Évaluation of *aegilops* for resistance to *Septoria tritici*

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Abstract

Good levels of resistance to *Septoria tritici* (perfect state: *Mycosphaerella graminicola*) have been identified in wheat species (*Triticum aestivum* and *T. durum*) but no immune reaction has been reported. The objective of the present study is to screen wild relatives of wheat for resistance to the pathogen. One hundred and sixty accessions of *Aegilops* species, close and distant relatives to wheat, were tested at the seedling stage against two geographically differing isolates of *S. tritici*. High level of resistance and possibly immune reactions were present in species that carry the D, S and U-genomes in ascending frequencies. Accessions of *T. speltoides* 45 and *T. tauschii* 72 showed no leaf necrosis when inoculated with the Arkansas isolate. *Triticum tauschii* and T. speltoides are direct ancestors of wheat. Identified resistance should be readily transferred to wheat due to the common genomes.

Key words : Hessian fly, Tan spot, Wheat, *Triticum aestivum*, Morocco, disease resistance

Résumé

Evaluation des *Aegilops* pour la résistance à la septoriose

Des niveaux appréciables de résistance à *Septoria tritici* (stade parfait : *Mycosphaerella graminicola*) ont été identifiées dans les blés (*Triticum aestivum* and *Triticum durum*) mais aucune réaction d'immunité n'a été rapportée. L'objectif du présent travail est de trier les espèces sauvages voisines du blé dur pour la résistance à ce pathogène. Cent soixante accessions des espèces *Aegilops* proches et lointaines du blé ont été testées au stade plantule avec deux isolats de *S. tritici* d'origine géographique différente. Des niveaux élevés de résistance et des réactions d'immunité ont été identifiés dans les espèces qui portent les génomes D, S ou U en fréquence croissante. Les accessions de *Ae. speltoides* 45 et *Ae. squarrosa* 72 ont manifesté une absence de nécrose quand elles ont été inoculées par un isolat d'Arkansas USA. *Aegilops squarrosa* et *Ae. speltoides* sont des parents lointains du blé. La résistance identifiée dans ces espèces sera relativement facile à transférer au blé grâce à la présence de génomes en commun.

Mots-clés : Cécidomyie, mouche de Hesse, tâche bronzée, blé, *Triticum aestivum*, Maroc, résistance aux maladies

ملخص

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أظهرت الأبحاث عن مستوى هام من مقاومة القمح الصلب و القمح الطري للتبقع السبتوري *Septoria tritici* لكن المناعة التامة لم يتم العثور عليها. تهدف هذه الدراسة إلى تقييم الأعشاب البرية إيجيلوبس *Aegilops* التي لها قرابة أصلية مع القمح لمقاومتها لهذا المرض. و قد تم تقييم مائة و ستين صنفا في مرحلة ثلاث ورقات من حيث مقاومتها لفوعتين من فوعات *Septoria tritici* ينتميان إلى أماكن مختلفة جغرافيا : الولايات المتحدة الأمريكية والمغرب. ثم كشف مستوى عال من المقاومة ونوع من المناعة الكاملة في بعض الحالات في هذه الأعشاب ولاسيما تلك التي تملك مجموع الكروموزومات من نوع D-S-U . الصنف رقم 45 من *Ae. speltoides* الصنف رقم 72 من *Ae. squarrosa* يعتبران أسلافا مباشرة للقمح. وهكذا، فالمناعة المحتملة في هذين الصنفين يمكن نقلها بدون صعوبة إلى القمح.

الكلمات المفتاحية : ذبابة الهس، التبقع السبتوري، القمح، القمح الطري، المغرب، مقاومة الأمراض

Introduction

Septoria tritici blotch is a world-wide disease causing substantial losses in wheat fields ranging from 10 to 80 % in Morocco (Jlibene 1991). Search for sources of resistance within the wheat species (*Triticum aestivum*) as a means to control this disease have been rewarding. Several sources of resistance have been identified after extensive screening of wheat germplasm collections maintained at different parts of the world (Rosielle 1972; Djerbi *et al.* 1976; Arjana *et al.*, 1975; Krupinsky *et al.* 1977; Eyal *et al.* 1983). However, presently identified sources of resistance do not provide full protection to the crop. Higher levels of resistance have been reported in close and distant relatives of wheat, particularly *Agropyron* (Rillo *et al.* 1970; Gough et Tuleen 1979) and *Secale* (May 1983) species. *T. monococcum* which is known to carry the A-genome, also present in wheat, has been reported resistant to several isolates from the United States and the Mediterranean Region (Yechilevich *et al.* 1983). However, no attention has been given to *Aegilops* species which carry the D or the B-genomes or equivalent. The objective of the present study was to evaluate 160 accessions belonging to 18 species of *Aegilops* for resistance to two geographically distant strains of *M.cosphaerella graminicola.*

