Powdery Mildew Resistance in Some *Aegilops* Species

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Abstract

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Resistance to powdery mildew (*Blumeria graminis* (DC.) E. O. Speer f.sp. *tritici* Em. Marchal) in *Aegilops crassa* Boiss., *Ae. ventricosa* Tausch, *Ae. biuncialis* Vis., *Ae. triuncialis* L. and *Ae. cylindrica* Host was tested at the stage of primary leaves in the years 2000 and 2001. All plants of *Ae. ventricosa, Ae. biuncialis* and sample No. 9 of *Ae. cylindrica* repeatedly showed a susceptible reaction after being inoculated by all powdery mildew isolates used. In contrast, plants of *Ae. crassa,* sample No. 8 of *Ae. cylindrica* and all samples (No. 13, 21, 22, 24 and 26) of *Ae. triuncialis* were resistant to all isolates. Samples No. 5, 6, 7, 19 and 23 of *Ae. cylindrica* contained resistant and susceptible plants in both years. Virulence to these samples ranged from 3% to 18%. Cluster analysis using DNA microsatellite markers showed that the accessions are arranged in groups based on taxonomic relationship but not on basis of resistance. Plants susceptible to powdery mildew at the juvenile stage showed satisfactory adult plant resistance.

Keywords: *Blumeria graminis* DC f.sp. *tritici*; *Aegilops* spp.; disease resistance; virulence analysis; DNA polymorphism; genetic resources

Wild relatives of common wheat, one of them the genus *Aegilops,* have become an important genetic resource of both resistance to various diseases and tolerance against abiotic factors. Powdery mildew resistance in hexaploid wheat is often governed by resistance gene *Pm2,* localised on chromosome 5DS (Nelson *et al.* 1995), and by resistance gene *Pm19* localised on chromosome 7D, translocated from *Ae. squarrosa* L. (Lutz *et al.* 1995). *Aegilops speltoides* Tausch is a donor of gene *Pm12*. Another species, *Ae. ventricosa,* is an excellent source of resistance to various wheat diseases, including rusts. A gene family comprising genes *Yr17*, *Lr37* and *Sr38* for resistance to rusts (Bariana & McIntosh 1994), *R* gene *Cre5* against cereal cyst nematode (Jahier *et al.* 2001), and to eyespot (gene *Pch1*) (Worland *et al.* 1988) were introgressed from *Ae. ventricosa*. Accessions of *Ae. cylindrica* exhibited resistance to snow mold similar to that of the most resistant winter wheat cultivars (Iriki *et al.* 2001). New resistance gene *LrTr* to leaf rust has been transferred from *Ae. triuncialis* to the D genome of wheat (Aghaeesarbarzeh & Barjitsingh Dhalival 2001). The aim of the present study was to test accessions of five *Aegilops* species for their reaction to wheat powdery mildew and determine whether any relationship between the species analysed on the basis of DNA polymorphism exists.

MATERIALS AND METHODS

***Origin of** Aegilops accessions.* Fourteen accessions of five *Aegilops* species were obtained from the Gene Banks in Prague and Piešťany (Table 1). Accessions of *Ae. ventricosa* originated from Libya and *Ae. crassa* from Turkmenia. Samples of *Ae. triuncialis* (except No. 13), *Ae. biuncialis* and accessions No. 19 and 23 of *Ae. cylindrica* were collected on the Crimean

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Table 1. Origin of *Aegilops* accessions

