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Morphological and molecular characterisation confirm that *Triticum monococcum* s.s. is resistant to wheat leaf rust

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Abstract The three diploid wheat species *Triticum monococcum*, *Triticum boeoticum* and *Triticum urartu* differ in their reaction to wheat leaf rust, *Puccinia triticina*. In general, *T. monococcum* is resistant while *T. boeoticum* and *T. urartu* are susceptible. However, upon screening a large collection of diploid wheat accessions, 1% resistant *T. boeoticum* accessions and 16% susceptible *T. monococcum* accessions were found. In the present study these atypical accessions were compared with 49 typical *T. monococcum*, *T. boeoticum* and *T. urartu* accessions to gain insight into the host-status of the diploid wheat species for wheat leaf rust. Cluster analysis of morphological data and AFLP fingerprints of the typical accessions clearly discriminated the three diploid species. *T. monococcum* and *T. boeoticum* had rather-similar AFLP fingerprints while *T. urartu* had a very different fingerprint. The clustering of most atypical accessions was not consistent with the species they were assigned to, but intermediate between *T. boeoticum* and *T. monococcum*. Only four susceptible *T. monococcum* accessions were morphologically and moleculary similar to the typical *T. monococcum* accessions. Results confirmed that *T. boeoticum* and *T. monococcum* are closely related but indicate a clear difference in host-status for the wheat leaf rust fungus in these two species.

Keywords AFLP fingerprint · Leaf rust resistance · Morphology · *Triticum monococcum*

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Introduction

Triticum monococcum s.l., or diploid wheat, consists of three closely related species: *Triticum monococcum*, *Triticum boeoticum* and *Triticum urartu* (Jakubziner 1958). Diploid wheat is a valuable source of resistance genes for wheat breeding. The stem rust resistance genes *Sr21*, *Sr22* (The 1973), *Sr35* (McIntosh et al. 1984), some undesignated leaf rust resistance genes (Hussien et al. 1997) and the powdery mildew resistance gene *Pm*Tmb (Shi et al. 1996) have been successfully introduced into polyploid wheat from diploid wheat.

In a recent study the occurrence of resistance to wheat leaf rust, *Puccinia triticina*, in 598 diploid wheat accessions was determined (Anker and Niks 2001). The diploid wheat species clearly differed in their reaction to wheat leaf rust. Resistance was confined to *T. monococcum* with 84% resistant accessions, whereas all *T. urartu* and 99% of the *T. boeoticum* accessions were susceptible to the wheat leaf rust isolate 'Felix'. The difference between the resistant and susceptible accessions was very clear with an infection type of 8–9 for the susceptible accessions and 0–3 for the resistant accessions, on a scale of 0–9 (Anker and Niks 2001).

T. urartu does not easily intercross with both other species but the morphologically quite-similar *T. boeoticum* and *T. monococcum* species intercross relatively easily allowing introgression of traits from one to the other species (Johnson and Dhaliwal 1976). Therefore, the identity of the accessions that reacted atypically to wheat leaf rust should be verified before a proper statement about the taxon-specificity of the resistance can be made.

The aim of this study was to distinguish the three diploid wheat species based on morphological characters and AFLP markers, and to verify the identity of the atypical susceptible *T. monococcum* and resistant *T. boeoticum* accessions. The results will provide more insight into the host status of the diploid wheat species for wheat leaf rust. The AFLP fingerprints will also provide information about the relatedness between the three diploid wheat species.

Material and methods

Plant material

Nineteen susceptible *T. boeoticum*, 15 resistant *T. monococcum* and 15 susceptible *T. urartu* accessions (hereafter referred to as 'typical accessions') were randomly chosen from at least 150 typical accessions of the same species. The typical accessions were compared to two atypically resistant *T. boeoticum* and 25 atypically susceptible *T. monococcum* accessions (hereafter called 'atypical accessions'), and *T. aestivum* accession 'Little Club'. All 76 diploid wheat accessions (Table 1) were obtained as part of a resistance study from the National Small Grains Collection, Idaho, U.S.A. (Anker and Niks 2001). Individual seeds were sown in 4-cm-diameter pots in the greenhouse in December. Two-week-old seedlings were vernalised in a climate room at 6°C and 14 h of light per day for 5 weeks. Two plants per accession were transferred to 12×12-cm pots and grown in a non-heated greenhouse from February to May.

