1990	A (9)
Vlasta	Hana/Brimstone/S13	1999	B

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*et al.* 1997; TERZI 1997) and it covers the whole genome variability. Several techniques are available for this purpose. RFLP is not suitable for routine identification of varieties, since RFLP requires too much of high quality DNA, experienced staff and because only low levels of polymorphism were detected by this approach in wheat (LANDRY & MICHELMORE 1987; KAM-MORGAN *et al.* 1989, DEVOS & GALE 1993). RAPD is a cost effective approach, but low reproducibility between laboratories was reported (JONES *et al.* 1997). AFLP analysis (VOS *et al.* 1994) has not been much used for wheat so far. Microsatellite analysis seems to be the most suitable tool for variety identification. Microsatellites (SSRs) consist of tandem-like repeated nucleotide motifs of 2 to 10 bp. When these regions are individually amplified by means of PCR, using a pair of flanking unique oligonucleotides as primers, they usually show high levels of polymorphism as a result of variation in the number of repeated

units (RUSSELL *et al.* 1997; CIOFI *et al.* 1998,). Once the primers are developed, the technique is easy to use and is suitable for automation.

The main aims of the work were to develop DNA fingerprints of selected registered wheat varieties and to prove the suitability of microsatellite analysis for the identification of varieties registered in the Czech Republic.

### MATERIAL AND METHODS

Fifteen registered wheat (*Triticum aestivum* L.) varieties were used in this study. They are listed and characterised in Table 1. DNAs were isolated from young bulked leaves harvested from at least 30 plants raised in the greenhouse, according to the protocol published by SAGHAI-MAROOF *et al.* (1984). Twenty-one pairs of fluorescently labelled microsatellite primers (Table 2) were

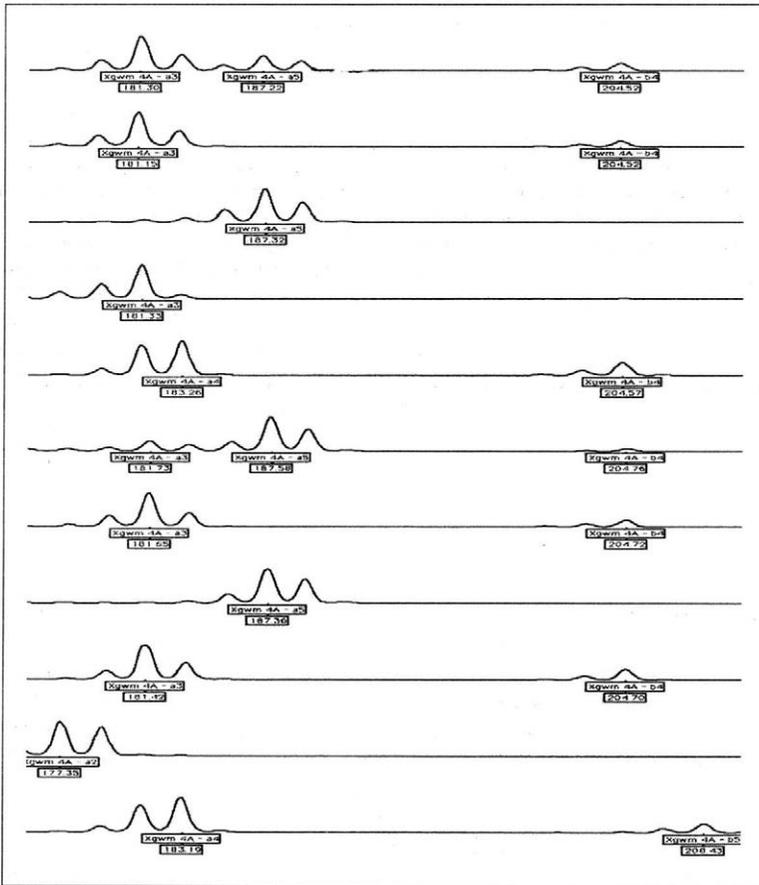


Fig. 1. DNA profiles of several wheat varieties amplified by the primer pair corresponding to the microsatellite Xgwm-160-4A. The alleles were numbered, catalogued and further used to plot the electronic profile of each variety

used for DNA profiling of the selected varieties under conditions described by RÓDER *et al.* (1998). Amplification was carried out in a MJ Research PTC 200 thermal cyclers. Amplification products were separated first in 1.5% agarose gel to control product quality and then analysed by capillary electrophoresis using a Genetic Analyser ABI PRISM 310 (PE Applied Biosystems). The Results were evaluated using GeneScan and Genotyper software. The potential of individual microsatellites to differentiate varieties was calculated as proposed by DAHLEEN (1997), using banding patterns identified by microsatellite length polymorphism:

$$DI = -\sum p_{ij} \ln p_{ij}$$

where:  $p_{ij}$  – frequency of the  $j^{\text{th}}$  pattern for the microsatellite  $i$  and the summation extends over all patterns

