Characterisation of Powdery Mildew Resistance Donors within *Triticum boeoticum* Accessions using RAPDs

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Abstract: Wide hybridisation has become a technique suitable for introgression of alien genes into modern wheat cultivars. Ten *Triticum boeoticum* Boiss. accessions resistant to powdery mildew were studied in more detail. Variability between the accessions was studied using resistance tests, RAPD and storage protein analysis. Potential gene recipients (*T. aestivum* and *T. durum* cultivars) were also included. Unlike protein analysis we were able to clearly distinguish among the individual accessions of *T. boeoticum* using RAPD. Computed dendrograms reflect phylogenetic relationships among the characterised species. All tests proved, that *T. boeoticum* is a valuable gene-pool. Three *T. boeoticum* × *T. durum* amphidiploid progenies were also analysed. They differ in the degree of resistance to powdery mildew. Both RAPD and storage protein analyses indicated that genome rearrangements occurred. We showed, that RAPD could be used to trace such events.

Keywords: *T. boeoticum*; wild wheat; RAPD; genetic diversity; resistance; wide hybridisation

Powdery mildew is one of the major wheat diseases in Central Europe and in other parts of the world, that can cause considerable yield losses (PRIESTLEY & BAYLES 1998). The disease is caused by the fungus *Blumeria graminis* (DC) Speer f.sp. *tritici* (= *Erysiphe graminis* (DC) f.sp. *tritici* Marchal).

Resistance breeding has been considered important for developing stable yielding cultivars. Several genes conferring resistance to different *B. graminis* isolates have been identified in wheat and closely related species. Some of them have been utilised in commercial wheat varieties. These genes were often soon overcome by new pathogen races, i.e. the resistance was not durable (BENETT 1984). Strategies such as cultivar mixing, diversification or gene pyramiding may increase resistance to powdery mildew (PRIESTLEY & BAYLES 1998). The superior performance of wheat cultivars with the T1BL-1RS or T1AL-1RS wheat-rye translocation (with *Pm8* and *Pm17*, respectively) based on *Secale cereale* L. cultivar Petkus, attributed to the disease resistance (ZELLER 1973), confirms such a possibility.

Sources of resistance genes from current wheat cultivars have been mostly exhausted. Wide hybridisation seems to be still promising for resistance breeding. Within the tribe *Triticeae*, with over 500 taxa and 37 genomically defined genera (DEWEY 1984), wide hybridisation presently belongs to methods enabling introgression of alien genes conferring desirable traits. e.g. pathogen resistance, from wild relatives to hexaploid wheat (*T. aestivum*) (GILL & RAUPP 1987; VALKOUN & BEDO 2001). Transfer of powdery mildew resistance from *Aegilops peregrina* (Hackel) Maire et Weiller (SPETSOV *et al.* 1997), *Aegilops speltoides* Tausch (JIA *et al.* 1996), *Aegilops markgrafii* (Greuter) Hammer (PEIL *et al.* 1997) to hexaploid wheat has been realised. Different techniques are available for such purpose – direct genetic transfer from diploid to hexaploid wheat for rapid introgression of the useful traits (GILL & RAUPP 1987) or tetraploid or tri-ploid bridge techniques (SHARMA & GILL 1983; VARDI 1971).

Potential donors of useful genes are screened by traditional approaches in field tests and also by molecular markers. Several techniques are available, e.g. RFLP, SSR, RAPD (WELSH & MCCLELLAND 1990; WILLIAMS *et al.* 1990; HARTL *et al.* 1993; MA *et al.* 1994a,b; MOHLER & JAHOOR 1996) or AFLP (VOS *et al.* 1995). Among them RAPD is a rapid and inexpensive method, though it has some limitations (JONES *et al.* 1997). Several markers have been also developed by means of RAPD (PEIL *et al.* 1997; SPETSOV *et al.* 1997). Availability of markers in hybrid genome studies reduces the number of progenies needed to

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obtain desirable individuals as it was proved with *Agropyron*-wheat addition lines (MA *et al.* 1994a,b) or *Thinopyrum bessarabicum* × *T. aestivum* amphiploids (WILLIAM *et al.* 1994; WEI & WANG 1995).