No. of accession	Name of species	Accession number at the Gene Bank	Donor institution	Acronym country of origin	Country of origin (region, locality, acronym collecting mission and number)
*	*Aegilops crassa*	01C2106032	VÚRV Prague	SUN	Soviet Union (Kopetdag Mountains, AE815)
*	*Aegilops biuncialis*	01C2101243	VÚRV Prague	LBY	Libyan Arab Jamahiriya (Akhdar, AE755)
*	*Aegilops ventricosa*	01C2100532	VÚRV Prague	LBY	Libyan Arab Jamahiriya (Akhdar, AE766)
13**	*Aegilops triuncialis*	01C2107091	VÚRV Prague	SUN	Soviet Union (Azerbaijan, Garar, SUNJAN-0-109)
21	*Aegilops triuncialis*	C2100021	VÚRV Piešťany	UKR	Ukraine (Crimean Peninsula, UKRKRY98-161)
24	*Aegilops triuncialis*	C2100024	VÚRV Piešťany	UKR	Ukraine (Crimean Peninsula, UKRKRY98-108)
26	*Aegilops triuncialis*	C2100026	VÚRV Piešťany	UKR	Ukraine (Crimean Peninsula, UKRKRY98-88)
5	*Aegilops cylindrica*	01C2104047	VÚRV Prague	CZE	Czechoslovakia (Burda, CSKHOL-89, E486)
6	*Aegilops cylindrica*	01C2104048	VÚRV Prague	CZE	Czechoslovakia (Burda, CSKHOL-89, E487)
7	*Aegilops cylindrica*	01C2104049	VÚRV Prague	CZE	Czechoslovakia (Burda, CSKHOL-89, E488)
8	*Aegilops cylindrica*	01C2104063	VÚRV Prague	CZE	Czechoslovakia (Dobre, CSKHOL-90, E578)
9	*Aegilops cylindrica*	01C2104064	VÚRV Prague	CZE	Czechoslovakia (Burda, CSKHOL-90, E579)
19	*Aegilops cylindrica*	C2100019	VÚRV Piešťany	UKR	Ukraine (Crimean Peninsula, UKRKRY98-169)
23	*Aegilops cylindrica*	C2100023	VÚRV Piešťany	UKR	Ukraine (Crimean Peninsula, UKRKRY98-102)

*only one accession from the species evaluated

**working designation based on the previous experiments

VÚRV Prague = Gene Bank at the Research Institute of Crop Production in Prague-Ruzyně

VÚRV Piešťany = Gene Bank at the Research Institute of Plant Production in Piešťany

Peninsula (Ukraine). Other samples of *Ae. cylindrica* (No. 5–9) were collected in the south and east of Slovakia. Accession No. 13 of *Ae. triuncialis* comes from Azerbaijan.

Phytopathological tests. Seeds of fourteen *Aegilops* accessions were sown in plastic pots in the greenhouse. The seedlings were protected against infection inside cellophane bags. Tests were carried out on 20mm segments of first leaves of the hosts, with at least two leaf segments from each accession. Leaf segments were laid out in Petri dishes (in 6 dishes in 2000 and in 60 dishes in the year 2001) on a medium containing 5% agar and 25 ppm benzimidazole. Each Petri dish with the leaf segments of 14 accessions of the *Aegilopses* in 2000 and with the segments of 19 samples (Figure 1) in 2001 was inoculated with the progeny of the single colony isolate by drawing spores into a pipet and blowing them into a settling tower. Because of the lack of plants in 2000, the segments of each accession were inoculated with conidia of only six highly virulent powdery mildew isolates. In 2001, 60 isolates possessing different virulence patterns to the known specific resistance genes (*Pm1*, *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3f*, *Pm3g*, *Pm4a*, *Pm4b*, *Pm5*, *Pm6*, *Pm7*, *Pm8*, *Pm1+2+9*, *Pm2+Mld*, *Pm17*, *MlAx*, *MlBr*, $MlCO_3$ and *MlFr*) were used for inoculation. The set of highly virulent powdery mildew isolates

was obtained from almost 3000 isolates collected from the territory of Slovakia and neighbouring countries in 1993–1998. Inoculated segments were incubated in a climate chamber at 18°C and under continuous light (960 lux). Inoculum density was approximately 250 conidia/cm^2. After 12 d each test set was scored for sporulation. Two types of reactions were distinguished: R – resistant and S – susceptible according to Limpert *et al.* (1987).

