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Resistance of Wild Barley (*Hordeum spontaneum*, *H. marinum* and *H. murinum*) to *Pyrenophora teres* and *Rhynchosporium secalis* causing Net Blotch and Scald Diseases in Tunisia

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ABSTRACT

Resistance to net blotch and scald in the seedling and in the adult growth stages were evaluated in 56 accessions of wild barley (*Hordeum spontaneum*, *H. marinum* and *H. murinum*) and seven varieties of *H. vulgare*. Generally, the screened barley genotypes were more resistant than the four checks for net blotch and scald diseases. Results of net blotch evaluation indicated that 88 and 51% of the barley genotypes were significantly more resistant than the most resistant check in the seedling and the adult growth stages, respectively. The *H. marinum* genotypes collected from Algeria and Egypt (accessions 4, 5 and 7) and the *H. spontaneum* genotypes collected from Afghanistan, Iraq and Syria (accessions 14, 25 and 43) were the most resistant in both growing stages. For both diseases (net blotch + scald), 36% of the evaluated barley genotypes were significantly more resistant than the most resistant check to a mixture of isolates. According to their reaction to both diseases, the accessions of *H. murinum* from Armenia, of *H. marinum* from Algeria, Egypt and Kazakhstan, and of *H. spontaneum* from Afghanistan, Azerbaijan, China, Egypt, Iraq, Lebanon, Libya, Syria, Turkmenistan, Turkey and Uzbekistan showed the highest level of resistance in the field. For scald, 45 and 15% of the evaluated barley genotypes exhibited more resistance than the mid check at seedling and adult growth stages, respectively. Among the evaluated barley genotypes, three accessions of *H. marinum* collected from Algeria and Egypt (accessions 4, 5 and 7) and two accessions of *H. spontaneum* collected from Afghanistan and Egypt (accessions 14 and 22) were resistant in both stages.

Keywords: cultivated barley, *Hordeum spontaneum*, *Pyrenophora teres*, resistant, *Rhynchosporium secalis*
Abbreviations: INAT, Institut National Agronomique de Tunisie; **LSD**, least significant difference; **LSI**, least significant increase

INTRODUCTION

Wild barley, *Hordeum vulgare* subsp. *spontaneum* (C. Koch) Thell (*H. spontaneum*), is the progenitor of cultivated barley (*Hordeum vulgare* L.). *H. spontaneum* is a common species in the Fertile Crescent (von Bothmer *et al.* 2003) that can be found across a wide geographic range extending from Israel and western Jordan to southeastern Turkey and covering areas from eastern Iraq and western Iran (Harlan 1992). *H. spontaneum* and *H. vulgare* have the same chromosomal number ($2n$ = 14). These two species could be easily crossed because both are fully inter-fertile, allowing the introgression of agronomically important genes from wild into cultivated barley (Lehmann 1991; Fischbeck and Jahoor 1992). Natural hybrids can occur when these two species coexist in the same field. In addition to *H. spontaneum*, von Bothmer *et al.* (1995) reported about 30 other wild species of *Hordeum* throughout the world. Among *Hordeum* species, *H. marinum* and *H. murinum*, commonly known as sea and wall barley, respectively are widespread in several areas including North Africa and the Middle East. However, the use of these two species in barley breeding programs is difficult due to strong sterility barriers and chromosomal instability between the wild species and *H. vulgare* (von Bothmer and Jacobsen 1990). In this case, the transfer of genes from wild species to cultivated barley is possible only by using transformation techniques.