Triticum boeoticum Boiss. with genome A^bA^b has been reported as a valuable source of desirable genes conferring protein quality, amino-acid content or resistance (BAHRAI *et al.* 1998; HAIRIT-SINGH *et al.* 2000; ANKER & NIKS 2001; JASMINDER-SINGH *et al.* 2001). Selected *T. boeoticum* accessions were studied at the RICP. A set of potential donors of resistance genes were studied in more detail also using RAPD markers to reveal the potential of RAPD for genetic diversity assessment and evaluation of wide hybrids.

MATERIALS AND METHODS

Plant material. Ten accessions of *T. boeoticum* Boiss. (*Triticum monococcum* L. ssp. *aegilopoides* (Link) Thell.) from the RICP Gene Bank, resistant to powdery mildew, were used. Their origin and properties are given in Table 1. In addition, four proposed recipient cultivars were included – the cv. Panondur and line 3310 of *T. durum* Desf. and the cvs. Regina and Florida of *T. aestivum* L., which are very susceptible to powdery mildew. Three lines derived from *T durum* × *T. boeoticum* crosses were also included: two sister-lines from cv. Panondur × line 5353 and one from cv. 3310 × line 5353.

DNA preparation. Genomic DNA was extracted from young leaves using the protocol of SAGHAI-MAROOF *et al*. (1984). The genomic DNA of each accession was extracted from bulked leaves of 10–20 individual plants. Quality and concentrations of DNA were detected electrophoretically and specrophotometrically. DNA was diluted to obtain a final concentration of 50 ng/µl.

Infection tests. *Triticum* accessions were screened for resistance to powdery mildew. Field resistance to powdery mildew and rust was recorded in the infection field. A row of the susceptible wheat cv. Michigan Amber served as a spreader of spores for the tested accessions. A detailed evaluation was performed in the greenhouse. Seedlings, grown in plastic pots, with about three leaves were dusted with spores, covered for 48 hours with a glass cylinder and evaluated 10 days after inoculation. The inoculum was composed of 9 different isolates collected from several locations in the Czech Republic. For the disease resistance evaluation a 5-point scale was used: 0 – resistant, no reaction, 1 – resistant, 2 – moderately resistant, 3 – moderately susceptible and 4 – susceptible.

RAPD assay. Decamer oligonucleotides used in this study were obtained from Advanced Biosystem Ltd. (England). Stoeffel fragments (Perkin-Elmer) were used for RAPD amplification. The amplification reaction mixture (25 µl) contained 1× buffer (Perkin-Elmer, for Stoeffel fragment), 3mM $MgCl_2$, 0.25mM dNTP, 50 ng primer, 50 ng template DNA and 1U of Stoeffel fragment. DNA amplification was performed using MJ Research 100 thermocycler with following program: one cycle with the sequence 94°C 1 min, 37°C 1 min, 72°C 1 min 30 s, and 35 cycles with the sequence 92°C 1 min, 37°C 1 min, 72°C 1 min 30 s, and a final cycle of 5 min at 72°C. The RAPD fragments were separated by agarose gel electrophoresis in 1× TAE buffer on 1.5% agarose (mol. biol. grade, Sigma-Aldrich). DNA fragments in gel were stained in water solution of ethidium bromide (3 mg/ml), visualised and photographed under UV light.

Storage protein analysis. Half of the kernels were used for gliadin extraction with 70% ethanol and PAGE separation according to METAKOVSKY and NOVOSELSKAYA (1991) (Al-lactate electrophoresis, pH 3.1). PAGE was performed using vertical electrophoresis unit Hoefer SE600 on 18 × 16 cm gels and Comassie blue stained gels were documented using the Lucia D (Bioimaging, USA) system.