## RESULTS AND DISCUSSION

Chromosome specific primer pairs for 21 microsatellites were tested for their ability to differentiate wheat varieties. Each primer pair amplified the microsatellite loci at a different chromosome, so that all chromosomes were covered by the markers. The number of amplified products corresponds in the case of microsatellites to the number of different alleles in the locus/loci (Fig. 1). The capacity of individual primer pairs to differentiate varieties was different (Table 2). Primer pairs producing only monomorphic products were not found, however, the microsatellite Xgwm 124-1B-HEX amplified only one type of product, which was only rarely present in the studied set of varieties. On average 6.3 alleles per primer pair were found in the evaluated varieties, the maximum was 13 alleles per primer pair (Xgwm 314-3D-TET). The presence

or absence of individual alleles was scored and treated as diagrams (Fig. 2), which formed variety specific profiles. The diversity index, calculated for all microsatellites, ranged from 0.934 to 0.126 (Table 2), with an average of 0.719. The microsatellites showed high potential for the monitoring of distinctness between the used wheat varieties. Although VARSHNEY *et al.* (1998) reported low capacity of microsatellites (SSR) to differentiate between wheat genotypes, in our case the microsatellite analysis was very efficient. The separation of amplification products by capillary electrophoresis provides probably more precise results in comparison with the hybridisation approach used by these authors. We were able to distinguish all studied wheat varieties using even one microsatellite primer pair localised at chromosome 3D. All pairs of the studied varieties differed in more than one allele. This shows a high potential of this microsatellite to differentiate varieties and also high variability of the evaluated genome region. The other primers had to be used in combinations to differentiate the varieties. The variation of discrimination capacity between primer pairs is largely explicable on the basis of the number of polymorphic fragments (alleles) a primer pair can detect. To ensure a sufficient level of distinctness, the number of polymorphic bands (= alleles) is recommended  $2n$ , i.e. twice the number of analysed varieties (LAW *et al.* 1998). Several combinations of four primer pairs, out of the 21 tested, exceeded this number. The microsatellites Xgwm 608-2D-TET, Xgwm 314-3D-TET, Xgwm 654-5D-TET, Xgwm 332-7A-FAM and Xgwm 611-7B-HEX were the most informative and it is possible to expect, that they can be efficiently used also to differentiate other varieties. It was rather surprising, that the D genome specific primers were the most polymorphic, although it is generally supposed, that the genomes A and B are due to evo-

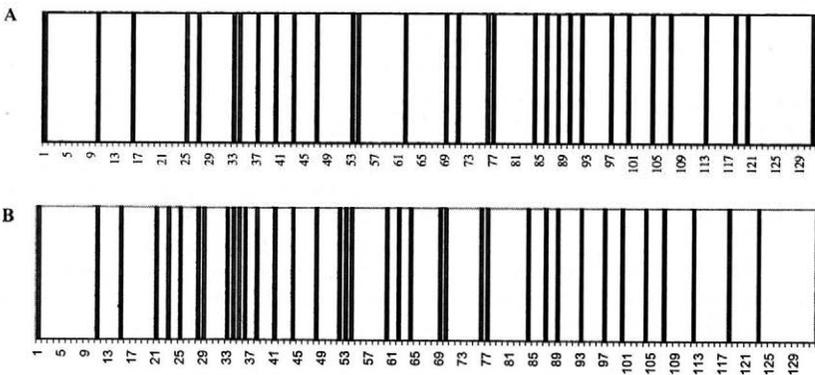


Fig. 2. Examples of electronic DNA profiles of two wheat varieties registered in the Czech Republic, based on polymorphism of 21 microsatellite loci and categorised polymorphic alleles. Profiles of cultivars Astella (A) and Alka (B) are presented. Black lines indicated the presence of individual alleles and form the typical profiles. The detailed description of categories (marker name, size) will be available on [www.vurv.cz](http://www.vurv.cz) after January 31, 2001