Morphological characterisation

A morphological description of each of the two plants per accession was made at anther dehiscence of the first ear per plant from April to May. The 17 characters observed were selected from Percival (1921), Johnson (1975) and Kimber and Feldman (1987) (Table 2).

DNA isolation and AFLP analysis

The two seedlings per accession were combined to collect one leaf sample of around 1 g for DNA isolation. DNA was extracted with the CTAB method (Van der Beek et al. 1992). The AFLP technique (Vos et al. 1995) was performed according to Van Eck et al. (1995) with modifications according to Qi and Lindhout (1997). The primer combination E33/M58 (AAG/CGT) was selected for its high number of amplification products and clear polymorphisms when tested on barley (Qi and Lindhout 1997) and on four diploid wheat accessions (Vaz Patto et al. 2000). Electrophoresis was performed in duplicate and only unambiguous fragments with a size range between 130 and 480 bp were scored as dominant markers.

Data analysis

Both the AFLP data and morphological data were analysed using principal co-ordinate analysis in NTSYS-pc (Rohlf 1997) version 2.02. Morphological data were standardised before using the SIMINT module, based on the average taxonomical distance, to

Table 1 Accessions of diploid wheat used for morphological and AFLP analysis. Accession numbers and indication of origin as used by the National Small Grains Collection, Idaho, USA. Leaf rust reaction according to Anker and Niks (2001)

Species	Accession number	Leaf rust reaction	Origin
Typical accessions, based on leaf rust reaction			
T. boeoticum	538526	S	Turkey
	538556	S	Iraq
	538587	S	Iraq
	538607	S	Iraq
	538619	S	Turkey, Yozgat
ssp. *boeoticum*	427484	S	Turkey, Mardin
	427503	S	Turkey, Mardin
	427522	S	Turkey, Mardin
	427536	S	Turkey, Mardin
	427554	S	Turkey, Mardin
	427637	S	Iraq
	427664	S	Iraq
	427799	S	Iran
	427903	S	Iraq
	427977	S	Turkey, Urfa
	428002	S	Lebanon
ssp. *thaoudar*	352503	S	Switzerland
	352504	S	Switzerland
	352505	S	Switzerland
T. monococcum	10474	R	USA, Washington
	17657	R	USA, Washington
	167589	R	Turkey, Canakkale
	168806	R	USA, Kansas
	221329	R	Yugoslavia
	266844	R	UK
	277135	R	Not known
	295058	R	Bulgaria
	330550	R	UK
	352479	R	Turkey
	355524	R	Germany, W-stephan
	377666	R	Yugoslavia
	427927	R	Iraq
	428152	R	Belgium
	503847	R	South Africa
T. urartu	17664	S	Lebanon
	428184	S	Turkey, Mardin
	428194	S	Turkey, Mardin
	428204	S	Turkey, Mardin
	428214	S	Turkey, Mardin
T. urartu	428234	S	Turkey, Urfa
	428254	S	Turkey, Mus
	428275	S	Lebanon
	428295	S	Lebanon
	428305	S	Lebanon
	428315	S	Lebanon
	428335	S	Lebanon
	428269	S	Syria
	538729	S	Turkey, Urfa
	538749	S	Lebanon
Atypical accessions, based on leaf rust reaction			
T. boeoticum			
ssp. *boeoticum*	427447	R	UK
ssp. *thaoudar*	352502	R	Turkey
T. monococcum	94741	S	Ukraine, Kharkiv
	182461	S	Turkey, Bolu
	345133	S	Serbia, S. Klisura
	345186	S	Serbia, D. Kormilovo
	407604	S	Turkey, Ankara
	554529	S	Turkey, Izmir
	560719	S	Turkey, Siirt
	560721	S	Turkey, Siirt
	560722	S	Turkey, Van
	560723	S	Turkey, Mus
	560724	S	Turkey, Mus
	560725	S	Turkey, Mus
	560726	S	Turkey, Mus
	560727	S	Turkey, Mus
	560728	S	Turkey, Mus
	573520	S	Turkey, Eskisehir
	573521	S	Turkey, Bilecik
	573523	S	Turkey, Bilecik
	573524	S	Turkey, Bolu
	573525	S	Turkey, Cankiri
	573526	S	Turkey, Cankiri
	573527	S	Turkey, Ankara
	573528	S	Turkey, Ankara
	573529	S	Turkey, Ankara
	591871	S	Georgia
T. aestivum	Little Club	S	Own collection