Data analysis. Electrophoreograms were used to score data for RAPD analysis. DNA fragment size was determined by comparisons with a DNA size marker (100 pb

Table l. Botanical determination and description of origin of the *T. boeoticum* Boiss. accessions

Accession No.	Variety	Origin
4086	var. *boeoticum*	Izgrev-Pčela
4087	var. *baydaricum* (Flaksb.) A. Filat. et Dorof.	Izgrev-Pčela
5087	var. *pseudoreuteri* (Thum.) A. Filat. et Dorof.	Garar nf. Džebraili
5078	var. *viridiboeoticum* (Jakubz.) A. Filat. et Dorof.	Gegadyr nf. Erebuni
5079	var. *aznaburticum* (Jakubz.) A. Filat. et Dorof.	Erebuni
5322	var. *viridiboeoticum* (Jakubz.) A. Filat. et Dorof.	Erebuni
5353	var. *pseudoboeoticum* (Flaksb.) A. Filat. et Dorof.	Bala
5354	var. *pantchitchii* (Flaksb.) A. Filat. et Dorof.	Bala
5355	ssp. *thaoudar* (Reut. ex Hausskn.) Grossh.	Guneykoy
5076	var. *abovjanii* A. Filat. et Dorof.	Bot. Gard. Bordeaux

marker, Sigma-Aldrich). Reproducible DNA bands were scored for their presence (1) or absence (0). The data were fed into the RAPDistance V1.04 program (Armstrong, Gibbs, Peakall & Weiller 1994). The RAPDistance Package' ftp://life.anu.edu.au/pub/software/ RAPDistance or http://life.anu.edu.au/molecular/software/rapd.html) and pairwise distances between the DNA samples were calculated using the RAPDALG module to generate Jaccard's or Dice's similarity coefficients ($2*n11/((2*n11) + n01 + n10)$, $n11/(n - n00)$. The distance matrix was used to construct dendrograms using UPGMA (unweighted pair group method with arithmetic average) by the STATISTICA Cluster analysis module.

The similarity matrix was analysed by principal co-ordinates using the STATISTICA program.

RESULTS

Resistance tests. Ten *T. boeoticum* accessions with a good field resistance, selected out of 47 previously screened accessions (HOLUBEC *et al.* 1992, 1993), were tested in more detail to reveal their resistances using several isolates of *B. graminis*. Five of the accessions were resistant to all isolates, the others were resistant only to some of them (Table 2). The included *T. aestivum* and *T. durum* cultivars were very susceptible. Three amphidiploid progenies (genome $2n = 42$, A^bA^bAABB) with stable chromosome numbers were tested mildew resistance. Two were resistant and one only medium resistant to the mixture of powdery mildew isolates in the leaf test. The two resistant amphidiploids were considered suitable for the transfer of resistance.

Protein analysis. Patterns of seed storage proteins (gliadins) were determined in the parental cultivars and lines. The protein patterns were specific for species and even cultivars. Detected variability within *T. boeoticum* accession was lower then expected. Not all accessions differed in their protein spectrum.

RAPD analysis and fragment identification. 40 decamer primers were tested (sets AB2 and ABN with 60% and 70%, respectively, of CG) for their ability to differentiate the accessions. Eight of them did not amplify any product, 7 amplified only 1–3 non-polymorphic bands. Finally ten decamers were selected (Table 3), producing on average 5.2 polymorphic bands per reaction. Their size ranged from 150 to 2200 bp (Fig. 1). Although 52 polymorphic bands were recorded, only 3 products distinguished between the two tested *T. aestivum* cultivars.

Signals specific for species. Among the detected signals, 12 were species-specific: two occurred only in the accessions of *T. aestivum*, 4 in those of *T. durum* and 5 only in all the *T. boeoticum* accessions. Three signals were recorded in both *T. durum* and *T. aestivum*, but never in the *T. boeoticum* eccessions, indicating possible specificity for the B genome.