Net blotch, incited by *Pyrenophora teres* [(Died.) Drechsl.] and scald, caused by *Rhynchosporium secalis* [(Oudem.) J.J. Davis], are the two most important foliar diseases of barley in Tunisia (Cherif *et al.* 1994) that are associated with high severity levels (70-80%) in some regions particularly during favorable weather conditions (pers. obs.). Among the various strategies to manage crop diseases, disease resistance is of immense practical importance. Although sources of resistance to these diseases have been identified from international and Tunisian material of *H. vulgare* (Bjørnstad *et al.* 2002; Afanasenko *et al.* 2004; Cherif *et al.* 2007; Silvar *et al.* 2009; Kamel *et al.* 2010), they do not represent the greatest potential diversity available for breeding purposes. Several studies have shown that *H. spontaneum* represents an alternative and rich reservoir of diverse alleles for disease resistance, which are rarely found in the cultivated barley germplasm. Sato and Takeda (1997) found that most accessions of *H spontaneum* evaluated at the seedling stage for their reaction to four *P. teres* isolates were highly resistant as they exhibited 1 to 2 on the rating scale, as described by Tekauz (1985). Also, Fetch *et al.* (2003) reported that resistance to net blotch at the seedling stage was frequent in *H. spontaneum*, where about 70% of accessions from both Israel and Jordan exhibited 1 to 5 on the rating scale. In the study of Steffenson *et al.* (2007), about 90% of the *H. spontaneum* accessions assessed were resistant to net blotch. Moreover, net blotch resistance is common in *H. marinum* and *H. murinum*. Sato and Takeda (1997) identified five accessions of *H. marinum* with an extremely high level of resistance to all isolates of *P. teres*. Wild barley also contains diverse resistance to barley leaf scald (Garvin *et al.* 1997; Genger *et al.* 2003). Since disease resistance is frequent in *H. spontaneum*, it may be possible to identify accessions with resistance to both net blotch and scald diseases that will be crossed with advanced barley breeding lines to simultaneously transfer resistance genes of

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Table 1 Data of wild and cultivated barley.

N°	Latin name	Origin country (wild barley) / Name (cultivated barley)	Province	Longitude	Latitude	Altitude
1	*H. murinum*	Armenia	Vayots Dzor	E045 11	N39 43 44	1,354
2	*H. marinum*	Azerbaijan	Abseron	E49 41 55	N40 30 06	50
3	*H. marinum*	Cyprus	Kyrenia	E33 04	N35 18	300
4	*H. marinum*	Algeria	Guelma	E07 24	N36 25	240
5	*H. marinum*	Algeria	Constantine	E07 01	N36 22	660
6	*H. marinum*	Egypt	Marsa Matruh	E27 13	N31 15	30
7	*H. marinum*	Egypt	Alexandria	E29 57	N31 10	10
8	*H. marinum*	Iran	Lorestan	E48 00	N32 45	-
9	*H. marinum*	Kazakhstan	Chimkent	E69 27.86	N42 23.59	500
10	*H. marinum*	Morocco	Nord Ouest	W06 18	N33 54	500
11	*H. marinum*	Morocco	Centre Nord	W004 54	N33 51 24	1,150
12	*H. marinum*	Russia	-	-	-	-
13	*H. marinum*	Russia	-	-	-	-
14	*H. spontaneum*	Afghanistan	Badakhshan	E70 34	N37 06	1,220
15	*H. spontaneum*	Afghanistan	Baghlan	E68	N36	637
16	*H. spontaneum*	Armenia	Ararat	E45 22	N39 47	-
17	*H. spontaneum*	Azerbaijan	Abseron	E49 28 49	N40 29 54	90
18	*H. spontaneum*	China	Tibet	E91 05	N29 41	-
19	*H. spontaneum*	China	-	-	-	-
20	*H. spontaneum*	Cyprus	Famaqusta	E34 01	N34 59 20	60
21	*H. spontaneum*	Cyprus	Famagusta	E34 03	N34 59	110
22	*H. spontaneum*	Egypt	Marsa Matruh	E27 10	N31 21	10
23	*H. spontaneum*	Iran	Fars	E050 50	N30 04	-
24	*H. spontaneum*	Iran	Hamadan	E48 29	N34 06	-
25	*H. spontaneum*	Iraq	As Sulaymaniyah	E44 50	N35 32	640
26	*H. spontaneum*	Iraq	Ninawa	E43 00	N35 35	250
27	*H. spontaneum*	Jordan	Irbid	E35 50	N32 32	590
28	*H. spontaneum*	Jordan	Ma'an	E35 29	N30 18	1,510
29	*H. spontaneum*	Kazakhstan	Chimkent	E69 41.85	N42 25.26	650
30	*H. spontaneum*	Lebanon	Rachaiya	E35 46	N33 31	1,050
31	*H. spontaneum*	Lebanon	Zahle	E36 01	N33 48	1,180
32	*H. spontaneum*	Libya	Al Fatih	E20 54	N32 33	320
33	*H. spontaneum*	Libya	Al Jabal al Akhdar	E21 43	N32 46	590
34	*H. spontaneum*	Pakistan	Baluchistan	E66 54	N30 18	1,400
35	*H. spontaneum*	Pakistan	Baluchistan	E66 54	N30 18	1,400
36	*H. spontaneum*	Palestine	Jerusalem	E35 03 53	N31 44	-
37	*H. spontaneum*	Palestine	Tel Aviv	E34 50	N32 10	-
38	*H. spontaneum*	Russia	Dagestan	E48 22	N41 56	40
39	*H. spontaneum*	Russia	-	-	-	-
40	*H. spontaneum*	Syria	Lattakia	E35 49 15	N35 36 20	20
41	*H. spontaneum*	Syria	Damascus	E36 05 40	N33 47 25	1,500
42	*H. spontaneum*	Syria	Dar'a	E36 10 30	N32 50 01	-
43	*H. spontaneum*	Syria	Aleppo	E37 46 12	N36 29 02	490
44	*H. spontaneum*	Syria	Homs	E36 38 07	N34 54 23	360
45	*H. spontaneum*	Syria	Idlib	E36 53 34	N35 33 50	420
46	*H. spontaneum*	Syria	Sweida	E36 46 25	N32 37 30	1,385
47	*H. spontaneum*	Syria	Al Hasakah	E41	N37	428
48	*H. spontaneum*	Syria	Al Hasakah	E41	N37	477
49	*H. spontaneum*	Tajikistan	Khudzhand	E69 20 40	N40 08 20	410
50	*H. spontaneum*	Tajikistan	Kulyab	70.05054	38. 17984	1,315
51	*H. spontaneum*	Turkmenistan	Ashkhabad	E57 07	N38 35	950
52	*H. spontaneum*	Turkmenistan	Krasnovodsk	E56 32 26	N38 53 11	-50
53	*H. spontaneum*	Turkey	Hakkari	E44 29	N37 15	1,125
54	*H. spontaneum*	Turkey	Gaziantep	E37 21 04	N36 52 55	610
55	*H. spontaneum*	Uzbekistan	Tashkent	E69 02	N41 10	410
56	*H. spontaneum*	Uzbekistan	Dzhizak	E068 04	N39 42 46	1,550
57	*H. vulgare*	'CI 14373'	-	-	-	-
58	*H. vulgare*	'CI 7584'	-	-	-	-
59	*H. vulgare*	'CI 1197'	-	-	-	-
60	*H. vulgare*	'CI 9776'	-	-	-	-
61	*H. vulgare*	'CI 2750'	-	-	-	-
62	*H. vulgare*	'CI 9819'	-	-	-	-
63	*H. vulgare*	'Compana'	-	-		-