Table 2 Characters used for the morphological description

Length of straw
Length of ear
Length of awns above the ear
Length of first awn of a spikelet
Length of second awn of a spikelet
Length of first tooth on sterile glume
Length of second tooth on sterile glume
Length of anthers
Presence of a third awn (present:1, absent:0)
Density (number of spikelets per 10 cm ear)
Number of flowers per spikelet
Number of rudimentary flowers
Pubescence leaves (strong:2, mild:1, absent:0)
Pubescence nodes (strong:2, mild:1)
Pubescence leaf ridges (longer hairs present:1, absent:0)
Anthocyanin at base of the stem (present:1, absent:0)
Flowering date (number of days till flowering of first ear)

produce the similarity matrix. The AFLP similarity matrix was calculated using the SIMQUAL module with the Jaccard coefficient. DCENTER and EIGEN procedures were employed for principal co-ordinate analysis. For comparison the data were clustered in dendrograms using the SAHN module with the UPGMA clustering method for both morphological and AFLP data.

Results

Principal co-ordinate analysis of the morphological data revealed three distinct groups for typical *T. monococcum*, *T. boeoticum* and *T. urartu* accessions (Fig. 1). In general, the two plants of each accession clustered close together.

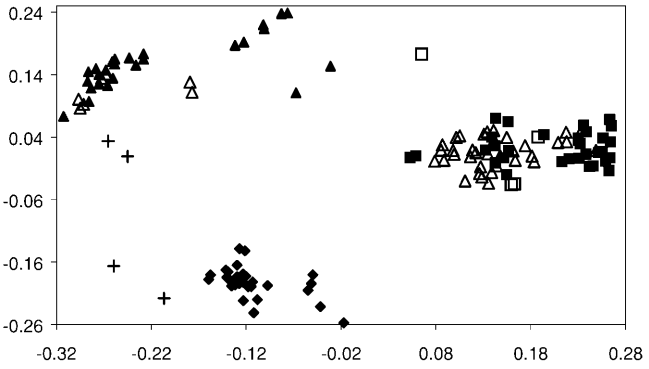

Fig. 1 Principal co-ordinate plot of 70 diploid wheat accessions and one tetraploid accession (two plants per accession) and two hexaploid wheat accessions (one plant per accession), based on morphological characteristics from Table 2 ; the first two principal co-ordinates accounted for 41.6% and 18.0% of the total variation respectively. ■ *T. boeoticum*, ▲ *T. monococcum*, ◆ *T. urartu*, + *T. durum* and *T. aestivum*; closed symbols typical accessions, open symbols atypical accessions

The average, minimum and maximum values for the morphological traits of the typical accessions are presented in Table 3. Three morphological characters (presence of the third awn, number of rudimentary flowers and leaf pubescence) were sufficient for the clustering of the typical accessions of the three diploid wheat species. Based on ear morphology (number of florets per spikelet and size of the teeth on the sterile glume) two atypical susceptible *T. monococcum* accessions were identified as *T. aestivum* (182461) and *T. durum* (94741) respectively. Four atypi-