Relationships between the accessions. The pairwise genetic distances among the analysed accessions, computed by RAPDistance, were used to differentiate the accessions. The resulting dendrogram (Fig. 2) and 3D plot of principal component analysis data (Fig. 3) clearly reflect expected distinctness between *T. aestivum* (hexaploid wheat), *T. durum* (tetraploid wheat) and *T. boeoticum* (diploid wild species). The same grouping was obtained by the use of Dice's and Jaccard's metrics. Variability among *T. boeoticum* accessions was high, as ex-

Table 2. Resistance of *T. boeoticum* Boiss. accessions to different races of *B. graminis* and field resistance to the mixture of isolates

T. boeoticum Acc. No.	Field resistance *B. graminis*	Isolate of *B. graminis*								
		89	205	45	164	93	222	187	102	203
4086	R	S	S	S	RS	S	MS			
4087	MR	R-MR	S	MS	R	S	R			
5087	MR			R			MS		MS	MS
5078	R			MR			R	R	R-MR	MR
5079	R			R			R	R	R	R
5322	R			R			R	R	R	R
5353	R			R			R	R	R	R
5354	R			R			R	R	R	R
5355	R			R-MR			R	R	R	R
5076	R			R			R	R	R	R

R = resistant (without symptoms)
MR = moderately resistant (mycelium slightly developed)
MS = moderately susceptible (mycelium developed, limited occurrence of necrosis and chlorosis)
S = susceptible (high sporulation, without necrosis)