both diseases. Thus, the objective of this study was to assess the reaction of several wild barley accessions from ICARDA and some known cultivars of *H. vulgare* to Tunisian isolates of *P. teres* and *R. secalis* in the seedling and in the adult growth stages.

MATERIALS AND METHODS

Plant materials

Plant material evaluated in this study consisted of 56 accessions of wild barley (*H. spontaneum*, *H. marinum* and *H. murinum*) pre-

served and provided by the International Center for Agricultural Research in the Dry Areas and seven resistant (Afanasenko *et al.* 1995) cultivars of *H. vulgare* (**Table 1**). The Tunisian commonly used cultivars 'Martin', 'Manel', 'Rihane' and 'Roho' were used as susceptible checks.

Pathogen isolates and inoculums preparation

Three Tunisian isolates of *P. teres* collected from 'Tunis', 'Morneg' and 'Mograne' and four Tunisian isolates of *R. secalis* collected from 'Jdidi', 'Boussalem', 'Krib' and 'Teboursouk' were used to screen the wild and the cultivated barley. These isolates were chosen according to their aggressiveness on barley genotypes. Nevertheless, their virulence was not tested. Therefore, they are considered as geographic isolates.

The isolates of *P. teres* were cultured on V8 juice agar (200 ml of V8 juice, Cambell Soup Co. Ltd.; 20 g agar; 3 g $CaCO_3$; 800 ml distilled water) in Petri dishes at 20°C under cool white and near ultra violet light with a 12-h photoperiod for 7 days (Steffenson *et al.* 1996). The *R. secalis* isolates were increased on LBA agar (20 g LBA, 5 g agar) in Petri dishes at 18°C for 13 days in the dark (Bouajila *et al.* 2006).

Inocula were prepared by scraping conidia from fungal cultures using a small volume of distilled water. The suspensions of conidia were filtered through double layers of gauze and the concentration was adjusted to10^4 conidia/ml for *P. teres* and to 10^6 spores/ml for *R. secalis*. Tween 20 was added as one drop/100 ml of distilled water (Steffenson *et al.* 1996; Bouajila *et al.* 2006).

