

## Phytoprotection



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### Évaluation de gènes de résistance à l'oïdium (*Blumeria graminis* f. sp. *hordei*) sur des variétés d'orge de Turquie

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#### Article abstract

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## Assessment of powdery mildew (*Blumeria graminis* f. sp. *hordei*) resistance genes in Turkish barley varieties

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Thirty-four Turkish barley (*Hordeum vulgare*) varieties were tested for the presence of resistance genes (R-genes) to powdery mildew (*Blumeria graminis* f. sp. *hordei*) using the “leaf segment test” with nine isolates of the fungus. The most commonly found R-gene was *Mla8*, while a combination of R-genes *Mlg* and *MI(CP)* was found in three varieties (Tokak 157/37, Beyşehir 98, Konevei 98). The gene *Mlh* was found in four varieties (Obruk 86, Anadolu 86, Çıldır 02, Özdemir 05), while no R-gene was found in three varieties (Hamidiye 85, Yesevi 93, Bülbül 89). No clear inferences could be made for the partly-resistant varieties Gemici 7243, Yea 793.12 and Akhisar 98 using the test isolates selected, which suggests the presence of still unidentified R-genes. Overall, 10 previously characterized R-genes for powdery mildew (*Mla8*, *MI(La)*, *Mlg*, *MI(CP)*, *Mlh*, *Mlat*, *Mla1*, *Mlh*, *Mla7*, *Mlra*) were present in Turkish barley varieties, along with some other(s) still to be identified.

Key words: Barley, *Blumeria graminis* f. sp. *hordea*, *Hordeum vulgare*, leaf segment test, powdery mildew, resistance genes.

### [Évaluation de gènes de résistance à l'oïdium (*Blumeria graminis* f. sp. *hordei*) sur des variétés d'orge de Turquie]

Trente-quatre variétés d'orge (*Hordeum vulgare*) cultivées en Turquie ont été testées pour la présence de gènes de résistance (R) à l'oïdium (blanc) (*Blumeria graminis* f. sp. *hordei*) à l'aide du « test des segments foliaires » et de neuf isolats du champignon. Le gène R le plus commun parmi les variétés testées était *Mla8*, alors qu'une combinaison des gènes *Mlg* et *MI(CP)* était trouvée chez trois variétés (Tokak 157/37, Beyşehir 98, Konevei 98). La présence du gène *Mlh* a été identifiée chez quatre variétés (Obruk 86, Anadolu 86, Çıldır 02, Özdemir 05), alors qu'aucun gène R n'a été détecté dans trois autres variétés (Hamidiye 85, Yesevi 93, Bülbül 89). Aucune inférence n'a pu être clairement établie pour les variétés Gemici 7243, Yea 793.12 et Akhisar 98 à l'aide des isolats fongiques utilisés, ce qui suggère la présence de gènes R encore non identifiés. Au total, 10 gènes de résistance à l'oïdium (*Mla8*, *MI(La)*, *Mlg*, *MI(CP)*, *Mlh*, *Mlat*, *Mla1*, *Mlh*, *Mla7*, *Mlra*) étaient présents dans les variétés d'orge cultivées en Turquie, en plus d'autre(s) gène(s) non identifié(s).

Mots clés: *Blumeria graminis* f. sp. *hordea*, gènes de résistance, *Hordeum vulgare*, oïdium, orge, test des segments foliaires.

### Dedicated to the memory of Dr. Şahin Dere.

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world, after wheat, maize and rice. Turkey is among the main barley producing countries, ranking ninth in the world (Anonymous 2005). Powdery mildew caused by the fungal pathogen *Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei* Em. Marchal (synonym *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal) is one of the most

destructive leaf diseases of this crop in regions with coastal climatic conditions, including the western and southern parts of Turkey. This disease lowers product quality and causes grain yield losses of up to 25-30%. Yield losses due to powdery mildew have been estimated at ~1 M ha yr<sup>-1</sup> in Europe (Czembor and Czembor 2001).

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In Turkey, powdery mildew epidemics usually occur in the western and southern parts of the country, especially when the spring season is cool and rainy. In recent years, epidemics have also become a problem under dry and hot climatic conditions due to the increased use of irrigation set-ups and nitrogen fertilizers. The control of powdery mildew in Turkey relies on fungicides - in some areas - and breeding for host resistance. The development of resistant cultivars, however, has not been pursued extensively as of yet, even though this control approach is generally considered cost effective and environmentally safe (Wolfe 1984; Wolfe and McDermott 1994).