DNA analyses. Total DNA was extracted from young fresh leaves by the method of Dellaporta *et al*. (1993). Analyses of DNA polymorphism were carried out by amplification of micro- and minisatellite polymorphism (Gupta *et al.* 1994) (Table 2). The PCR reactions were performed in a 20 µl volume containing: 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.25mM of each dNTP, 1mM primer, 0.75 U Taq-DNA polymerase, 25 ng DNA. The starting denaturation, lasting for 2 min at 94°C, was followed by 40 cycles: 1 min at 94°C, 1 min at temperature calculated due to sequence of primer and 5 min at 72°C. The last step was 7 min at 72°C. Amplification products were loaded in 1.5% agarose gels in 0.5 × TBE buffer with ethidium bromide and separated by electrophoresis. For each sample the polymorphic bands were scored as present (1) or absent (0). The data were used to calculate the Jaccard coefficient of genetic similarity and to construct a dendrogram by hierarchical cluster analysis (UPGMA method) by SPSS Professional Statistics v. 6.0.1. for Windows (SPSS inc., Chicago, I11. USA).

RESULTS AND DISCUSSION

In 2000, the plants of different *Aegilops* species and accessions were classified into two groups: resistant (R) or susceptible (S) to the six highly virulent powdery mildew isolates. All plants of *Ae. crassa* and *Ae. triuncialis* were completely resistant, while all of *Ae. biuncialis* and *Ae. ventricosa* were susceptible. Accessions of *Ae. cylindrica* collected in the south and east of Slovakia differed from each other. While the plants of accession No. 8

Table 2. Primers used to study *Aegilops* accessions

Primer name	Primer sequence	Type of sequence	Reference
LBHB01	5′-(ACTG)4-3′	microsatellite	–
LBHB02	5′-(GACA)4-3′	microsatellite	–
LBHB04	5′-(GACAGATA)2-3′	microsatellite	–
LBMB-A	5′-(GACA)4TA-3′	ISSR	–
LBMB-B	5′-(GACA)4TT-3′	ISSR	–
LBMB-C	5′-(GACA)4GT-3′	ISSR	–
LBMB-D	5′-(GACA)4AA-3′	ISSR	–
LBMB-E	5′-(GATA)4AT-3′	ISSR	–
HVR-	5′-CCCTCCTCCTCCTTC-3′	minisatellite	Winberg *et al.* (1993)
HVR+	5′-AGGAGGAGGGGAAGG-3′	minisatellite	Winberg *et al.* (1993)
YNZ22	5′-CTCTGGGTGTGGTGC-3′	minisatellite	Nakamura *et al.* (1987)
FVIIexB-C	5′-TACGTGTGTGTGTCC-3′	minisatellite	Murray *et al.* (1988)
FVIIex8	5′-ATGCACACACACAGG-3′	minisatellite	Murray *et al.* (1988)
HBV5	5′-GGTGTAGAGAGGGGT-3′	minisatellite	Nakamura *et al.* (1987)
HBV3	5′-GGTGAAGCACAGGTG-3′	minisatellite	Nakamura *et al.* (1987)
14C2	5′-GGCAGGATTGAAGC-3′	minisatellite	Vergnaud (1989)
33.6	5′-AGGGCTGGAGGAGGGC-3′	minisatellite	Jeffreys *et al.* (1985)
33.15	5′-AGAGGTGGGCAGGTGG-3′	minisatellite	Jeffreys *et al.* (1985)
M13 phage	5′-GAGGGTGGXGGXTCT-3′	minisatellite	Vassart *et al.* (1987)