Table 2. List of selected primers (RÖDER *et al.* 1998) used for the analysis of wheat microsatellites in 15 varieties registered in the Czech Republic

Primer names chromosome specificity	DI	Number of		Primer names chromosome specificity	DI	Number of	
		alleles	profiles			alleles	profiles
Xgwm 99-1A	0.765	6	5	Xgwm 194-4D	0.776	6	7
Xgwm 124-1B	0.126	1	2	Xgwm 410-5A	0.675	5	4
Xgwm 232-1D	0.816	7	7	Xgwm 554-5B	0.632	2	3
Xgwm 312-2A	0.754	8	9	Xgwm 654-5D	0.899	9	10
Xgwm 120-2B	0.701	4	6	Xgwm 427-6A	0.777	5	6
Xgwm 608-2D	0.934	13	15	Xgwm 70-6B	0.32	2	2
Xgwm 480-3A	0.425	3	3	Xgwm 325-6D	0.77	5	5
Xgwm 181-3B	0.685	7	9	Xgwm 332-7A	0.899	12	12
Xgwm 314-3D	0.918	9	13	Xgwm 611-7B	0.881	9	11
Xgwm 160-4A	0.788	7	7	Xgwm 121-7D	0.794	8	8
Xgwm 6-4B	0.775	4	5	Average means	0.719	6.3	7.1

DI – diversity index (DAHLEEN 1997); number of alleles – number of alleles detected in the studied varieties; number of profiles – number of different patterns recorded over studied varieties

lution much more variable (LIU & TSUNEWAKI 1991; TALBERT *et al.* 1995). The number of microsatellites used in the study might be, however, too low to confirm this.

The described work confirms, that microsatellite analysis can be successfully applied to produce polymorphic DNA profiles of wheat varieties. The profiles could be useful for several applications, especially to measure distinctness of varieties. Their potential for routine variety identification will be further investigated using comparable numbers of individual plants from a variety as it is in the case of storage protein analysis. All developed DNA profiles are documented more in detail with allele sizes and frequencies at the RICP and can be also visited at web-site [www.vurv.cz](http://www.vurv.cz) after January 31, 2001.

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## Abstrakt

LEIŠOVÁ L., OVESNÁ J. (2001): **Použití mikrosatelitní analýzy pro identifikaci odrůd pšenice.** *Czech J. Genet. Plant Breed.*, **37**: 29–33.

V práci je popsáno využití analýzy délkové variability mikrosatelitních oblastí pro rozlišení odrůd pšenice. U 15 odrůd registrovaných v ČR bylo identifikováno celkem 132 odlišných alel v rámci 21 mikrosatelitních lokusů. Pro každý mikrosatelitní marker byl spočten index diverzity (DI), jehož hodnota se pohybovala v rozsahu 0,126 až 0,934. Průměrná hodnota 0,719 je považována za dostatečně vysokou pro rozlišování genotypů. Prokázali jsme, že mikrosatelitní lokusy jsou dostatečně polymorfni a na základě jejich analýzy je možné rozlišit odrůdy pšenice pěstované v ČR.

**Klíčová slova:** pšenice, mikrosatelity, DNA fingerprinting, rozlišení odrůd

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## Genetic Diversity in a Group of Recent Elite Faba Bean Lines

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**Abstract:** Genetic diversity among faba bean inbred lines descending from a group of elite cultivars belonging to both European and Mediterranean regions was measured based on amplified fragment length polymorphisms. Genetic similarities were calculated from these data using Jaccard's coefficient, a cluster was constructed and a Principal Coordinate Analysis was applied. The reliability of the genetic similarity estimates was investigated. Clustering of genotypes indicated consistency with relationships based on pedigree data, and as yet unknown relationships were detected. Several promising parental combinations for breeding purposes were tentatively proposed. Further studies on the precision of the genetic similarity estimates are in preparation.

