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Évaluation de la résistance à l'oïdium dans des populations d'orge sauvage (Hordeum spontaneum L.) de la région égéenne de la Turquir

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Résumé de l'article

In a study of resistance to powdery mildew, we investigated three populations of *Hordeum spontaneum* using leaf segment test. For comparison of the reactions, we used 44 barley lines as differentials representing most of the resistance used in barley breeding. The *H. spontaneum* accessions were infected with 21 isolates selected for their reactions on the differential barleys. The results of *H. spontaneum* collections did not show any similarities with differential barleys used. Resistance reactions were very rare. There was no resistance to 13 out of the 21 isolates. Horizontal resistance was noticed in most of *H. spontaneum* lines.

Assessment of powdery mildew resistance in wild barley (*Hordeum spontaneum* L.) populations in the aegean region of Turkey

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[Évaluation de la résistance à l'oïdium dans des populations d'orge sauvage (*Hordeum spontaneum* L.) de la région égéenne de la Turquie]

Dans une étude sur la résistance à l'oïdium, nous avons évalué trois populations de l'*Hordeum spontaneum* à l'aide d'un test sur des portions de feuilles. Afin de comparer les réactions, nous avons utilisé 44 lignées différentielles d'orge qui représentent l'essentiel de la résistance utilisée dans les programmes d'amélioration génétique de l'orge. Les obtentions de l'*H. spontaneum* ont été infectées par 21 isolats choisis en fonction de leurs réactions sur les orges différentielles. Les résultats avec les échantillons de l'*H. spontaneum* ont été très différents de ceux obtenus avec les orges différentielles. Les réactions de résistance ont été très rares. Il n'y a pas eu de résistance envers 13 des 21 isolats. Une résistance horizontale a été remarquée pour la plupart des lignées de l'*H. spontaneum*.

INTRODUCTION

Powdery mildew caused by *Blumeria graminis* f. sp. *hordei* Em. Marchal is one of the principal foliar diseases of barley (*Hordeum spontaneum* L.) in Europe and Turkey. This disease has been controlled in Europe by the use of host resistance genes and of fungicides. In order to have resistance to the disease, varieties with race-specific resistance genes have been regularly introduced since early sixties (Löwer *et al.* 1997). Generally, the most extensively used resistance genes have remained effective for only a few yr until an increase in the frequency of corresponding virulence genes of the pathogen was observed, then the varieties with the resistance genes in question became increasingly more susceptible to powdery mildew (Brown and Joergensen 1991; Fischbeck and Jahoor 1991; Jahoor and Fiscbeck 1987a; Jiang *et al.*

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1984; Moseman *et al.* 1985). On the other hand, natural populations of wild progenitors of crops have coevolved with their parasites in the origin, so they have been regarded as potentially valuable sources for disease resistance, due to continuous assaults by the plant pathogens. In order to improve the strategic use of resistance, it is necessary to find a new resistance gene in wild barley populations (Jahoor and Fischbeck 1987a; Nevo 1986; Nevo *et al.* 1979). Wild barley is abundant and widespread over many regions in Turkey (Braun and Turgut 1995) and is an important source for resistance to obligated parasites (Jahoor and Fischbeck 1987b; Moseman and Joergensen 1973; Segal *et al.* 1987).

Large number of *Hordeum spontaneum* accessions collected from natural populations in Israel were studied and it was reported that many of them were either resistant or showed low infection to field infection by powdery mildew in Germany (Fischbeck *et al.* 1976). Jahoor and Fishbeck (1987a) searched resistance genes in 42 barley lines derived from the F_7 generation of crosses, between barley cultivars and different accessions of *H. spontaneum* collected in Israel and concluded that natural populations of *H. spontaneum* formed a large pool for mildew resistance which has not been used yet against virulence genes of powdery mildew (Bothmer and Joergensen 1986).

The objective of this study was to describe occurrence and frequency of resistance genes in natural population of *H. spontaneum* at three different micro-sites in the Aegean region in Turkey.

MATERIALS AND METHODS

The material of study consisted of 200 seed samples of *Hordeum spontaneum* collected at different locations on the West Coast of Turkey in September 1998. The seeds were planted in January 1999 at the experimental field of the Dalaman Plant Production Center and harvested in May.

Brief descriptions of the populations at three micro sites

Bornova population: The population was collected on the slope of dranage canal that was 1.5 m wide and 150 m long, near the experimental field of the Field Crop Department of Agricultural Faculty at Aegean University. The distance from the micro site to Lzmir bay was approximately 10 km with altitude of 40 m.

Meryemana population: This population was established in the Virgin Mary House in the National Park located on the top of a hill at Selçuk-Izmir province. The distance from this micro site to Selçuk was approximately 15 km and altitude was 500 m. The area of the population was 1 m wide and 140 m long.