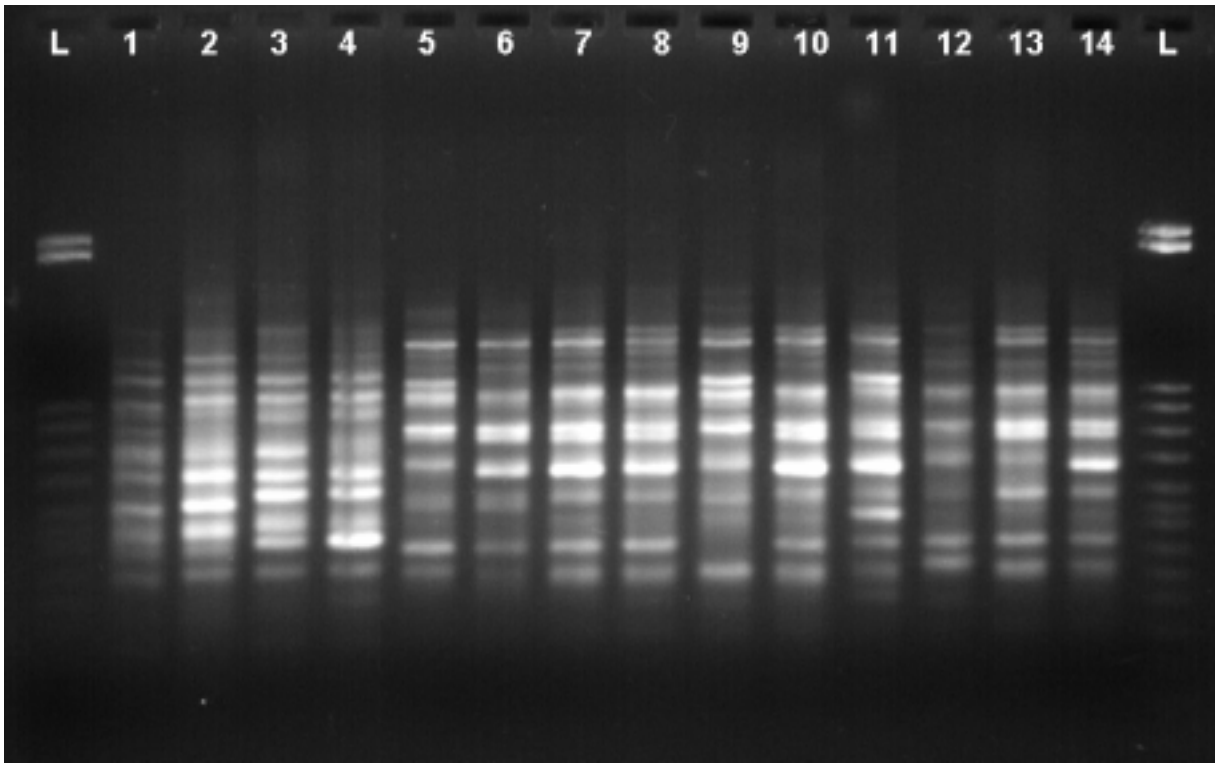

Fig. 1. RAPD profiles of *T. aestivum* cv. Regina, Florida (1, 2), *T. durum* cv. Panondur, line 3310 (3, 4) and *T. boeoticum* accessions (5–14) amplified using decamer ABN 15 (Advanced Biosystem Ltd., England) and separated on 1.5% agarose gel. L = DNA ladder 100 pb (Sigma-Aldrich)

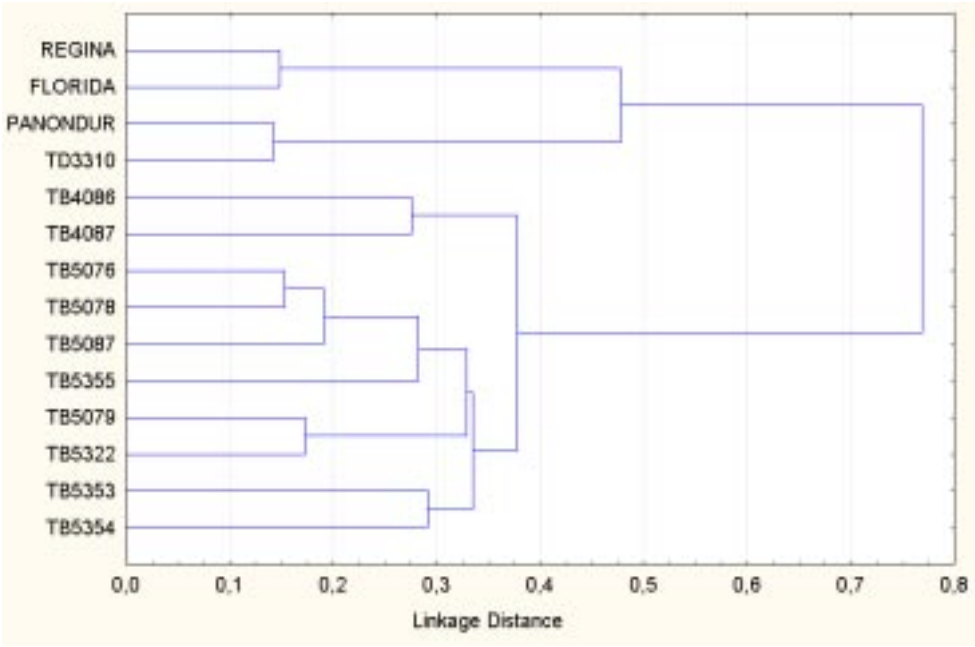

Fig. 2. Dendrogram generated by the UPGMA analysis calculated from RAPD markers using Jaccard's metrics showing relative genetic distances among analysed species and varieties of *T. aestivum* (cvs. Regina, Florida), *T. durum* (TD – cv. Panondur and line 3310), *T. boeoticum* (TB + accession number)