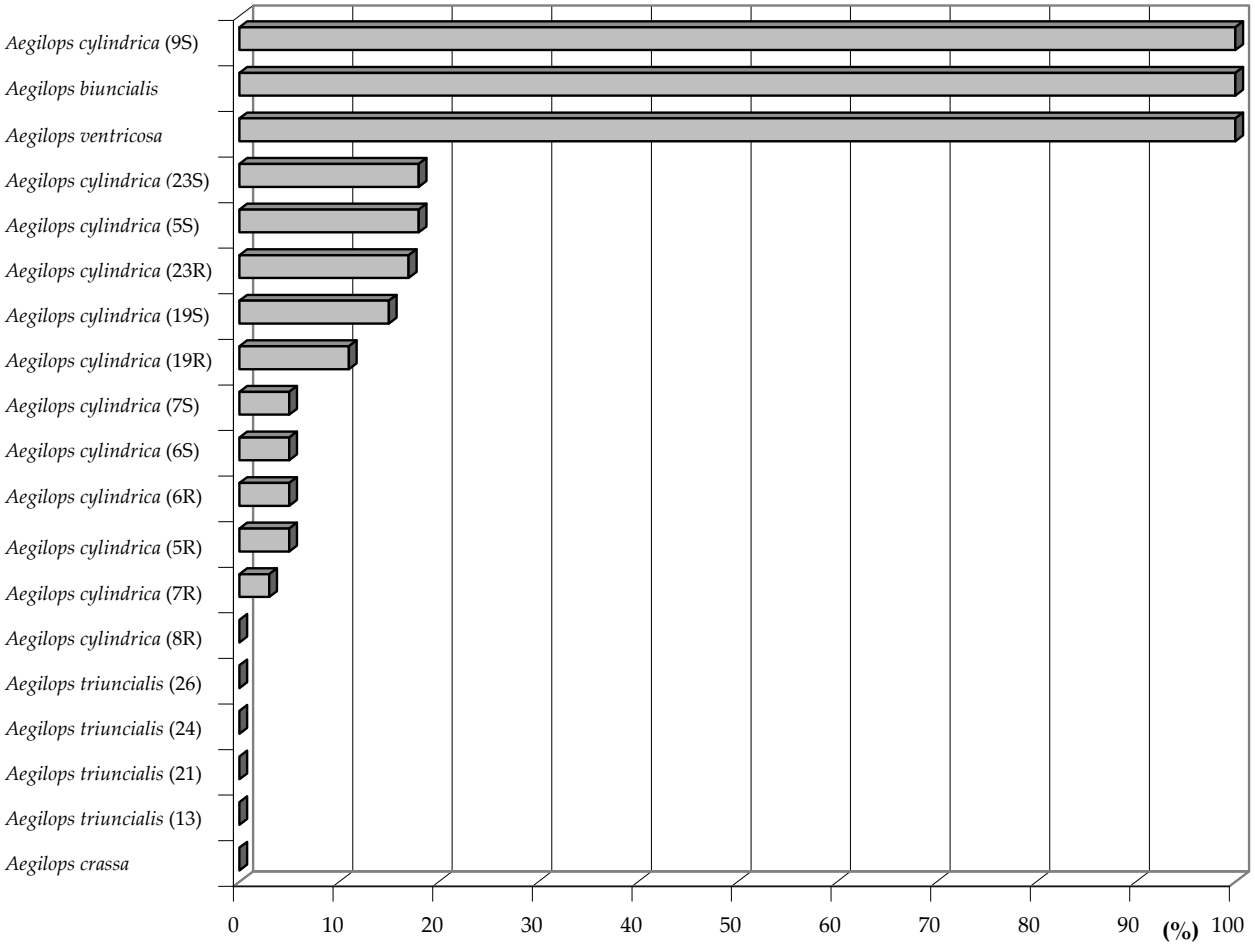

Figure 1. Percentage of virulent powdery mildew isolates in 2001 (R – progeny of plants resistant in 2000, S – progeny of plants sensitive in 2000)

from the east of Slovakia were completely resistant, accession No. 9 from southern Slovakia was completely susceptible. Other accessions (No. 5, 6 and 7) from southern Slovakia contained both resistant and susceptible plants.

Seed of the tested accessions was produced, grown out and inoculated again in 2001, this time by 60 powdery mildew isolates. The same results as in 2000 were observed in 2001, which means that each accession fell again into the same category (R or S) (Figure 1).

Virulence frequencies ranging from 3% to 18%, observed in the accessions No. 5, 6, 7, 19 and 23 of *Ae. cylindrica,* indicated that some plants of these accessions, coming from different localities, carry a resistance gene to which a pathogen population has already adapted. Lower virulence frequencies observed in the accessions No. 5 and 19 in the R group when compared to the S group are probably a result of selection that occurred in the previous year. High virulence frequency (100%) against *Ae. biuncialis, Ae. ventricosa* and *Ae. cylindrica* No. 9 could be caused or by absence of resistance genes or by uneffective resistance gene(s) in these host genotypes. In contrast, the zero virulence against accessions of *Ae. triuncialis, Ae. crassa* and *Ae. cylindrica* No. 8 can be considered as a result of the effective resistance gene(s) operation in these potential resources of powdery mildew resistance. Our observations of plants of fourteen *Aegilops* accessions at the higher ontogenetic stage showed high degree of resistance against powdery mildew (adult plant resistance – APR) in the field conditions.