Race-specific resistance to powdery mildew is governed by major resistance genes (or R-genes) that can be introgressed from resistant varieties into susceptible cultivars of agronomic interest. The best potential sources of new resistance genes for cultivated barley most likely are landraces from the centre of origin (Ceccarelli *et al.* 1987, 1995). The Fertile Crescent area, covering the southeastern part of Turkey, is considered as the centre of origin for barley and wheat (Czembor 1996; Willcox 1995; Zohary 1999), which suggests that barley varieties, landraces and wild relatives from Turkey might represent an

interesting source of R-genes for powdery mildew (Jahoor and Fishbeck 1987). Until now, nearly all European barley cultivars have been assessed for the presence and identity of R-genes to powdery mildew, making it possible to conclude to the presence of one or two R-genes in most varieties. Although the interaction between powdery mildew and barley is often regarded as one of the best characterized host-pathogen systems, and although many resistance alleles have already been identified (Kolster *et al.* 1986), the identification of R-genes for this disease in Turkish barley varieties and landraces still remains incomplete.

As a first step towards the effective use of powdery mildew R-genes in breeding programs, it is essential to test for the presence of those genes in registered cultivars and for the virulence of the pathogen through periodical surveys (Czembor and Bladenopoulos 2001; Czembor and Czembor 1998, 2000a; Czembor and Gacek 1990; Czembor and Johnston 1999). In practice, such surveys are conducted based on the gene-for-gene hypothesis, i.e. by inoculating plants with pathogen isolates that present a defined virulence spectrum (Flor 1942, 1955; Moseman 1959). The infection spectra observed make it possible to

**Table 1. Registration number (RN), variety name, and botanical name of the 34 Turkish barley varieties assessed in this study**

Number	RN	Variety name	Botanical name
1	TR41009	Zafer 160	<i>Hordeum vulgare vulgare</i>
2	TR41010	Yesilköy 387	<i>Hordeum vulgare vulgare</i>
3	TR41011	Gemici 7243	<i>Hordeum vulgare vulgare</i>
4	TR41012	Kaya 7794	<i>Hordeum vulgare distichon</i>
5	TR45288	Tokak 157/37	<i>Hordeum vulgare distichon</i>
6	TR45289	Cumhuriyet 50	<i>Hordeum vulgare distichon</i>
7	TR45290	Yerçil 147	<i>Hordeum vulgare distichon</i>
8	TR50882	Hamidiye 85	<i>Hordeum vulgare distichon</i>
9	TR50883	Obruk 86	<i>Hordeum vulgare distichon</i>
10	TR50884	Anadolu 86	<i>Hordeum vulgare distichon</i>
11	TR50885	Bülbül 89	<i>Hordeum vulgare distichon</i>
12	TR57795	Efes-1	<i>Hordeum vulgare distichon</i>
13	TR57796	Efes-2	<i>Hordeum vulgare distichon</i>
14	TR57797	Efes-3	<i>Hordeum vulgare distichon</i>
15	TR57786	Sahin 91	<i>Hordeum vulgare distichon</i>
16	TR57790	Yea. 793.12	<i>Hordeum vulgare distichon</i>
17	TR68592	Bornova 92	<i>Hordeum vulgare nutans</i>
18	TR68593	Serife hanım 98	<i>Hordeum vulgare</i>
19	TR69697	Vamık hoca 98	<i>Hordeumvulgare agriacanthom</i>
20	TR69698	Akhisar 98	<i>Hordeum vulgare agriacanthom</i>
21	TR69699	Süleyman bey 98	<i>Hordeum vulgare nutans</i>
22	TR69700	Bilgi 91	<i>Hordeum vulgare</i>
23	TR72333	Beyşehir 98	<i>Hordeum vulgare</i>
24	TR72334	Konevi 98	<i>Hordeum vulgare</i>
25	TR72338	Basgöl	<i>Hordeum vulgare</i>
26	TR72340	Çıldır-02	<i>Hordeum vulgare</i>
27	TR72342	Avcı-2002	<i>Hordeum vulgare</i>
28	TR72343	Yesevi-93	<i>Hordeum vulgare</i>
29	TR72344	Orza-96	<i>Hordeum vulgare</i>
30	TR72345	Aydan hanım	<i>Hordeum vulgare</i>
31	TR76583	Özdemir 05	<i>Hordeum vulgare</i>
32	TR76584	Ince 04	<i>Hordeum vulgare</i>
33	TR76585	Kalaycı 97	<i>Hordeum vulgare</i>
34	TR76586	Erginel 90	<i>Hordeum vulgare</i>

**Table 2. Isolates of *Blumeria graminis* f. sp. *hordei* and their infection types on 'Pallas' differential near-isogenic lines (Kölster *et al.* 1986) and 12 additional cultivars**