Moreover, for net blotch disease, barley seeds inoculum was produced in order to increase chances of such infection at the adult growth stage. This inoculum was prepared by growing 1 ml of *P. teres* suspension adjusted to 10^4 conidia/ml on 200 g of moistened and autoclaved barley seeds that were incubated at 20 ± 0.5°C under a 12-h photoperiod for 20 days.

Disease assessment

For seedling test, five seeds of each accession of wild barley and variety of cultivated barley were planted in a plastic tray (60 × 40 × 4 cm) using a completely randomized design with two replications. The trays were placed in a growth chamber maintained at 20-23°C and a 16-h photoperiod. Fifteen days after sowing, seedlings were inoculated separately with 'Morneg' isolate of *P. teres* and 'Krib' isolate of *R. secalis*. Inoculum was applied with an atomizer until the plants were uniformly wet. Inoculated plants were incubated in a mist chamber at 100% RH at 20°C for 48 h and then returned to the growth chamber. The infection responses of barley seedlings to *P. teres* were rated 12 days after inoculation on the qualitative 1 to 10 scale of Tekauz (1985). Symptoms on the second leaves of inoculated plants with *R. secalis* were scored 21 days after inoculation using the numerical disease (0 to 5) described by Salamti and Tronsmo (1997).

For the adult growth stage, tests for disease resistance were carried out at INAT (Institut National Agronomique de Tunisie). Barley lines were sown on December 7, 2009, using an augmented design with three replications. Each line was sown in single 1 m long row spaced 0.4 m apart. No spreader rows were planted. The trial was conducted following optimal cultural practices, but without applying fungicides. At the early-tillering stage of growth (GS 22-26) (Zadoks *et al.* 1974), plants were artificially inoculated by both net blotch and scald pathogens. Net blotch inoculation was made by spraying a spore suspension and by scattering pre-infected barley seeds (approximately 30 g/m row line) with a mixture of the three isolates ('Tunis', 'Morneg' and 'Mograne' isolates) of *P. teres*. However, artificial inoculation of scald has been achieved by spraying a spore suspension of the four isolates of *R. secalis*: 'Jdidi', 'Boussalem', 'Krib' and 'Teboursouk'. The mixture of isolates was used in order to identify genotypes with general resistance as in the natural conditions. Inoculated plants were then covered for at least 24 h with transparent polyethylene sheets in order to provide a high relative humidity to promote infection. Net blotch and scald symptoms on the foliage were recorded at the flowering (GS 61-65) (Zadoks *et al.* 1974) growth stage using the percent disease severity (Burleigh and Loubane 1984) on five randomly selected plants per line.

Statistical analysis

The frequency distributions of disease reactions among the barley genotypes were generated using Excel. An analysis of variance (ANOVA) was performed to determine differences among wild and cultivated barley genotypes for their reaction to net blotch and scald in the seedling and adult growth stage using PROC GLM (SAS institute 1988). Models I and II were used for data estimated in the growth chamber and in the field, respectively.

Model I: $Y_{ij} = \mu + G_i + \varepsilon_{i(j)}$

where Y_{ij} is the observation of genotype i in replication j, μ is the general mean, G_i is the effect of genotype i and $\varepsilon_{i(j)}$ is the residual effect.

Model II: $Y_{ij} = \mu + b_j + c_i + X_i(C_i) + \varepsilon_{ij}$

where Y_{ij} is the observation of genotype i in block j, μ is the general mean, b_j is the block effect, c_i is the check, $X_i(C_i)$ is the genotype effect and ε_{ij} is the residual effect. Then, barley genotypes were compared to the most resistant check and to the mid check (the mean of the four checks) using the $LSD_{0.05}$ (least significant difference) and the $LSI_{0.05}$ (least significant increase) for the growth chamber and the field data, respectively.

RESULTS AND DISCUSSION

For the growth chamber experiment, net blotch development progressed rapidly and symptoms appeared on susceptible accessions and cultivars seven days after inoculation. However, scald symptoms appeared 10-12 days after inoculation. Therefore, net blotch and scald reactions on barley genotypes were recorded 19 and 21 days after inoculation, respectively.