**Keywords:** genetic diversity; AFLP; *Vicia faba*

Faba bean (*Vicia faba* L.) is an old world grain legume with high seed protein content. It is also characterized by a potential for high yield and low input requirements. The total area devoted to faba bean production has decreased over the last decades. A main reason for this decline has been insufficient breeding progress for yield and yield stability. Broadening the genetic base and systematic exploitation of heterosis in faba bean have been suggested as means to overcome these problems (KITTLITZ *et al.* 1993; BOND 1993). Information on the amount and structure of the genetic diversity in elite germplasm is far from being adequate for breeding purposes (LINK *et al.* 1995; POLIGNANO *et al.* 1993). Estimation of genetic relationships is useful in organizing germplasm, especially when selecting parents for line and hybrid breeding. Earlier, many studies devoted to faba bean and based on morphological and biochemical observations as well on geographical data have been dedicated to these objectives (e.g., AMET 1986; BOZZINI & CHIARETTI 1997; DE PACE *et al.* 1987; HANELT 1972; KÄSER & STEINER 1983; POLIGNANO *et al.* 1993; SERRADILLA *et al.* 1993; SABRAH & EL-METAINY 1985; SUSO *et al.* 1993). LINK *et al.* (1995) have employed the random amplified polymorphic DNA (RAPD) technique to study the genetic diversity within and between European minor and major germplasm and Mediterranean germplasm. They were able to group the European large seeded lines as an intermediate group between the Mediterranean and the European small seeded groups. Amplified Fragment Length Polymorphism (AFLP) has emerged lately as an important technique for genetic diversity studies (ZABEAU & VOS 1993). The advantage of the AFLP assay over

the other DNA markers is the high number of polymorphisms amplified for a single PCR reaction, consequently increasing the speed of data generation.

The study presented here was conducted to (1) investigate the reliability of the AFLP technique for genetic studies in faba bean, and to (2) analyze genetic diversity among a group of inbred faba bean lines descending from elite cultivars originating from various geographical origins.

### MATERIALS AND METHODS

**Plant material:** A total of 22 *Vicia faba* elite cultivars that were released in the European and the Mediterranean markets (18 European spring cultivars, one European winter bean and three Mediterranean cultivars; Table 1) were selected. All cultivars were inbred in Germany at the Institute of Agronomy and Plant Breeding, Göttingen, and at the State Plant Breeding Institute, Stuttgart-Hohenheim, for two to more than 12 generations. One F<sub>1</sub> hybrid resulting from crossing K25 and 34M was included, hence, the material comprised 23 genotypes.

**DNA isolation and AFLP analysis:** Approximately 0.2 g of young leaves harvested directly from 15 days old seedlings (one individual per genotype except for the line K25) was ground in liquid nitrogen to obtain a fine powder. DNA was extracted according to DOYLE and DOYLE (1990). AFLP reactions were performed using the GIBCOBRL AFLP System I (Cat. No. 10544) as described in the manufacture's manual.

**Data scoring and analysis:** A successful AFLP assay of a genotype results in amplification products separat-

Table 1. Information on the faba bean lines used in the present study

Cultivar	Faba bean cultivar	Country of origin	Year of release	Inbreeding generation
European	Gloria	Austria	1983	6
	Karna	Austria	1994	4
	Styria	Austria	1996	10
	Merkur	Czech republic	1997	2
	Mars	Denmark	1993	2
	Maris Bead	England	1965	2
	Maya	France	1995	2
	Music	France	1995	2
	Herz Freya (H22)	Germany	1935	10
	Kristall (K25)	Germany	1973	>12
	Troy	Germany	1985	9
	Gobo	Germany	1987	6
	L1 M × CE	Germany	–	5
	L2 M × CE	Germany	–	5
	Scirocco	Germany	1992	5
	Hiverna (winter bean)	Germany	1986	7
	Alfred	Netherlands	1983	5
	Victor	Netherlands	1988	8
Pistache	Netherlands	1990	5	
Mediterranean	Giza 3	Egypt	~1979	9
	Morocco (34M)	Morocco	–	>8
	Peleponnes (Pel)	Greece	–	>11

ed by gel electrophoresis along the genotype's lane, located at positions on the gel that strictly correspond to the products' length. Any position on the gel which, when scored across genotypes, contains at least once a DNA amplification product will be called herein "fragment". Whenever a given fragment contains a scoreable amplification product, this will be termed "band". Hence, the number of fragments per genotype is a constant number for the experiment, whereas the number of bands per genotype varies across the genotypes. Monomorphic fragments contain an amplification product, a band, in each inbred line, polymorphic fragments don't. Bands were scored as (1), and absent bands were scores as (0). In cases where a band's intensity was markedly less strong than normal but not convincingly absent, a score of (9) was given. It was considered as missing data point and termed "doubtful band". Fragment scoring was performed visually from X-ray films and the resulting 1/0/9 matrix was then used to calculate similarity coefficients. Genetic similarity values (GS values) according to JAC-CARD (1908) were used throughout, applying the software NTSYS-pc version 1.8 (ROHLF 1993).