No	Differential lines <sup>1</sup>	R-genes	Differential test isolates <sup>2</sup>								
			B4(C15)	B95(53/01)	B100(60/01)	B121(26/04)	B120(20/04)	B97(57/01)	B91(98AF066)	B21(R86/01)	B103(64/01)
0	Pallas	Mla8	4	4	4	4	4	4	4	4	4
1	P01	Mla1, ML (A12)	0	4	4	4	0	0	0	0	0
2	P02	Mla3,	4	0-1n	0	0	4	4	0-1n	4	0-1n
3	P03	Mla6, Mla14	0	0	0-1n	3n-4	4	4	0	4	4
4	P04B	Mla7, MI(NO3)	0	4	4	3-4	1-2n	3n	4	1n	4
5	P08B	Mla9	0	0	4	4	4	0	4	0	0
6	P09	Mla10, MI (Du2)	0	4	3n	0	4	0	4	0	0
7	P10	Mla12, MI (Em2)	0	4	0-1n	1n	3n-4	4	1n	0-1n	3n-4
8	P11	Mla13, MI (Ru3)	0	0	0	4	0	4	4	0	4
9	P12	Mla22 Mic)	4	0	0	3n-4	0	4	0	4	0
10	P14	Mira	4	4	4	0	4	4	4	4	4
11	P16	Mlk	2cn	2cn	4	1-2cn	4	1-2cn	3n-4	1-2cn	3n-4
12	P20	MI	2n	2n	2n	2-3n	2n	4	1-2n	1-2cn	2n
13	P21	Mlg, MI (CP)	2-3n	4	4	4	4	4	4	0	4
14	P23	Mlla	1-2n	4	4	4	4	2n	4	4	2n
15	P24	Mlh	4	4	0	0	4	4	4	4	4
16	ISO2R	Mlg	4	4	4	4	4	4	4	1-2n	4
17	SI-1	SI 1	0	1-2n	3n	0	0	0	0	0	0
18	GUNNAR	Mla3, MI (Tu2)	1-2n	0	0	0	2n	4	2-3n	4	0
19	SV83380	Mlab	2n	3n	2-3n	3n	4	4	3n-4	2n	4
20	MELTAN	Mla13, Mli 8lm9),+	0	0	0	4	0	4	0	0	4
21	GOLDIE	Mla 12, U	0	2n	0	4	1n	4	4	0	4
22	STEFFI	MI (St)	0	0-1n	0-1n	3n	4	2n	4	0	1n
23	HENNI		0	1-2n	0-1n	4	4	1n	4	0	1-2n
24	PUNTO	Mla3, MI (Tu2), MI (lm9),+	0	0	0	0	1n	4	1-2n	1n	0
25	BENEDIKTE	Mla9, MI(lm9)	0	0	2n	4	0-1n	0	1n	0	0n
26	SCARLETT		0	0-1n	0	3cn	4	2n	4	0	0-1n
27	CARLSBERG	Mla8	4	4	4	4	4	4	4	4	4
28	Bülbül 89 / control		4	4	4	4	4	4	4	4	4

<sup>1</sup> P01-P24: Pallas differential near-isogenic lines.<sup>2</sup> Scale 0-4: 0 = not compatible; 4 = compatible; n = necrosis; c = chlorosis.

determine a 'reaction spectrum' for each interaction, which then makes it possible to identify the resistance phenotype of the tested plant (Czembor and Czembor 1998, 1999, 2001; Dreiseitl and Jørgensen 2000). Although Turkish varieties probably possess a number of unidentified and/or still uncharacterized R-genes for powdery mildew, monitoring has not yet been done on a systematic basis. As an attempt to provide useful information for future breeding efforts, the objective of this study was to identify major powdery mildew R-genes in barley varieties grown in Turkey.

Seed samples from 34 barley cultivars were provided by the Aegean Agricultural Research Institute of Turkey. A list of the varieties tested and their origin is presented in Table 1. All barley cultivars in this study were of the spring type, with six- and two-row heads, covered kernels, and an intermediate heading date. These cultivars are grown in the Aegean and Mediterranean coastal regions. In this experiment, the plants were grown at 20-22°C in a growth room under a 14h:10h light/dark photoperiod, until they reached the second leaf stage. The leaves of these seedlings were used for the "leaf segment test" (Lutz *et al.* 1992) (see below).