However, cluster analysis using DNA microsatellite markers show that the accessions are arranged in groups based on taxonomic relationship and not on basis of resistance. A dendrogram (Figure 2) constructed for the *Aegilops* accessions tested for resistance against powdery mildew showed five

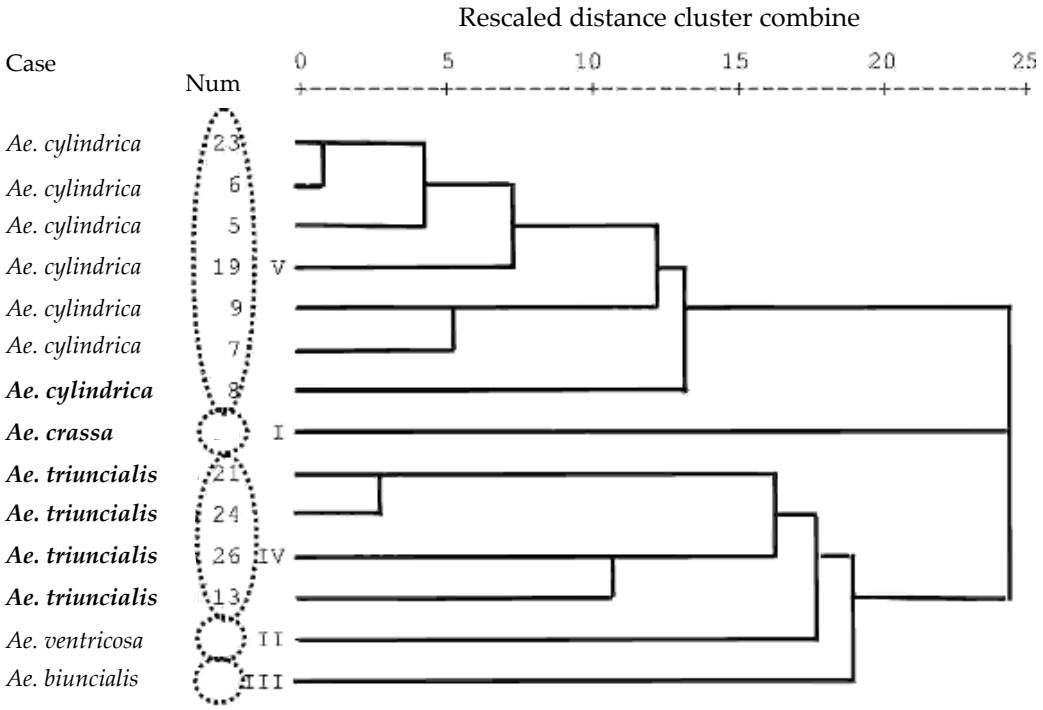

Figure 2. Grouping of *Aegilops* accessions tested for resistance to powdery mildew (resistant accessions in bold print; those in cursive contained both resistant and susceptible plants; values 0–25 = Index of similarity, i.e. Jaccard coefficient))

main branches, with differentiation reflecting the species. Group IV contains four *Ae. triuncialis* and group V seven *Ae. cylindrica* accessions. At a lower level of differentiation in the dendrogram, sample No. 8 within group V, tends to separate from other *Ae. cylindrica* accessions and converges to samples in the lower part of the dendrogram (groups I and IV) which were resistant against powdery mildew. Almost all accessions in group V differed in their reaction to this pathogen. Guadagnuolo *et al.* (2001) have also discriminated populations of *Ae. cylindrica* using microsatellites.