Table 3 Average values and range for the morphological characteristics of the typical *T. boeoticum* (Tb), *T. monococcum* (Tm) and *T. urartu* (Tu) accessions. Traits are presented in the same order as in Table 2. In bold character-values that distinguished one species from the other two

Item	Av. **Tb**	Range	Av. **Tm**	Range	Av. **Tu**	Range
Length,cm						
Straw	81	54–108	81	50–108	60	39–86
Ear	7.0	5.5–8.5	5.7	4.0–7.0	7.8	5.0–9.5
Awn	8.1	3.0–12.0	5.2	1.0–9.0	4.5	3.0–9.0
Awn1/sp	10.2	6.0–14.5	5.1	2.3–8.8	5.5	2.0–9.3
Awn2/sp	4.1	0.6–8.8	0.2	0.1–1.1	5.0	2.0–8.8
Length,mm						
Tooth1/gl	1.8	1.3–2.1	**0.7**	0.5–1.1	2.0	1.8–3.0
Tooth2/gl	0.8	0.5–1.0	0.5	–	0.5	–
Anthers	4.0	3.5–4.3	3.5	3.0–4.0	**2.1**	2.0–3.0
Othertraits						
3rdawn	0	–	0	–	0.8	0/1
Density	2.4	2.0–2.7	4.1	3.3–4.9	2.6	2.0–3.4
No.fl/sp	2	–	**1**	–	2	–
No.rudfl	1	–	**2**	–	1	–
Leaves	2	–	0.3	0/2	**1**	–
Nodes	2	–	1.2	1/2	1	–
Ridges	1	–	0.3	0/1	0	–
Base	0.1	0/1	0	–	0	–
Flowering	82.4	75—91	91.8	88–102	84.6	75–91