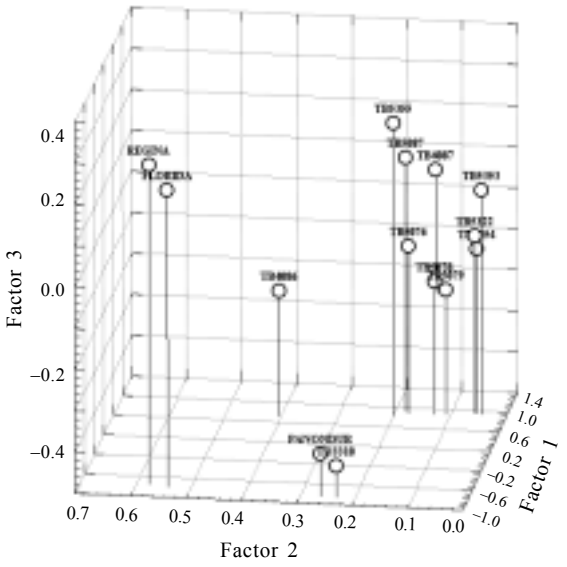

Fig. 3. Principal component analysis of relative genetic distances calculated from RAPD markers using Jaccard's metrics between accessions of *T. aestivum* (cv. Regina, Florida), *T. durum* (cv. Panondur, line 3310) and *T. boeoticum* (TB + accession number), showing grouping of the three species

pected for wild species. No correlation between the relative genetic distance and geographical distance of places of origin was found.

Amphiploid analysis. Three amphiploid progenies were analysed using (1) selected RAPD primers and (2) gliadin spectra. Not all the bands amplified by RAPD primers in the parents were detected in the progenies (Fig. 4). On average 38% of RAPD bands were missing. This indicates a high level of rearrangements in amphiploids. 35% of the missing RAPD bands belonged to the diploid *T. boeoticum* and 65% to the tetraploid *T. durum*, indicating the same ratio of rearrangements in both the parental genomes (1:2 – diploid:tetraploid). Gliadin analysis showed the presence of all α- and β-gliadins from the parental lines (*T. durum*, $2n = 28$, AABB and *T. boeoticum,* $2n = 14$, A^bA^b) in the amphidiploids, but some α- and β-gliadins signals were missing (Fig. 5).

DISCUSSION

We have investigated diversity within the set of ten *T. boeoticum* accessions from the Gene Bank, RICP Pra-

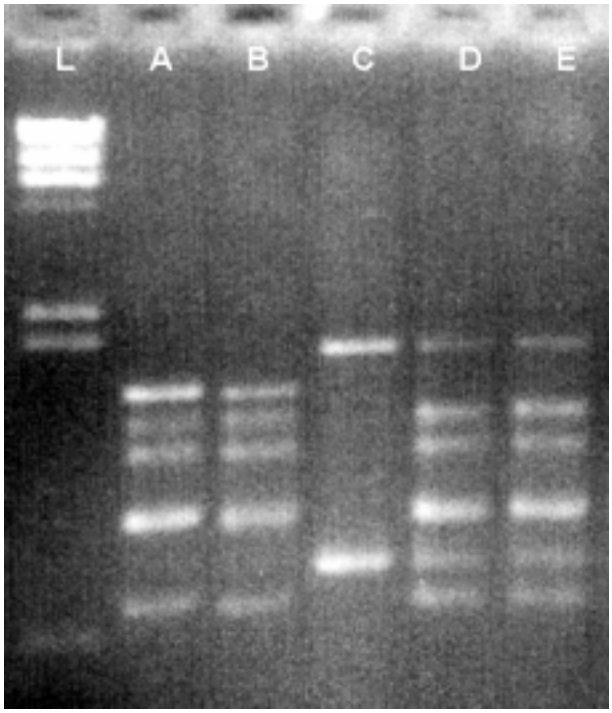

Fig. 4. RAPD profiles of *T. durum* 3310 (A, B), *T. boeoticum* 5353 (C) and their two amphiploid progenies (D, E) indicating that one parental band is missing. Primer ABN 01 was used for amplification and λHindIII digest was used as a size standard (L)