Monomorphic fragments were excluded from the analysis. The resulting similarity matrix was used to construct

a cluster based on the unweighted pair-group method with arithmetic means (UPGMA); additionally, a Principal Coordinate Analysis (PCoA) was performed. The heterozygous  $F_1$  genotype was excluded from these approaches. To test the precision of the generated GS values, the total data set of 526 polymorphic fragments was randomly subdivided into disjoint data subsamples. Thus five subsamples comprising 100 fragments each, i.e. each representing 19% of the data, three subsamples comprising 150 fragments each (28.5% of the data) and two subsamples, each harbouring 50% of the total data set were taken from the total data set. For all subsamples of AFLP fragments, estimates of all pairwise genetic similarities among the 22 inbred lines were determined, and for each subsample, the coefficient of variation among its GS values was calculated. Furthermore, the corresponding GS values of these subsamples were compared. Comparing only subsamples of equal size, ten comparisons among the five subsamples of 100 fragments were possible. Three were possible among the three subsamples of size 150, and one comparison between the two subsamples each comprising half of the fragments. Spearman rank correlations were calculated to quantify the similarity of GS values of pairs of subsamples.

An estimation of the error variance for the genetic similarity values was performed. For this purpose, for two genotypes two replicates were used. First, two individuals of the inbred line K25 entered into the AFLP assays and scoring procedure. Second, from the scored banding pattern of the genotypes K25 and 34M, the expected scores of the hybrid  $F_1(K25 \times 34M)$  were generated by combining the parental scores, assuming dominant inheritance of the AFLP bands. Thus, a hypothetical genotype  $F_1(\text{hyp})$  was constructed. Since an individual of the hybrid  $F_1(K25 \times 34M)$  has been included in the material, a second case of two replicates was available:  $F_1(\text{hyp})$  vs.  $F_1$ . The GS of the first K25 individual to all other lines was compared with corresponding GS values of the second K25 individual; the GS of the  $F_1$  individual to all other lines was compared with corresponding GS values of the  $F_1(\text{hyp})$  genotype. The GS values among the replicates of the same genotype ( $0.968 < \text{GS} < 0.986$ ) were excluded from the analysis. An ANOVA was conducted to estimate the least significant differences when comparing GS values based on scores of non replicated individuals.

## RESULTS

Screening 15 *EcoR I/Mse I* primer combinations indicated the usefulness of 12 combinations that resulted in a total of 662 fragments, 526 of which were polymorphic (Table 2). With the primer combinations E-ACC/M-CTC, /M-CTG and /M-CTT, the production of scorable fragments was not possible under the employed conditions. Primer combinations varied in the number of polymorphic fragments that were produced and feasible for scoring. Excluding the primer combination E-ACA/M-CAT that resulted in a very low number of scorable frag-

Table 2. Primer combinations (*EcoR I/Mse I*) used for the AFLP analyses

Primer combination	Total number of fragments	% of polymorphic fragments
E-ACA/M-CAC	68	77.94
E-ACA/M-CAG	82	85.37
E-ACA/M-CAT	18	27.78
E-ACA/M-CTA	58	81.03
E-ACC/M-CAG	76	85.53
E-ACC/M-CAT	28	42.86
E-AGC/M-CAC	73	90.41
E-AGC/M-CAG	47	89.36
E-AGC/M-CTA	53	77.36
E-AGG/M-CAC	41	78.05
E-AGG/M-CTA	67	91.04
E-AGG/M-CTC	51	62.75

ments, an average of 43.8 polymorphic fragments were produced per primer combination. AFLP fragment sizes ranged from 50–450 base pairs (bp). Scorable fragments however, were detected between 60–250 bp.