**Table 3. Infection types (IT) based on the symptoms observed (Welz 1988)**

IT	Symptoms
0	No visible symptoms (immunity)
1	Necrotic flecks, usually minute; no mycelial growth; no sporulation (hypersensitivity)
2	Frequent chlorosis; reduced mycelial growth; no or very scarce sporulation
3	Moderate mycelial growth; moderate sporulation; sometimes chlorosis
4	Profuse sporulation of well-developed colonies and sometimes green islands

Nine isolates of *Blumeria graminis* were used as differentiating races for resistance tests (Table 2). These isolates were obtained from collections of the Riso National Laboratory of Denmark, and chosen based on their virulence spectrum as observed on the Pallas isogenic differential line set (Kolster *et al.* 1986). The fungi were provided by Dr. M.S. Hovmøller (Royal Agricultural and Veterinary University, Denmark) as purified single spore isolates, maintained and propagated on young seedlings of the powdery mildew-susceptible cultivar Cartegana.

The infection type of the isolates was assessed on host leaves using 3-cm-long leaf segments cut from the middle part of the primary leaf of 12-d-old seedlings laid on benzimidazole-containing agar (35 ppm benzimidazole in 1.5% agar). Inoculations were performed using a homemade mini-settling tower. The leaf segments inoculated on agar plates were placed in a growth chamber at 17-18°C under a 12h:12h light/dark photoperiod. Infection types were scored after 10 d according to the 0-4 scale of Welz

(1988) (Table 3). Leaf segments with infection types (or scores) of 0, 1 or 2 were classified as resistant to the fungus; leaf segments with scores of 3 or 4 were classified as susceptible.

Specific R-genes associated with each genotype were inferred by comparing their reaction spectrum with that of previously characterized differential lines (Brown and Jørgensen 1991; Czembor and Bladenopoulos 2001). Identifications were done based on the gene-for-gene hypothesis (Flor 1942, 1955). When a compatible reaction was observed with a given isolate (scores 3 and 4), it was inferred that the tested cultivar did not possess the resistance allele(s) for which the isolate was avirulent. Incompatible reactions (scores 0-2) with isolates possessing only one avirulence allele among the remaining possible resistance alleles then made it possible to postulate that the matching resistance allele was present (Czembor and Czembor 2001; Dreiseitl and Jørgensen 2000). Table 4 presents putative resistance alleles for the 34 barley varieties tested.

**Table 4. Resistance alleles and infection types of 34 Turkish varieties challenged with nine isolates of *Blumaris graminis* f. sp. *hordei***

No	Cultivars	Differential test isolates																Postulated alleles
		B4(C15)	B95(53/01)	B100(60/01)	B121(26/04)	B120(20/04)	B97(57/01)	B91(98AF066)	B21(R86/01)	B103(64/01)								
1	Zafer 160	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Mla8
2	Yesilköy 387	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Mla8
3	Gemici 7243	3	3	1	0	2	2	4	4	4	2	2	4	4	3	3	3	+?
4	Kaya 7794	0	1	4	3	3	2	4	4	3	3	2	2	4	2	2	4	Mla (La)
5	Tokak 157/37	3	2	4	4	2	2	4	4	4	4	4	4	4	0	0	4	Mlg, MI (CP)
6	Cumhuriyet 50	3	3	4	4	2	2	4	4	3	2	4	4	4	3	3	4	+?
7	Yerçil 147	0	0	2	3	2	2	4	4	4	4	2	2	4	4	0	4	+?
8	Hamidiye 85	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	None
9	Obruk 86	4	3	4	3	1	0	0	0	4	4	4	4	4	4	4	4	Mlh
10	Anadolu 86	4	4	4	4	0	0	4	4	2	2	4	4	4	4	4	3	Mlh +?
11	Bülbül 89	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	None
12	Efes-1	4	3	4	3	2	0	4	4	0	1	2	2	4	4	4	3	Mla1 +
13	Efes-2	3	4	4	3	3	4	4	4	4	3	4	4	4	4	4	4	Mla 8
14	Efes-3	3	3	4	3	2	2	4	4	2	2	4	4	4	4	4	2	Mla +?
15	Sahin 91	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	Mla8
16	Yea. 793.12	4	4	4	4	4	4	3	3	3	2	2	2	4	4	4	4	+?
17	Bornova 92	0	0	4	4	4	4	4	4	1	0	0	0	0	1	0	1	Mla 1+ ?
18	Serife hanım 98	0	0	3	3	3	2	4	4	1	2	4	4	4	2	2	4	Mla 7+?
19	Vamık hoca 98	2	3	4	4	2	2	2	2	2	1	2	3	4	4	3	2	MIAb
20	Akhisar 98	3	2	4	4	3	3	3	2	2	1	4	4	4	4	4	3	+?
21	Süleymanbey 98	1	2	3	3	4	4	4	4	4	4	4	4	4	4	3	4	MIAb+?
22	Bilgi 91	4	4	4	4	4	4	4	4	4	4	4	4	4	0	1	4	Mlg
23	Beyşehir 98	3	2	4	4	4	4	4	4	4	4	4	4	4	0	0	4	Mlg, MI (CP)
24	Konevi 98	3	3	4	4	4	4	4	4	4	4	4	4	4	0	0	4	Mlg, MI (CP)
25	Basgöl	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Mla 8
26	Çıldır-02	3	3	4	4	0	0	0	0	4	4	4	4	4	4	4	4	Mlh
27	Avcı-2002	0	2	4	4	3	4	4	4	4	4	4	4	4	4	4	4	Mla7
28	Yesevi-93	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	None
29	Orza-96	4	4	4	4	0	2	4	4	4	4	4	4	4	4	4	4	Mlra
30	Aydan hanım	4	4	4	4	4	4	0	0	4	4	4	4	4	4	4	2	Mlg
31	Özdemir 05	4	4	4	4	0	0	0	0	4	4	4	4	4	4	4	3	Mlh
32	Ince 04	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	Mla 8
33	Kalaycı 97	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Mla 8
34	Erginel 90	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Mla 8