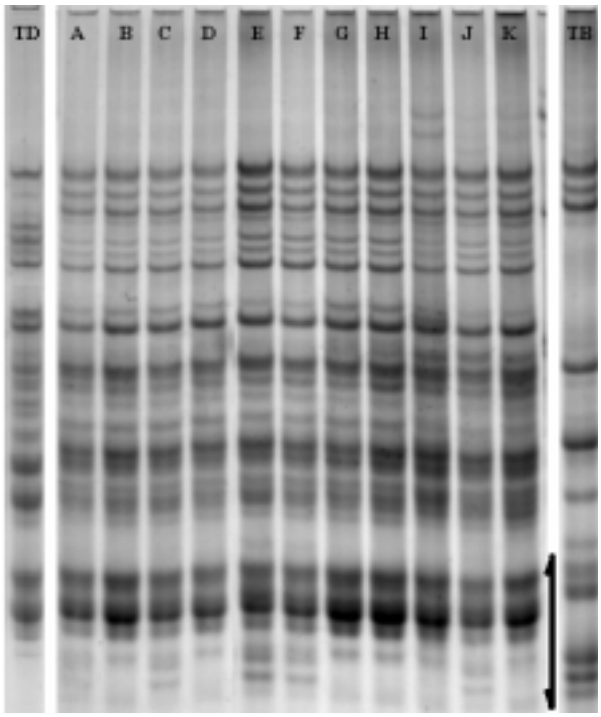

Fig. 5. Electrophoretic patterns of gliadins in amphidiploid progenies (A–K) and parental lines of *T. durum* 3310 (TD) and *T. boeoticum* 5353 (TB), showing variability and, consequently, genome rearrangements, especially in α- and β-gliadin loci of the amphidiploids. Single seeds from the three different progenies were used. The arrow shows the α-gliadin region

Table 3. Sequence of oligonucleotide primers used for variability analysis (Advanced Biosystem, Ltd., England)

Primer name	Sequence	Primer name	Sequence
ABN 01	5′-CTCACGTTGG	ABN 09	5′-ACCTCAGCTC
ABN 04	5′-GACCFACCCA	ABN 12	5′-CACAGACACC
ABN 05	5′-ACTGAACGCC	ABN 14	5′-TCGTGCGGGT
ABN 06	5′-GAGACGCACA	ABN 15	5′-CAGCGACTGT
ABN 08	5′-ACCTCAGCTC	ABN 16	5′-AAGCGACCTG

gue-Ruzyně. Different degrees of mildew resistance and race specificity were found, confirming that *T. boeoticum* can be a rich source of new genes.

The accessions were characterised on DNA level using RAPD to estimate (1) the diversity within the selected set, (2) relative genetic distance among *T. boeoticum* accessions, *T. durum* and *T. aestivum* cultivars as possible receptors of the resistance gene(s) and, finally, to find (3) signals specific to species and accessions and to determine their occurrence in amphiploid progenies of *T. durum* and *T. boeoticum* interspecific crosses.

WEI and WANG (1995) and DUBCOVSKY *et al. (*1997) have shown, that RAPD technology is very suitable for studying diversity in wild species and is cheap, fast and easy to perform. In our investigation only 25% of decamer primers provided polymorphic amplification products suitable for diversity studies. However, unlike protein analysis, RAPD differentiated all accessions of *T. boeoticum*. On the other hand, only 3 polymorphic signals differentiated the two hexaploid *T. aestivum* cvs. Regina and Florida. This means, that only two primers out of 40 were able to distinguish between them, even though Florida carries a rye-wheat translocation on the short arm of chromosome 1B (T1BL/1RS). Storage protein analysis clearly identified the presence of this translocation in wheat cultivars. Other authors have also reported a low variability of DNA sequences in hexaploid wheat (VARSHNEY *et al.* 1998). It was proved by BEUNINGEN and BUSCH (1997) that post-1975 cultivars have a narrow genetic diversity, probably due to strict quality requirements.