In conclusion it can be stated that the accessions of *Ae. crassa, Ae. triuncialis* and sample No. 8 of *Ae. cylindrica* represent potential genetic resources of resistance against wheat powdery mildew. According to literature some accessions of *Ae. tauschii* could also be a good resource of such resistance (Assefa & Fehrmann 1998).

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Súhrn

Švec M., Miklovičová M., Šudyová V., Hudcovicová M., Hauptvogel P., Kraic J. (2004): **Odolnosť niektorých druhov mnohoštetu** (*Aegilops* sp.) **voči múčnatke trávovej.** Plant Protect. Sci., **40:** 87–93.

V rokoch 2000 a 2001 sme sledovali rezistenciu viacerých druhov mnohoštetu (*Aegilops crassa* Boiss., *Aegilops ventricosa* Tausch., *Aegilops biuncialis* Vis., *Aegilops triuncialis* L. a *Aegilops cylindrica* Host) voči izolátom múčnatky trávovej na pšenici (*Blumeria graminis* f.sp. *tritici*), pri ktorých bola zistená virulencia voči väčšine známych génov špecifickej rezistencie (*Pm1, Pm2, Pm3a, Pm3b, Pm3c,Pm3d, Pm3f, Pm3g, Pm4a, Pm4b, pm5, Pm6, Pm7, Pm8, Pm1+2+9, Pm2+Mld, Pm17, MlAx, MlBr, MlCO$_3$, MlFr*). Fytopatologické testy sme vykonali na 20mm segmentoch primárnych listov každého druhu, resp. genotypu uložených na 6% agarovej pôde s prídavkom benzimidazolu. V roku 2000 sme mali k dispozícii iba malé množstvo rastlín, ktoré sme inokulovali šiestimi vysokovirulentnými izolátmi. Vzorkám (genotypom) druhu *Aegilops cylindrica* s rôznym číselným označením (5, 6, 7, 8, 9, 19, 23 – pracovné označenie v rámci predchádzajúcich laboratórnych testov) sme podľa výsledkov testu pridelili označenie R (rezistentné) a S (senzitívne) a pod týmto označením sme ich testovali aj v roku 2001. V roku 2001 sme namnožené vzorky otestovali 60-timi vysokovirulentnými izolátmi múčnatky trávovej. Príbuzenské vzťahy medzi jednotlivými druhmi a vzorkami mnohoštetu sme sledovali na základe DNA analýz pomocou mikro- a minisatelitového polymorfizmu. Výsledky získané analýzou DNA sme spracovali pomocou programu SPSS Professional Statistics, v rámci ktorého sme vypočítavali Jaccardov koeficient genetickej podobnosti a na jeho základe následne zhotovili dendrogram. Získané frekvencie virulencie voči genotypom č. 5, 6, 7, 19 a 23 druhu *Aegilops cylindrica* kolíšuce v rozsahu 3–18 % naznačujú, že tieto vzorky obsahujú nejaký gén rezistencie, voči ktorému sa časť populácie patogéna adaptovala formou vzniku a rozšírenia virulentných izolátov. Nižšia frekvencia virulencie voči vzor-

kám č. 5 a 19 v skupine rezistentných (R) oproti skupine senzitívnych (S) je pravdepodobne výsledkom selekcie uskutočnenej v predchádzajúcom roku. Spomedzi viacerých vzoriek druhu *Aegilops cylindrica* iba vzorka č. 8 bola úplne rezistentná podobne ako aj všetky rastliny druhu *Ae. crassa* a všetky vzorky druhu *Ae. triuncialis*. Naopak vzorka č. 9 *Ae. cylindrica* a všetky rastliny druhov *Ae. biuncialis* a *Ae. ventricosa* vykazovali v juvenilnom štádiu vývoja kompatibilnú reakciu po inokulácii sadou 60 izolátov. Zhluková analýza na báze DNA mikrosatelitového polymorfizmu ukázala, že vzorky mnohoštetu boli usporiadané do zhlukov nie na základe rezistencie, ale na základe taxonomickej príbuznosti.

Kľúčové slová: múčnatka trávová na pšenici; rezistencia k chorobám; analýza virulencie; DNA polymorfizmus; genetické zdroje

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