Different approaches to judge the reliability of the AFLP data produced for the studied faba bean lines were used. For the possible 253 GS values, the mean coefficient of variation (CV %) was calculated for a number of subsamples. The mean CV decreased from 13 to 9.48% when employing 19 and 50% of the data respectively (Fig. 1) and reached 8.2% upon considering the whole data set. The effect of the doubtful bands harboured by some fragments on the analysis was also investigated. Doubtful bands amounted to 7.5% of the data, and being

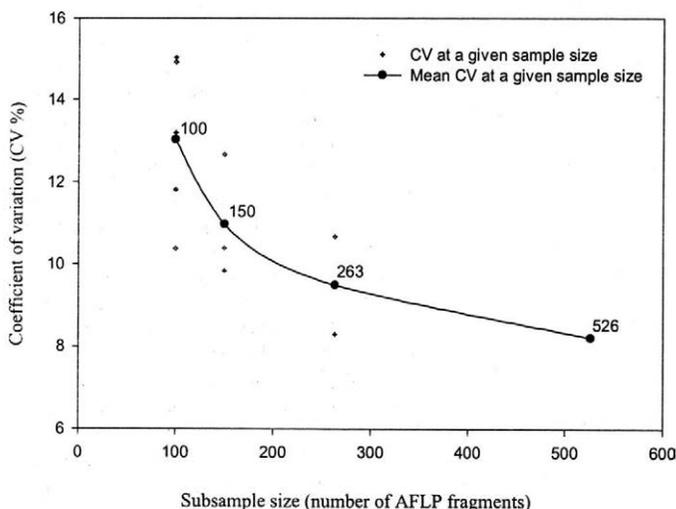


Fig. 1. Coefficient of variation of 253 GS values in disjoint, random subsamples of AFLP fragments

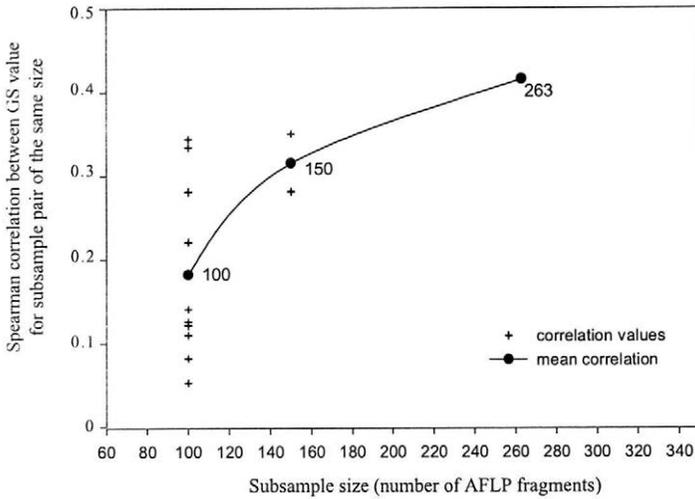


Fig. 2. Correlation of 253 pairwise comparisons (GS values) between any two data subsamples of the same size (for details refer to the text)

distributed across the 526 scored fragments, 297 fragments were scored with one to several doubtful bands. Those 297 fragments were analysed as a disjoint fragment sample and compared to the remaining 229 fragments. On correlating the 253 GS values of both, the 297 and the 229 sample, the Spearman rank correlation coefficient amounted to  $r_s = 0.429$ . Fig. 2 illustrates the relation between the number of fragments considered in a given subsample and the Spearman rank correlation coefficients for the 253 corresponding GS values in pairs of subsamples. A continuous increase in the mean correlation was shown as a result of increasing the number sampled fragments.

The number of differently scored fragments in the case of the two individuals of genotype K25 was two, the direct similarity between these two individuals was  $GS = 0.986$ ; in the case of the  $F_1$ , a total of eleven fragments were scored to be different, and the direct comparison yielded  $GS = 0.968$ . The ANOVA (Table 3) revealed that the standard deviation among the regarded GS values (averages across two replicates) amounted to 10.20% ( $[(10.56 + 82.23 + 12.18)^{1/2}]$ ) on a scale of  $0\% < GS < 100\%$ . This variation is markedly inflated due to the inclusion of the  $F_1$  into this consideration. The standard deviation in the total data