Seedling response of barley genotypes to *P. teres* and *R. secalis* isolates was variable. In general, higher disease scores were observed on susceptible check of cultivated

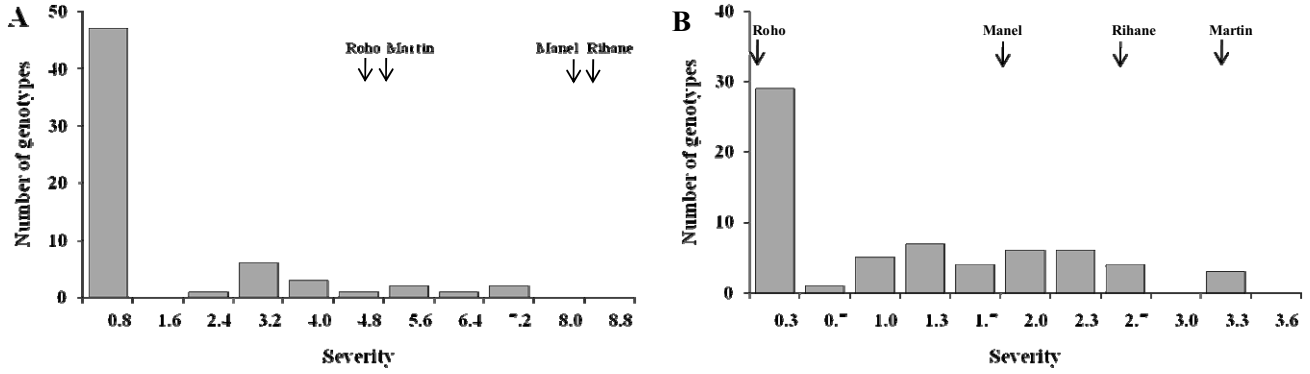

Fig. 1 Frequency distributions of disease reactions among the 56 accessions of wild barley and the seven cultivars of cultivated barley evaluated at the seedling stage. (**A**) Net blotch reaction. (**B**) Scald reaction.

Table 2 Mean squares of net blotch and scald reaction on the seedling stage.

Source of variation	df	Net blotch reaction	Scald reaction
Genotype	66	10.73**	2.14**
Error	67	0.84	0.65
CV (%)		66.00	81.70

** Significant at $P < 0.01$

barley. The reaction of all 56 accessions of wild barley and the seven cultivars of cultivated barley to 'Morneg' isolate of *P. teres* and to 'Krib' isolate of *R. Secalis* is presented in **Fig. 1**. For net blotch reaction, the majority of genotypes were highly resistant since 73% of them exhibited less than 1 on the 1-10 rating scale. Infection responses of 'Roho', 'Martin', 'Manel' and 'Rihane' were 4.7, 5.0, 8.2 and 8.3, respectively. Thus, approximately 93% of the barley genotypes were more resistant (to 'Morneg' isolate of *P. teres*) than 'Rihane' and 'Manel', and 85% were more resistant than 'Martin' and 'Roho' (**Fig. 1A**). Scald reaction varied from zero to 3.3 on the 0-5 rating scale. About 94, 91 and 76% of the barley genotypes were more resistant than 'Martin', 'Rihane' and 'Manel', respectively; but none of the genotypes were more resistant than 'Roho' (**Fig. 1B**).

ANOVA showed highly significant differences between barley genotypes for their reactions to net blotch and scald at the seedling stage (**Table 2**). The mean comparison test indicated that 88% (59 genotypes) of the evaluated barley genotypes were significantly more resistant to net blotch ('Morneg' isolate) than the most resistant check ('Roho'). 60% of these genotypes are *H. spontaneum* whereas 19% are *H. marinum* and 9% are *H. vulgare* (**Table 3**). All the evaluated accessions of *H. marinum*, 63% of the evaluated accessions of *H. spontaneum* and 71% of the evaluated cultivars of *H. vulgare* were highly resistant with a zero infection response. This would imply that the resistant genes of these accessions are effective in controlling Tunisian isolates. The most resistant accessions of *H. spontaneum* originated from various geographic areas, mainly the Fertile Crescent and Central Asia. In a previous study, Sato

Table 3 The most resistant genotypes to net blotch on the seedling and adult growth stages using the LSD and LSI tests.