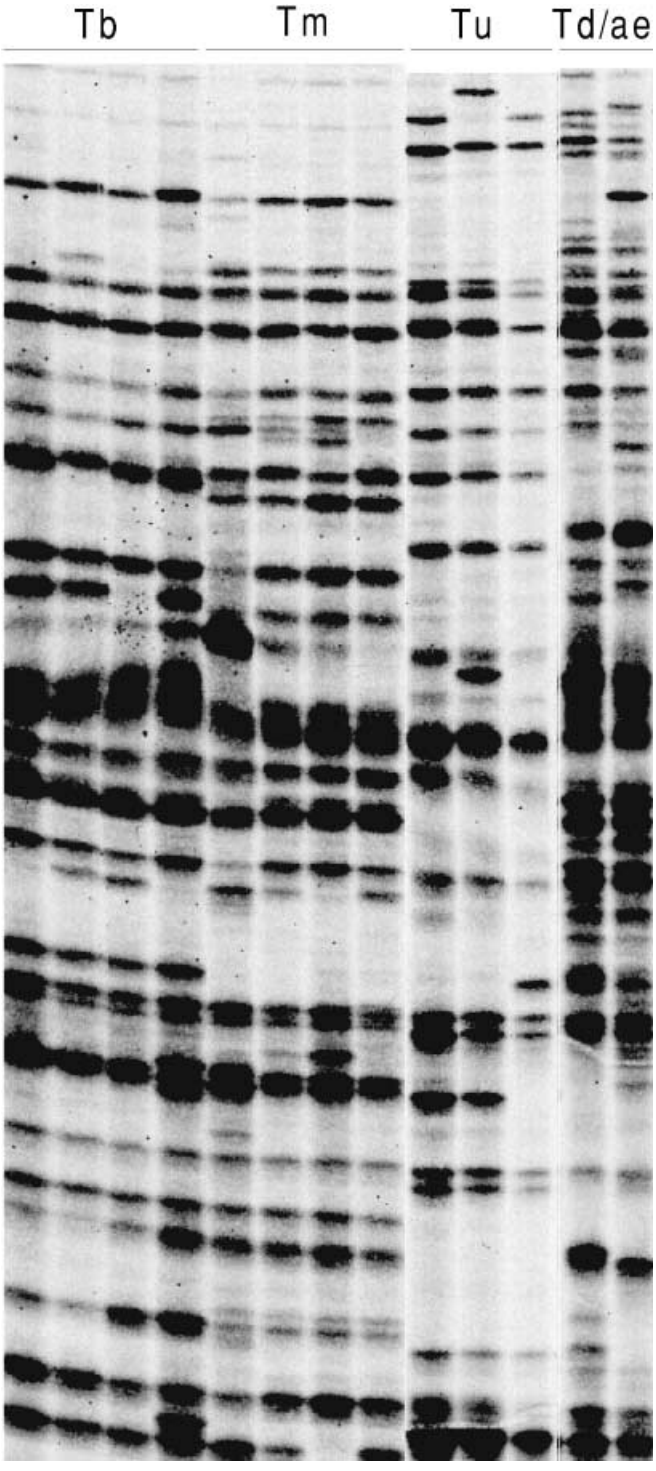

Fig. 2 AFLP pattern of *T. boeticum* (*Tb*), *T. monococcum* (*Tm*), *T. urartu* (*Tu*), *T. durum* (*Td*) and *T. aestivum* (*Tae*)

cal *T. monococcum* accessions clustered with the *T. monococcum* group and the remainder with *T. boeoticum*. One atypical *T. boeoticum* accession (352502) resembled typical *T. boeoticum* morphologically. The two plants of the other atypical *T. boeoticum* accession (427447) differed morphologically and did not cluster together.

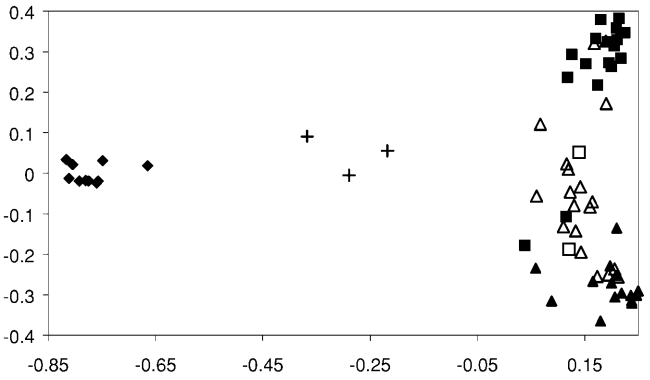

Fig. 3 Principal co-ordinate plot of all accessions based on one AFLP fingerprint per accession; the two principal co-ordinates accounted for 29.7% and 12.7% of the variation respectively. ■ *T. boeoticum*, ▲ *T. monococcum*, ◆ *T. urartu*, + *T. durum* and *T. aestivum*; closed symbols typical accessions, *open symbols* atypical accessions

AFLP patterns of typical *T. monococcum* and *T. boeoticum* were relatively similar while *T. urartu* was clearly different from these two species. As expected, the tetra- and hexa-ploid accessions showed a different pattern with more fragments than did accessions of the diploid species (Fig. 2). With primer combination E33/M58 between 47 and 52 AFLP fragments were amplified per species. In total, 64 fragments were used for the analysis of the diploid species and another eight fragments when the polyploid accessions were included. The number of monomorphic markers (present in all accessions of one species and absent in the other species) ranged from 20 to 24 per diploid species, and of polymorphic markers (present in some accessions of one species and absent in the other species) from 26 to 32. None of the monomorphic markers present in typical *T. monococcum* accessions were absent in all typical accessions of *T. boeoticum*, and vice versa. Therefore, no species-specific markers for these species were generated using this primer combination. The distinction between these two species was restricted to polymorphic markers. Between *T. urartu* and typical *T. boeoticum* and *T. monococcum*, respectively, the percentage of polymorphism based on monomorphic markers was 21% and 25%. The rate of polymorphism within *T. boeoticum* was 59%, in *T. monococcum* 56% and in *T. urartu* 42%. The three typical *T. boeoticum* ssp. *thaoudar* accessions had highly polymorphic AFLP patterns (43% polymorphism); two of these three AFLP patterns were also very different from the other *T. boeoticum* accessions. If the three typical *T. boeoticum* ssp. *thaoudar* were not taken into account 54% polymorphism was found in typical *T. boeoticum* accessions.