Dendrograms based either on Jaccard's similarity coefficients (Fig. 2) or Dice's similarity coefficients were constructed. Clustering reflected the phylogenetic classification of *T. boeoticum*, *T. durum* and *T. aestivum.* The dendrogram based on Dice's coefficients provided similar results, only the relative genetic distance of three *T. boeoticum* accessions was slightly different, resulting from a different mathematical formula for the pair-wise distances. Relative genetic distances between *T. boeoticum* accessions, expressed by the dendrogram, were relatively high. This could be expected for a wild species, indicating that *T. boeoticum* represents a large gene pool. However, the measured relative genetic distances among accessions was not correlated with geographical distances of places of their origins. This reflects probably both varietal differences and influence of climatic conditions as it was proposed also by PAGNOTTA *et al.* (1995). Other effects (e.g. accidental seed transfer with crops) could contribute to the spreading of genotypes to more distant regions.

Amphidiploids are valuable for wheat breeding (RAMSEY & SCHEMSKE 1998). However, it is known, that genome rearrangement can occur in the genome of wide hybrids (QI-ZENGJUN *et al.* 2000) and after selfing of amphidiploids. LIU *et al.* (1998) reported genomic changes in 3–6 generations old amphidiploids, indicating extensive rearrangements in low copy, non-coding sequences. It is therefore essential, to have a tool suited to monitor such changes in breeding. C-banding and *in situ* hybridisation techniques have been mostly used for this purposes (HARJIT-SINGH *et al.* 2000; MA-YOUZHI *et al.* 2000). We have tested, if RAPD can also serve as such a tool.

The produced amphidiploids were used to show, if some RAPD products can be used for progeny testing. Most of species-specific amplification products were found in the amphidiploids, but some of them were missing. The missing signals were mostly the intensively stained ones. DEVOS and GALE (1992) have shown, that such products most likely arose from repetitive sequences. Moreover, it is known that more than 70% of the DNA in *Triticeae* genomes are repetitive sequences and about 16–45% represent species specific sequences (FLAVELL *et al.* 1981; ANAMTHAWAT-JÓNSSON & HESLOP-HARRISON 1992). Repetitive sequences change rapidly and produce a high degree of variation. But no "loss/gain" (e.g. loss of a band of one parent and gain of a new one) events were recorded in any of the amphidiploids. Changes in the α- and β-gliadin pattern do as well not exclude rearrangement in coding sequences. Both RAPD and protein analysis could be further used to study the structure and stability of selfed amphidiploid progenies and especially expected rearrangement.

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Abstrakt

OVESNÁ J., KUČERA L., BOCKOVÁ R., HOLUBEC V. (2002): **Charakterizace linií *Triticum boeoticum* jako donorů odolnosti vůči padlí travnímu s využitím RAPDs**. Czech J. Genet. Plant Breed., **38**: 117–124.

Bylo prokázáno, že vzdálená hybridizace je vhodnou technikou pro introgresi vybraných genů z planých příbuzných druhů do hexaploidní pšenice. Studovali jsme detailněji vybrané linie *Triticum boeoticum* Boiss. rezistentní vůči padlí travnímu. V rámci vybrané sady *T. boeoticum* jsme s využitím testů odolnosti, RAPD a analýzy zásobních proteinů sledovali variabilitu. Zahrnuli jsme i tetraploidní a hexaploidní odrůdy, do kterých se bude odolnost přenášet. Analýza zásobních proteinů neumožnila odlišit jednotlivé linie. RAPD analýza se však pro tyto účely ukázala jako vhodná. Dendrogram odvozený na základě RAPD dat odrážel fylogenetické vztahy v rámci studovaného souboru. Všechny typy testů prokázaly, že kolekce *T. boeoticum* je velmi bohatý genový zdroj. Rovněž bylo analyzováno amphidiploidní potomstvo *T. boeoticum* × *T. durum*. Jednotlivé linie se lišily ve stupni odolnosti k padlí travnímu. Jak RAPD, tak proteinové analýzy naznačily, že v genomu amfidiploidů došlo k určitému přeuspořádání. Prokázali jsme, že RAPD může být využita pro sledování takových změn.

Klíčová slova: *T. boeoticum*; RAPD; genetická diverzita odolnosti; padlí travní; vzdálená hybridizace

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