Genotypes	Disease reaction at the seedling stage	Genotypes	Adjusted disease severity at the adult growth stage
1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10 - 11 - 12 - 13 - 14 - 15 - 16 - 17- 23 - 25 - 27 - 28 - 29 - 30 - 31 - 33 - 35 - 36 - 38 - 39 - 41 - 43 - 44 - 46 - 47 - 50 - 51 - 52 - 53 - 54 - 55 - 58 - 59 - 61 - 62 - 63	0.00	4 - 5 - 7 - 14 - 25 - 43	-1.53
49 - 56	0.16	32	-1.13
40 - 42	0.25	22	-1.03
60	0.33	31 - 49 - 52 - 56	0.00
32	0.67	9 - 17 - 29 - 42 - 48 - 51	0.75
48	0.75	10 - 13 - 16 - 19 - 36 - 38 - 50 - 53 - 55	0.77
18	1.00	12	1.27
19	1.58	54	1.75
22	1.67	1	2.00
26	1.83	37	2.77
34	2.00	41 - 45 - 47	3.25
21	2.33	**'Roho'**	**10.94**
45	3.00		
'Roho'	**4.70**		

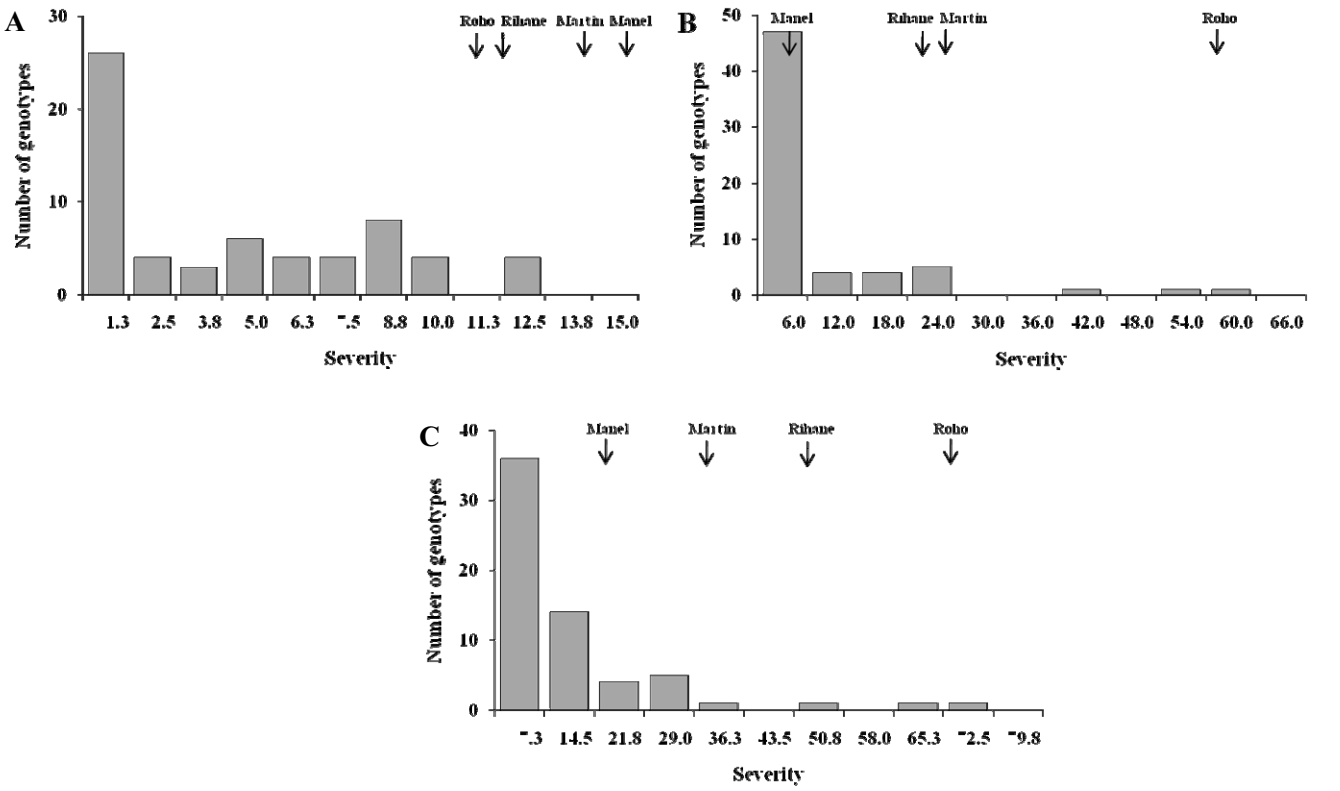

Fig. 2 Frequency distributions of disease reactions among the 56 accessions of wild barley and the seven cultivars of cultivated barley evaluated in the adult growth stage. (**A**) Net blotch reaction. (**B**) Scald reaction. (**C**) Net blotch + scald reaction.