In the PCO analysis based on the AFLP data of all species, the three sets of typical diploid accessions and the polyploid accessions each formed a distinct cluster (Fig. 3). However, two typical *T. boeoticum* ssp. *thaoudar* accessions (352504 and 352505) and most of the atypical *T. boeoticum* and *T. monococcum* accessions were placed in between the two typical clusters of these

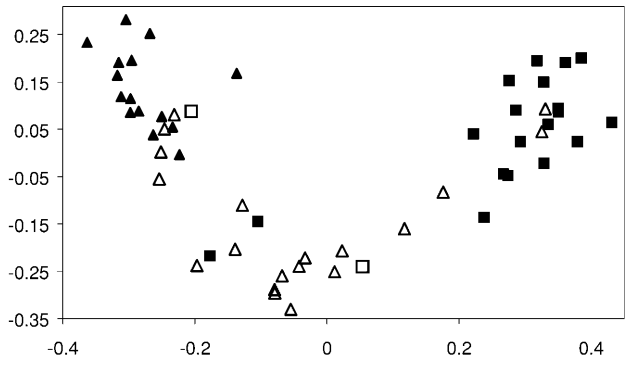

Fig. 4 Principal co-ordinate plot of typical and atypical *T. monococcum* and *T. boeoticum* accessions, based on the AFLP fingerprint as in Fig. 3. The two principal co-ordinates accounted for 22.2% and 8.9% of the variation respectively. ■ *T. boeoticum*, ▲ *T. monococcum*; *closed symbols* typical accessions, *open symbols* atypical accessions

two species (Fig. 4). Four atypical *T. monococcum* accessions (345133, 345186, 573529 and 591871) clustered within *T. monococcum* and two within *T. boeoticum* (560719 and 560721). These six accessions are the only atypical accessions for which the morphological data result in the same clustering as the molecular data. The groups formed in PCO analysis were confirmed with UPGMA analysis both for morphological and AFLP data (data not shown). The UPGMA analysis also showed that all accessions, save two *T. urartu* accessions, could be unambiguously discriminated based on the 72 amplified fragments (data not shown).

In summary, all three diploid wheat species, represented by the typical accessions, could be distinguished with PCO analysis based on morphology and on molecular data. The clustering of most atypical accessions, susceptible *T. monococcum* and resistant *T. boeoticum*, was not consistent with the species they were originally assigned to.

Discussion

The data presented here showed that the 72 AFLP fragments obtained with one primer combination selected for high numbers of amplification products and high polymorphism rate, gave a clear separation of the three diploid wheat species *T. monococcum*, *T. boeoticum* and *T. urartu*. The use of DNA markers for the classification of different taxa is a relatively new development. In general, the clustering of accessions based on AFLP markers corresponds well with previous taxonomic classifications of the plant species studied (e.g. *Lactuca* spp.: Hill et al. 1996; *Manihot* spp.: Roa et al. 1997; *Solanum* spp.: Kardolus et al. 1998; *Gossypium* spp.: Pillay and Myers 1999; *Oryza* spp.: Aggarwal et al. 1999). Different numbers of polymorphic amplified fragments have been used to establish relatedness: from 273 by Mace et al. (1999) in the Solanaceae to 1,191 by Aggarwal et al. (1999) in rice. In the former study, major clusters obtained with each individual primer combination (nine to 49 markers) were similar to that with all primer combinations together.