Emiralem population: The popu-lation was along Emiralem-Manisa highways side slope, nearby Göktepe Village. The area of population was 1 m x 130 m and altitude 40 m.

Differential set of monogenic resistant barley lines

Pallas near isogenic lines of barley (Koelster *et al.* 1986) and other lines from RisØ National Laboratory, Denmark, were used as a differential set for comparison with the resistance reaction of the wild barleys. In addition, we included 14 lines with resistance derived from *H. spontaneum* collected in Israel (Jahoor and Fischbeck 1987b).

Fungal material

Ten test isolates of *Blumeria graminis* f. sp. *hordei* provided by J.H. Jørgensen, six isolates produced from cleistothecia of the pathogen and five isolates from three pathogen populations examined in this study were used for differentiation. These test isolates were selected according to their infection types (Table 1) to the differential barley set (Table 2).

Resistance test

Test isolates were used to identify resistance in wild barley populations. The leaf segment test was carried out using detached primary leaf segments of 10 d old *H. spontaneum* seedlings. Primary leaves of young barley plants were

Table 1. Infection types of *Erysiphe graminis* isolates on barley lines (according to Welz (1986))

Infection score	Symptoms
0	No infection, no necrotic lesions
1	Necrotic flecks, few mycelium, no sporulation
2	Few necrotic flecks, little sporulation
3	Medium sporulation
4	Full sporulation

clipped and cut in three segments. One segment of each line was laid into one Petri dish, giving 25 segments with different *H. spontaneum* plants and the control variety, Manchuria. The leaf segments were put on water agar containing 30 μL L^{-1} Benzimidazole (and 5 g agar, allowing the leaves to stay green for approximately 3 wk), and kept under standard conditions (17-18 °C; 14/10 h day/night). These were also the incubation conditions for the pathogen when inoculated onto the leaf segments in the Petri dishes.

Inoculations were performed at a sterile clean bench using a settling tower (tin cylinder diam 180 mm, 300 mm height, open at one side) with a small hole close to the upper side of the tower. This cylinder was put over an opened Petri dish containing 26 line segments. Through the hole at the upper side of settling tower one segment with one single spore colony was held and its conidia were blown into the tower. There the conidia could settle down in still air on the leaf segments. After incubating the closed Petri dishes for nine d under standard conditions, the leaf segments were evaluated for infections. If any mycelium were visible on leaf segment of the control variety, we assigned this as a successful infection, indicating the presence of the respective virulence factor in the tested isolate.

The reactions were evaluated according to infection types as described by Welz (1986) (Table 1). The list of the barley lines that had been used as dif-

Table 2. List of the barley lines used as differentials in the determination of the virulence of the test isolates and resistance genes

	Line	Gene(s)		Line	Gene(s)
1	P 01	Mla1, Mla(Al2)	23	Nigrate	Mla30
2	Manchuria Iso 1R	Mla1, Mla(Al2)	24	Turkey 290	Mla31
3	Black Russian	Mla2, Mla(BR2)	25	Sv 83380	Ml(Ab)
4	P 02	Mla3	26	Atlas	Mlat
5	Gunnar	Mla3, Mla(Tu2)	27	Goldfoil	Mlg
6	Gopal	Mla5	28	Deba Abed	Mlg, Ml(CP)
7	P 03	Mla6, Mla14	29	P21	Mlg,?
8	P 04A	Mla7, Mlk1	30	P24	Mlh
9	P 04B	Mla7	31	Line 81882	Mlhb
10	Manchuria Iso 26R	Mla7, Mla(LG2), Mla(LG3)	32	Herta	Ml(He), Mla8
11	Manchuria Iso 10R	Mla7, Mla(Mu2)	33	P17	Mlk1
12	Triumph	Mla7, Mla(Tr3), Ml(Ab)	34	Nakaizumi -Zairai	Mlk2
13	Calsberg II	Mla8	35	Kairyo Bozu	Mlkb
14	P 08B	Mla9	36	Pallas 23	MlLa
15	P 09	Mla10, Mla(Du2)	37	Pallas 18	Mlnn
16	A222	Mla11	38	Pallas 22	Mlo5
17	P10	Mla12, Mla(Em2)	39	Pallas 19	Mlp
18	P11	Mla13, Mla(Ru3), Mla(Ru4)	40	Russian 74	Mlr74
19	P12	Mla22	41	Russian 81	Ml81
20	P13	Mla23	42	Pallas 14	Mlra
21	Engledow India	Mla24	43	Pallas 15	Ml (Ru2)
22	RS1-8	Mla27	44	Manchuria	Control

ferentials in the determination of the virulence of the test isolates and of the presence of resistance genes is presented in Table 2.

For the identification of the infection, the infection assesment was transformed into a binary code (Habgood 1970). Infection types 3 and 4 (compatible) were transformed to 1, while the rest were transformed to 0. Comparing these infection reading with Table 3, we inferred which resistance genes were effective most probably in the wild barley plants.