Table 4 Mean squares of net blotch, scald and net blotch + scald reactions on the adult growth stage.

Source of variation	df	Net blotch reaction	Scald reaction	Net blotch + scald reaction
Bloc	2	6.18^{ns}	40.55^{ns}	55.33^{ns}
Genotype	60	25.60^{ns}	192.04^{ns}	309.24^{ns}
Check	3	11.77^{ns}	1389.59^{ns}	1277.60^{ns}
Error	6	8.81	362.18	435.91
CV (%)		55.46	219.44	148.84

^{ns}: not significant at P<0.05

and Takeda (1997) found that the average resistance of *H. spontaneum* accessions was higher in accessions from Afghanistan, Iran, Pakistan and Russia (Central Asia). In addition, Jana and Bailey (1995) and Sato and Takeda (1997) as well as Fetch *et al.* (2003) reported that resistant accessions were frequent in the Middle East. However, in general, net blotch resistance was more often found in germplasm from the humid (e.g., Mediterranean coast) than these from the arid areas (e.g., Negev Desert) (Fetch *et al.* 2003). On the other hand, accessions from Morocco were found to be susceptible (Sato and Takeda 1997). Therefore, resistance to *P. teres* at the seedling stage is widespread in *H. spontaneum* accessions of different provenance. Moreover, the cultivated barley cultivars 'CI 7584', 'CI 1197', 'CI 2750', 'CI 9819' and 'Compana' showed a highly resistant level to net blotch at the seedling stage. For scald disease, the LSD test showed that no barley genotype was significantly more resistant to the 'Krib' isolate than the most resistant check ('Roho'), which exhibited an infection response of zero. However, five accessions of *H. marinum* (42%), 21 accessions of *H. spontaneum* (49%) and 3 cultivars of *H. vulgare* (43%) (data not shown) were significantly more resistant than the mid check, which had an infection response of 1.85%. Resistance to scald disease at the seedling stage was previously reported by Abbott *et al.* (1992) who observed that 77% of the evaluated accessions of *H. spontaneum* from Israel, Iran and Turkey were highly resistant to *R. secalis* according to the 0-4 scale of Jackson and Webster (1976).

The frequency distributions of net blotch, scald and the sum net blotch + scald reactions in the adult growth stage of wild and cultivated barley genotypes can be observed in **Fig. 2**. Net blotch severity varied from 0 to 13%. Approximately, 85% of the evaluated barley genotypes were more resistant than 'Roho' and 'Rihane' and 91% of the genotypes were more resistant than 'Martin' and 'Manel' (**Fig. 2A**). Scald severity varied from 0 to 60% (**Fig. 2B**). About 71% of the evaluated barley genotypes had a severity < 6%. The severities of 'Manel', 'Rihane', 'Martin' and 'Roho' were 5, 21, 24 and 57%, respectively. Thus, about 71, 80, 86 and 89% of the barley genotypes were more resistant than 'Manel', 'Rihane', 'Martin' and 'Roho', respectively. **Fig. 2C** shows that net blotch + scald reaction varied from 0 to 73%. Almost 74, 86, 87 and 89% of the genotypes were more resistant than 'Manel', 'Martin', 'Rihane' and 'Roho', respectively.