Although the AFLP patterns of *T. monococcum* and *T. boeoticum* were distinct, species-specific monomorphic markers were not found, indicating a close relatedness between these two species. Nevertheless, the two species could be unambiguously distinguished in the principal co-ordinate analysis based on polymorphic markers. *T. monococcum* and *T. boeoticum* patterns were quite different from the *T. urartu* pattern. This is in agreement with the findings by, amongst others Castagna et al. (1994), Ciaffi et al. (1997), Dvorak et al. (1988) and Hammer et al. (2000), who applied gliadin patterns and RFLP markers based on single and repetitive sequences and microsatellite markers respectively. The poor crossability between *T. urartu* and either *T. monococcum* or *T. boeoticum*, almost always resulting in sterile F_1s, also indicates the distant relatedness between *T. urartu* and the other two species (Johnson and Dhaliwal 1976).

Based on the morphological and molecular data, two atypical accessions received as *T. monococcum* were actually polyploid wheats. The other atypical *T. monococcum* accessions could be divided into three groups. Two accessions resembled *T. boeoticum* morphologically and molecularly. Most likely these accessions are typical susceptible *T. boeoticum* assigned to the wrong species in the germplasm collection.

The use of AFLP markers provided insight into the intermediary status of the 13 atypical *T. monococcum* accessions that morphologically resembled typical *T. boeoticum* accessions but molecularly clustered between *T. boeoticum* and *T. monococcum*. These accessions may represent the progeny of hybrids that arose in the past. The offspring may have retained some of the *T. boeoticum* genotype, viz. for most of the morphological characters listed in Table 2, and for reaction to leaf rust, but also be genetically similar to *T. monococcum*. As both diploid wheat species cross relatively easily giving fertile offspring, hybridisation and hence genetic exchange could easily have taken place in the region of common origin or even during propagation in the germplasm collection. Gene flow between closely related sympatric species has been observed for other genera as well (e.g. *Daucus*, Wijnheijmer et al. 1989; *Chenopodium*, Wilson and Manhart 1993).

Only four accessions resembled typical *T. monococcum* accessions both morphologically and molecularly but were susceptible to the wheat leaf rust fungus. Thus, 98% of the *T. monococcum* accessions previously tested were resistant to wheat leaf rust instead of 84% (Anker and Niks 2001). The susceptible *T. monococcum* accessions could lack the resistance gene(s) to (one of) the avirulence gene(s) in the isolate. Niks (1988) and Heath (1991) presented a model and cited evidence that non-host resistance in plants may be based on a very high allele frequency of one or a few resistance genes in the plant species and an equally high frequency of corresponding avirulence alleles in the pathogen. Histological observations in *T. monococcum* have shown that the majority of the accessions reacted to wheat leaf rust with a hypersensitive response

(Anker and Niks 2001). This indicates that the species-specific reaction to wheat leaf rust in diploid wheat is most likely based on a high allele frequency of one or more effective major genes in *T. monococcum*, and the absence thereof in the closely related *T. boeoticum*, in the four susceptible *T. monococcum* accessions and in *T. urartu*. Hence, *T. monococcum* has almost a nonhost status to the wheat leaf rust (see Niks 1987).

To verify the model of a high allele frequency in both *T. monococcum* and the fungus for resistance genes and corresponding avirulence genes, large-scale tests with different isolates would be necessary. A small-scale test with three leaf rust isolates and a mixture of isolates from two accessions each of typical and atypical *T. monococcum* and *T. boeoticum* showed that the typical *T. monococcum* and *T. boeoticum* were resistant and susceptible respectively. The atypical *T. boeoticum* were in general resistant and the atypical *T. monococcum* accessions showed a variable reaction depending on the inoculum used (data not shown; work by D.L. Long, Cereal Disease Laboratory). These results support the model described above.

The results presented here support the assumption of Anker and Niks (2001) that *P. triticina* isolate 'Felix' has species-specific pathogenicity to the three diploid wheat species and that *T. monococcum* has almost a non-host status to the pathogen. The results emphasise the need for verification of the identity of accessions in resistance surveys as was indicated by Niks (1987). This is especially true if seedlings of closely related species are tested and no distinction can be made based upon seed and seedling morphology.

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