RESULTS AND DISCUSSION

When accessions of *H. spontaneum* from three different locations in Turkey were inoculated with isolates of *B. graminis* f. sp. *hordei*, it was found that the progenies were susceptible; slow mildewing occurred frequently. The reaction pattern of all *H. spontaneum* accessions was clearly different from reaction of the known genes for mildew resistance included in this study. Our infection data confirmed that the resistance factor(s) in wild barley were non-specific for the pathogen. Since the majority of the *H. spontaneum* accessions showed distinctive variation in resistant reaction types against mildew cultures, the study did not support the assumption that differences in resistant reaction types against distinct mildew cultures are sufficient to indicate the presence of supplementary genes for mildew resistance in a given genotype of the host. The natural population of *H. spontaneum* in Turkey is regarded as a large gene pool for powdery mildew that is not yet used in cultivated barley. Il has been concluded that *H. spontane-*

Table 3. Examples of some *Hordeum spontaneum* accessions and their reactions against 21 isolates, 16 days after inoculation

Population[a]	Plant number	BR3a	BR6a	BR9a	BR40b	BR43a	BR50b	M46c	E49a	E101j	B44b	B64b	A6	58-74	C15	MH21	R86-1	FP4-13	Race1 HL3/5	HL3/5	GE3	A27
M	119	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	4	4	4
M	121	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
M	123	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4
M	125	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
M	129	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
M	135	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
M	137	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
M	139	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
M	141	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
B	20	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4
B	28	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
B	86	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4
B	118	4	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4
B	156	4	4	4	4	4	4	4	4	4	0	0	4	3	4	4	4	4	4	4	4	4
B	160	4	4	4	4	4	4	4	4	4	1	4	4	4	4	4	4	4	4	4	4	4
B	169	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
B	172	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
B	191	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
B	193	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4
E	111	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4
E	112	4	4	4	4	4	4	4	4	4	4	4	4	4	1	4	4	4	4	4	4	1
E	114	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	1	4	4	4	4	3
E	120	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
E	121	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3
E	130	1	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3	2	4	4	4	3
Control		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

[a] M: Meryemana; B: Bornova; E: Emiralem.

um may be a useful genetic resource for breeding resistance to powdery mildew.

Only few resistance reactions to the test isolates could be observed. None of these reactions were identical with the reactions on the differential barley lines, suggesting unknown resistance alleles were present. The *H. spontaneum* samples showed a reduced number of colonies compared with the susceptible control Manchuria. The infection of wild barleys was also slowed down significantly, with the assessment having to be done 14-16 d after inoculation, instead of 8-9 d with commercial varieties.

After 14-16 d of incubation, few resistance reactions indicate some major gene reactions, which are most likely unknown resistance genes. Even resistance reactions like those derived from Israeli *H. spontaneum* collections could not be found, indicating a different coevolution in Israel and Turkey. These resistant reactions from Israel *H. spontaneum* showed important differences mainly in comparison with the frequent susceptibility found in the Turkish samples. Neither the corresponding resistance to the virulence found in the mildew tests from cleistothecia of populations were detected nor any other resistance from the large differential set of barley lines.

The observations of delayed infection and reduced colonies clearly indicate the presence of horizontal resistance in these wild barley populations. The assessment of leaf segment test was delayed compared with the control variety, Manchuria under the same conditions due to slow infection and growth of the colonies. The reduced number of colonies was clearly visible, but due to the high infection density, an exact quantification was impossible. For assessment of horizontal resistance it may also be better to use seedling test rather than leaf segment test in which the leaves decay rapidly when incubated for more than 2 wk.

Wild plant populations are probably protected from the severe effects of parasite attacks by their genetic diversity in relation to the various forms of resistance and tolerance.

One possible hypothesis for the presence of a high amount of horizontal resistance and its advantage may be the climatic conditions that these populations experience. The vegetation period of wild barley in this region is very short, only about 10 wk. During this period, there are about 5-6 wk of favourable conditions for powdery mildew, due to increasing temperatures at the end of April. This means that only a very limited number of generations with limited amount of offsprings can be produced successfully, which may be enough to avoid a mildew epidemic. Horizontal resistance to local isolates is more stable under selection than vertical resistance. Because of this reason, horizontal resistance may be a better defence strategy under the ecological conditions in wild barley growing areas in Turkey.

In conclusion, horizontal resistance can be effectively used to barley breeding programs. There are no crossing barriers between cultivated barley and *H. spontaneum* and they also share the same genome (Bothmer and Joergensen 1986).

Furthermore, this presents plant breeders with particular difficulties when deploying new sources of resistance. In the future, it will be interesting to observe the effect of horizontal resistance on selection for common virulences. The area of barley varieties with horizontal resistance can be increased rapidly.

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