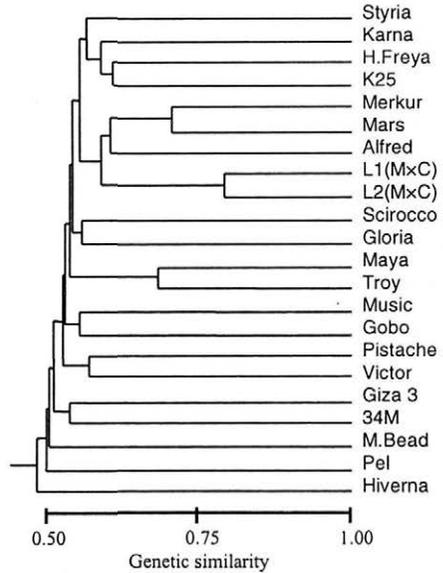


Fig. 3. Grouping of 22 faba bean lines based on 526 AFLP fragments using Jaccard's coefficient of similarity and UPGMA clustering

Table 3. Analysis of variance for two genotypes (G; K25 and F1) in two replicates (R), regarding their GS values to [21 + 2 × 2] = 25 other lines (L); GS values transformed to percent, i.e.,  $0\% < GS < 100\%$

Source	df	Mean squares	Variance components	F
G	1	529.54	10.56	357**
L	24	330.42	82.23	223**
LG	20	25.85	12.18	17**
LRG	46	1.48	1.48	

\*\*significant at 0.01 level of probability



inal GS values that entered this ANOVA; but it is considered to be valid for the whole data set as well. It considers the error that may be caused by minor genetic differences between individuals within the same inbred genotype, partly by non-dominant inheritance of AFLP bands, and by errors that evolve after DNA extraction, probably mainly when scoring.

SNEATH and SOKAL (1973) raised the important question of what is a sufficient number of markers to obtain a stable classification of genotypes. Here, this question was approached twice, by (1) considering the decrease in the variance between the 253 GS values when increasing the fragment subsample size, and by (2) considering the correlations between the GS values in all pairs of data subsamples of same size. As to the first approach, the variance between the GS values is expected to be inflated by error, the more, the smaller the subsample size. The asymptotic decline of the CV in Fig. 1 indicated that already with a number of 263 fragments (i.e. 50% of the total data set), the CV contains only a small residual error variance. As to the results given in Fig. 2 it seems doubtful whether with larger number of AFLP fragments, a correlation near to unity can be attained at all. Our full data set has shown to be very informative, nevertheless, increasing the number of AFLP fragments is advisable. Still this will not eliminate errors. Here some errors obviously occurred, leading to a standard error term of s.e. = 1.22% for the GS values.

Irrespective of these restrictions, the data clearly showed that (1) genotypes such as winter types and exotic genotypes were identified as being distant and that (2) there does not seem to be a very marked structure within the elite, European gene pool as represented with the given genotypes. It might be promising for breeders to establish such structures. A direct, though tentative result may be the identification of a promising parental combination for hybrid or line breeding, such as Scirocco × Gobo, that yielded a significantly lower similarity than, e.g., Scirocco × Mars ( $0.486 < 0.603$ ). Further combinations of elite European lines with low GS values ( $0.477 < GS < 0.493$ ) are Maris Bead × Victor, Troy × Victor, Troy × Merkur, and Styria × Victor. These proposals have of course to be confirmed by further studies, especially by running adequate field trials.

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## Abstrakt

ZEID M., SCHÖN C.C., LINK W. (2001): **Genetická diverzita ve skupině současných elitních linií bobu.** Czech J. Genet. Plant Breed., 37: 34–40.

Genetická diverzita mezi inbredními liniemi bobu pocházejícími ze skupiny současných odrůd z evropského i ze středo-zemního regionu byla zjišťována na základě AFLP (amplified fragment length polymorphism). Z těchto dat byly pomocí Jaccardova koeficientu vypočteny genetické podobnosti, byl vytvořen klaster a podroben analýze hlavních komponent. Zkoumala se spolehlivost odhadů genetické podobnosti. Shlukové seskupování genotypů odpovídalo poměrům založeným na rodokmenových údajích a byly objeveny i dosud neznámé vztahy. Bylo navrženo několik slibných rodičovských kombinací pro šlechtění. Další studie k přesnosti odhadů genetické podobnosti jsou připravovány.

**Klíčová slova:** genetická diverzita; AFLP; *Vicia faba*

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