Material and methods

Eighty seven entries of 9 species of Aegilops, kindly supplied by Dr. Kimber (University of Missouri) were planted, 10 seeds per entry in trays, November, 8, 1985, and vernalized at 4 °C for 6 weeks. The seedlings in the 1-2 leaf stage were removed from the vernalizer and inoculated twice with an Arkansas inoculum (kindly supplied by P.J. Raymond, Monsanto Chemical Co., St. Louis, Missouri) at a rate of 10^6 spores/ml. The suspension was uniformly spread using a pressurized atomizer. Inoculated seedlings were put in a dew chamber at 24 °C day (16 hours), 22 °C night (8 hours) for 48 hours with no light during the first 24 hours. A susceptible (cv 'Nasma') and a resistant wheat (cv 'BT908') were included as checks. Three weeks later, percent area infected of the two top leaves was recorded. Resistant seedlings and seedlings of the susceptible wheat check were transferred into pots to the greenhouse and inoculated at boot stage. Plants were covered with plastic during the 72 hours following inoculation to ensure high relative humidity.

The same entries and others supplied by Dr. Amri, a total of 160 entries belonging to 19 species were grown in a growth chamber at the Centre Régional de la Recherche Agronomique, Settat, Morocco (Aridoculture Program) and inoculated at the tillering stage, with an isolate from Morocco. A concentrated solution of spores increased in a liquid medium was spread using an atomizer. Seedlings were put for incubation in a dew chamber for 48 hours. Upon removal from the incubator, seedlings were put in a growth chamber where disease rating was done three weeks later. Seedlings were rated 'R' if no apparent symptoms were observed, 'MR' if some discoloration on lower leaves was visible, 'MS' when up to 25 % necrosis occurred and 'S' with more than 25% necrosis. Species and their genomic formulae are given in table 1.

Results

Reaction to Arkansas isolate

A wide variation in reaction to the disease was observed among species and among accessions within the same species (Table 2). Two thirds of the accessions scored less than 20 % disease necrosis, among which 75 % were in the range 0-10 %. Because we seek a high level of resistance and because of the abundance of resistance in *Aegilops* species tested, accessions showing more than 20 % necrosis were considered as susceptible. Entries were considered resistant 'R' if a disease score was less than 10 % necrosis and moderately resistant 'MR' if more than 10 % and less than 20 %. The resistant bread wheat check 'BT908' scored 15.25 % and the susceptible check 'Nasma' scored 28.24 %. Only one third of the accessions tested scored more than 20 % infection. Also 60 % of the accessions showed higher levels of resistance than the resistant wheat check cv 'BT908', stressing the importance of wild species as a reservoir of valuable sources of resistance to this disease. Resistance was found in 7 of 8 species (*T. bicorne* was the exception), with high frequencies in *T. crassum, T. cylindricum, T. tauschii, T. ventricosum* and *T. speltoides*. Two accessions (C 45 and CI 72) of *T. speltoides* and *T. tauschii* respectively showed no symptoms of the disease.

Table 1. *Aegilops* species tested for reaction to *Mycosphaerella graminicola* (Morocco and Arkansas isolates), their genomic formulae and origin of cytoplasm

Species	Genome[1]	Plasma[1]
T. cylindricum	CD	D
T. tauschii	D	D
T. ventricosum	DN	D
T. juvenale	DMU	D2
T. crassum	DM	D2
T. crassum	DDM	D2
T. aestivum	ABD	B
T. monococcum	A	A
T. beoticum	A	A
T. speltoides	S	S, G
T. longissima	Sl	S^l
T. bicorne	Sb	Sb
T. kotschyi	US	S2
T. dichasians	C	C
T. triaristatum 4X	UM	U
T. triaristatum 6X	UMN	U
T. triunciale	UC	C, U
T. columnare	UM	U2
T. comosum	M	M
T. ovatum	UM	M^o
T. uniaristatum	N	N

[1]underlining indicates modified genomes (source : Kimber and Tsunewaki 1988)

45 and CI 72) of *T. speltoides* and *T. tauschii* respectively showed no symptoms of the disease (Table 3). All the three *T. crassum* lines were resistant and all 16 *T. cylindricum* lines tested were either resistant or moderately resistant (Table 2). Seventy five percent of the 31 *T. tauschii* accessions and 56% of the 16 *T. speltoides* were either resistant or moderately resistant (Table 2). Accessions of *T. kotschyi*, *T. juvenale* and *T. bicorne* were mostly susceptible (Table 2).

Some resistant accessions were transferred into pots and inoculated at heading stage with the same isolate. The results showed that lines whose seedlings were selected as resistant were also resistant at adult plant stage. Pycnidia developed on the susceptible check 'Nasma'.