ANOVA revealed that the genotype effect was not significant for net blotch, scald and the sum net blotch + scald reactions at the adult growth stage (**Table 4**). The LSI test indicated that 51% (34 genotypes) of the evaluated barley genotypes were significantly more resistant to a mixture of isolates of *P. teres* than the most resistant check ('Roho'). 76% of these genotypes were *H. spontaneum*, 26% were *H. marinum* and 3% were *H. marinum* (**Table 3**). However, all the cultivated barley genotypes known for their resistance to *P. teres* (Afanasenko *et al.* 1995) were susceptible under field conditions at INAT probably due to the high aggressiveness of the mixture of the three isolates used (isolates of 'Tunis', 'Morneg' and 'Mograne') and the favorable climatic conditions to net blotch expansion. The most resistant *H. marinum* genotypes originated from North Africa (Algeria and Egypt) and the most resistant *H. spontaneum* genotypes originated from several areas in the Middle East and Central Asia (Afghanistan, Egypt, Iraq, Lebanon, Libya, Syria, Tajikistan, Turkmenistan and Uzbekistan). Thus, no relationship was noted between environmental conditions of temperature, rainfall or altitude and disease resistance of *H. spontaneum*. Similar results were obtained by Fetch *et al.* (2003), who identified accessions with high levels of net blotch resistance in the higher moisture areas of the Mediterranean coast to the arid region of the Negev Desert. Moreover, the Middle East and Central Asia, identified in this study as the origin of the source of resistance, could be considered as important centers of diversity for *P. teres* and those co-evolutionary forces are operating in this pathosystem. Finally, the *H. marinum* genotypes collected from Algeria and Egypt (accessions 4, 5 and 7) and the *H. spontaneum* genotypes collected from Afghanistan, Iraq and Syria (accessions 14, 25 and 43) were the most resistant to net blotch in the seedling and adult growth stages as they exhibited a zero infection response and percent disease severity at both growing stages. For scald disease, the LSI was equal to 47.66, indicating that no barley genotype was significantly more resistant to a mixture of isolates than the most resistant check ('Manel'). However, three accessions of *H. marinum* (25%), five accessions of *H. spontaneum* (12%) and two cultivars of *H. vulgare* (29%) (data not shown) were significantly more resistant than the mid check. Among the evaluated barley genotypes, three accessions of *H. marinum* collected from Algeria and Egypt (accessions 4, 5 and 7) and two accessions of *H. spontaneum* collected from Afghanistan and Egypt (accessions 14 and 22) were resistant to *R. secalis* in the seedling and adult growth stages. For the cultivated barley cultivars, 'CI1197' and 'CI9776' exhibited a 0 infection response at the seedling stage and cultivars 'CI9819' and 'Compana' exhibited severities equal to 0 in the adult growth stage. For the sum net blotch + scald, the LSI test indicated that 36% (24 genotypes) of the evaluated barley genotypes were significantly more resistant to a mixture of isolates than the most resistant check ('Manal') (**Table 5**). 79% of these genotypes were *H. spontaneum*, 17% were *H. marinum* and 4% were *H. marinum*. However, all the evaluated *H. vulgare* cultivars were significantly more susceptible than the most resistant check for both diseases. It seems therefore that resistance to both net blotch and scald is more frequent in wild than in cultivated barley genotypes. Jana and Bailey (1995) found that 4.5% of *H. spontaneum* accessions and only 0.3% of *H. vulgare* cultivars were resistant to *Cochliobolus sativus.* In addition, they found that 22.0% of *H. spontaneum* accessions and only 0.5% of *H. vulgare* varieties were resistant to *P. teres.* According to their reaction to both diseases, the accessions of *H. marinum* from Armenia, of *H. marinum* from Algeria, Egypt and Kazakhstan, and of *H. spontaneum* from Afghanistan, Azerbaijan, China, Egypt, Iraq, Lebanon, Libya, Syria, Turkmenistan, Turkey and

Table 5 The most resistant genotypes to the sum net blotch + scald reaction on the adult growth stage using the LSI test.

Genotype	Adjusted severity
1 - 9 - 17 - 29 - 31 - 41 - 42 - 45 - 47 - 48 - 51 - 54	-0.90
56	-0.40
4 - 5 - 7 - 14 - 22 - 25 - 32 - 43	-0.19
52	0.09
53	0.42
49	0.49
'Manel'	**7.34**

Uzbekistan showed the highest level of resistance. These results support the effectiveness of resistance genes to Tunisian isolates of *P. teres* and *R. secalis* among these accessions. Gustafsson and Claesson (1988) indicated that resistance in the wild species they sampled was due to a combination of different characters, including waxy layers of leaves, leaf pubescence or biochemical factors.

The obtained results indicated that accessions 4, 5 and 7 of *H. marinum* and accessions 14, 25 and 43 of *H. spontaneum* were highly resistant to net blotch and scald at seedling and adult growth stages. However, the resistance of the determined genotypes should be confirmed by carrying out the screening trials for at least two generations for better credibility of the findings. The selected accessions of wild barley could be useful parents in barley breeding programs for net blotch and scald resistance. Nevertheless, the transfer of genes from *Hordeum* species, other than *H. spontaneum*, into cultivated barley is difficult, due to the strong incompatibility barriers between wild species and cultivated barley (Pickering and Johnston 2005). Therefore, introgression of resistant factors will be more advantageous using *H. spontaneum*. Resistant accessions of this specie could then be crossed with superior barley lines in order to generate new populations of barley and to simultaneously transfer genes of resistance for both diseases into cultivated barley. Furthermore, a DNA marker study would be developed to map new resistance loci provided by wild barley. Then, the identified markers could assist barley breeders to generate cultivars with high level of resistance.

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