Reaction to Morocco isolate

The percentage of resistant lines to the moroccan isolate identified for each species was less than that obtained with the Arkansas isolate (Table 4). For example, only 82 % (vs 100 %) of *T. Cylindricum*, 51 % (vs 75 %) of

T. Tauschii and 50 % (vs 56 %) of *T. Speltoides* entries were found resistant. This could be due to higher aggressiveness of the Moroccan isolate or to the use of concentrated inoculum of the moroccan isolate or both. Many reports have indicated differences in virulences of the pathogen. Accessions caracterized as MS with the Morocco isolate may in fact be considered as resistant. However, 85 accessions out of 185 (46 %) belonging mostly to *T. Cylindricum, T. tauschii* and *T. triunciale* were rated resistant (Table 4). Accessions of *T. triunciale* were not tested with Arkansas isolate due to shortage of space. Nasma, used as check, was highly susceptible to the Moroccan isolate.

Table 2. Frequency of accessions of *Aegilops* species grouped into four classes of reactions, resistant (R), medium resistant (MR), medium susceptible (MS) and susceptible (S) to an Arkansas isolate of *Mycosphaerella graminicola* at seedling stage.

	Number of Accessions	Classes of reaction			
Species		R	MR	MS	S
T. crassum	3	100			
T. cylindricum	16	88	12		
T. tauschii	31	65	10	3	22
T. ventricosum	8	13	50	25	13
T. speltoides	16	31	25	19	25
T. kotschyi	5	20		60	20
T. juvenale	3			67	33
T. bicorne	3				100
Total	85	52	15	13	20

Entries resistant to Morocco and Arkansas isolates

Accessions tested under both isolates and showing resistance in both situations are presented in Table 5. Among 32 entries tested, 20 were resistant to both isolates (ie, 62 %). The frequency of resistant lines was higher in the *T. cylindricum* (88 %) than in the *T. tauschii* (53 %) species, both carrying the D-genome. The bread wheat check 'Nasma' was susceptible to both isolates, but less so to Arkansas isolate.

T. tauschii (DD) is a direct ancestor of wheat, contributing to the D-genome. The wheat group can be divided into three clusters of species, the A, D and U-genome clusters (Kimber, G, 1988). The A-genome cluster included accessions of *T. monococcum* and *T. beoticum*. The 12 *T. monococcum* and the 3 *T. beoticum* entries carrying the A-genome did not show good resistance to the Moroccan isolate. Yechilevich *et al.* reported *T. monococcum* as having high frequency of resistance to seven geographically differing isolates including some from the Mediterranean region. However, the high level of stress imposed in this study, ie concentrated inoculum, may be responsible for the observed results. The U-genome cluster included *T. ovatum, T. columnare, T. triunciale*

Table 3. *Aegilops* accessions resistant to Arkansas isolate of *M. graminicola* (% necrosis less than 10%) at seedling stage

Species	PI/CI number	Number of plants	% Necrosis	Standard error
T. speltoides	000045	5	0.00	0.00*
T. tauschii	000072	5	0.00	0.00*
T. tauschii	000025	3	0.33	0.33
T. cylindricum	172357	8	0.37	0.18*
T. cylindricum	374353	2	0.50	0.50
T. cylindricum	374347	7	0.71	0.18*
T. tauschii	000026	6	0.83	0.48*
T. cylindricum	-	4	1.00	0.71
T. tauschii	000017	9	1.11	0.39*
T. tauschii	000021	8	1.12	0.44*
T. cylindricum	330484	7	1.14	0.83*
T. cylindricum	172358	11	1.18	0.55*
T. tauschii	000009	9	1.22	0.36*
T. tauschii	000018	9	1.22	0.36*
T. crassum	276972	9	1.22	0.36*
T. cylindricum	374345	3	1.33	0.88
T. tauschii	000027	7	1.57	0.75*
T. cylindricum	266814	7	1.71	0.52*
T. tauschii	000010	8	1.75	0.37*
T. cylindricum	428561	9	1.78	0.40*
T. tauschii	000019	9	1.78	0.57*
T. cylindricum	344778	5	1.80	1.07*
T. tauschii	000020	7	1.86	0.40 *
T. tauschii	000013	8	1.87	0.44*
T. cylindricum	276977	11	1.91	0.51*
T. speltoides	369583	5	2.60	2.36
T. tauschii	000022	7	2.71	0.71*
T. cylindricum	276974	8	3.00	1.46
T. speltoides	393493	6	3.17	1.28
T. tauschii	000011	6	3.83	1.01
T. crassum	392330	8	3.87	1.80
T. tauschii	000008	5	4.40	1.03
T. tauschii	000024	9	4.44	2.60*
T. tauschii	276985	4	4.50	0.87
T. cylindricum	374320	6	5.17	3.99
T. tauschii	000004	7	5.71	1.29
T. cylindricum	276976	10	7.40	2.97
T. kotschyi	000036	7	7.43	3.22
T. ventricosum	276999	11	7.73	2.19
T. tauschii	300489	10	8.20	2.26
T. crassum	330483	7	8.43	3.06
T. speltoides	000061	6	8.50	1.63
T. tauschii	452130	7	9.29	3.05
T. speltoides	449340	5	9.60	5.24

* Seedlings transplanted to test adult plant reaction to the disease

and *T. kotschyi*. High frequency of resistance to the Moroccan isolate was observed in this group. However, the number of entries tested did not exceed 5 except for *T. triunciale* species which included 40 accessions. The D-genome cluster was represented by accessions of *T. tauschii, T. cylindricum, T. ventricosum, T. crassum* and *T. juvenale*, all of them had high level of resistance to the *Septoria tritici* blotch disease.

Resistance to *M. graminicola* appears to be present in high frequencies in the D, U, and S-genome carrying species and with less frequency in A-genome carrying species. Methods to transfer resistance genes from *T. tauschii* to wheat have been devised and used with relative success to transfer Hessian fly resistance genes (Amri 1989). Among accessions resistant to both isolates, five *T. tauchii* (CI 8, 9, 19, 24 and 27) and three *T. cylindricum* (CI 172357, CI 172358, CI 374353), all carrying the D-genome, have been reported to be resistant to Moroccan population of Hessian fly (*Mayetiola destructor*) (Amri *et al.* 1992). The three entries of *T. cylindricum* were also found resistant to a US isolate of *Pyrenophora tritici-repentis,* the causal organism of Tan-spot (Alam and Gustafson 1988). These entries should constitute excellent sources for multiple resistance to a complex of parasites. Our experience in the field indicated that wheat with resistance derived from *Triticum tauschii* possesses good adaptation to Moroccan environments. The source parent used was developed at Kansas State University and supplied by Dr. R. Sears to Amri as part of the PhD thesis material (Amri, pers. comm.). Some entries, resistant to H. fly and rusts (*Puccini spp.*), have been identified and are in the stage of advanced yield tests. Release of some of these lines is expected.

Tableau 4. Frequency of resistance to *M. graminicola* found in Aegilops species seedlings (Morocco isolate)

Species	N	% R	% MR	% MS	% S
T. cylindricum	11	9	27	45	18
T. tauschii	37	3	24	24	49
T. triaristatum	16	13	25	25	38
T. triunciale	40	18	25	30	28
T. juvenale	3	30	30		30
T. ovatum	8		12	38	50
T. monococcum	12		17	8	75
T. speltoides	4		25	25	50
T. bicorne	2		50		
T. aestivum	4		25		75
T. dichasians	2		50		50
T. beoticum	3			67	33
T. crassum	2			50	50
T. Kotschyi	4			75	25
T. columnaris	3			100	
T. comosum	1			100	
T. ventricosum	5				5
T. uniaristatum	2			2	100
T. longissimum	1			100	100
Total	160	8	21	28	43

R, MR, S and MS refer to Resistant, Medium Resistant, Susceptible and Medium Susceptible, respectively.

Table 5. *Aegilops* accessions resistant to both Morocco and Arkansas isolates of *Mycosphaerella graminicola*, at seedling stage

Species	CI/PI number	Reaction to isolate from	
		Arkansas	Morocco
T. cylindricum[12]	172357 *	R	MR
T. cylindricum[12]	374353	R	MR
T. cylindricum[2]	330484	R	R
T. cylindricum[12]	172358	R	R
T. cylindricum	344778	R	MR
T. cylindricum[2]	374320	R	R
R. *cylindricum*	000044	MR	MR
T. cylindricum[2]	349035	MR	MR
*T. tauschii*1	000008	R	R
*T. tauschii*1	000024	R	R
*T. tauschii*1	000009	R	R
T. tauschii[2]	000005	MR	R
T. tauschii[2]	000026	R	MR
T. tauschii[2]	000021	R	MR
T. tauschii[1]	000027	R	MR
T. tauschii[1]	000019	R	MR
T. tauschii	000020	R	MR
T. tauschii[2]	276985	R	MR
T. speltoides	393493	R	R
T. crassum[2]	392330	R	MR
T. aestivum (check)	Nasma	S	S[+]

R, MR, S : Resistant, Moderately resistant and Susceptible, respectively.
(+) indicates the presence of pycnidia;
1 : Resistant to Hessian fly (Amri et al., 1993)
2 : Resistant to Tan-spot (Alam and Gustafson, 1988)

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