Each cultivar was tested as two replicates with each test isolate.

This study is, to our knowledge, the first to identify R-genes for powdery mildew in commonly grown barley cultivars bred in the National Agricultural Research Institutes of Turkey. The presence of several R-genes was inferred among the varieties tested, including *Mla8*, *MiLa*, *Mlg*, *Mi(CP)*, *Mlh*, *Mlat*, *Mla1*, *Mlh*, *Mla7*, *Mlra*, and a few uncharacterized genes. The most common R-gene was *Mla8*; it was found in nine cultivars out of 34 (Zafer-160, Yesilköy 387, Efes-2, Sahin-91, Başgöl, Yesevi-93, Ince-04, Kalaycı-97, and Erginel 90). Several cultivars contained a single (known) R-gene for powdery mildew [namely Orza-96 (*Mlra*), Serife hanım (*Mla7*), Bornova-92 (*Mla1*), Kaya-7794 (*Mla(La)*), Bilgi 91 (*Mlg*), Vamık hoca-98 (*Mi(Ab)*), Sülayman bey-98 (*Mi(Ab)*), Obruk-86 (*Mlh*), Özdemir 05 (*Mlh*), Çıldır 02 (*Mlh*), and Anodulu-86 (*Mlh*)], while three cultivars (Tokak-157/37, Beysehir-98, and Konevi-98) contained both *Mlg* and *Mi(CP)*. Interestingly, the varieties Gemici-7243, Yea-793.12, and Akhisar-98 were partly resistant to the fungus, but no R-gene could be inferred because their reaction spectra with the test isolates were not conclusive. By contrast, the cultivars Hamidiye-85, Yesevi-93, and Bülbül-89 showed no resistance to any of the isolates tested, as also observed by Lower *et al.* (1997).

Compared with other studies on R-genes in barley cultivars or landraces, the number of distinct R-genes postulated here is relatively large considering the limited number of cultivars tested (10 different genes in 34 cultivars). Of these genes, none had previously been detected in barley landraces from Morocco (Czembor and Czembor 2000a, b), and the previously described R-genes detected in landraces from Greece, such as *Mla6*, *Mla14* or *Mlat*, were not found in the varieties tested in the present study (Czembor 2001). By contrast, the genes *Mlg*, *Mlak*, *Mla7*, and *Mla(Ab)* have also been detected in North American cultivars (Dreiseitl and Steffenson 2000). Likewise, the genes *Mlg*, *Mlk*, *Mla7*, *Mi(CP)*, and *Mla1* were previously detected in a population of 108 Baltic spring barley cultivars and breeding lines (Tueryapina *et al.* 1996), the genes *Mla1*, *Mla7*, and *Mlk* were detected in Tunisian landraces (Czembor and Johnston 1999), and the genes *Mlg* and *Mla7* were detected in a population of 20 cultivars from Greece (Czembor and Bladenopoulos 2001). Based on these previous reports and on the present study, the most commonly found R-genes for powdery mildew in barley appear to be *Mlg*, which has been introduced into many European cultivars a long time ago, and *Mla7*, which has also been introduced into common barley varieties over the years.

The identification of R-genes based on tests performed using leaf segments and fungal isolates with different virulence spectra is an effective and useful tool for plant breeders (Russel 1978). Based on our data, it can be concluded that Turkish barley cultivars possess several genes for resistance to powdery mildew that can be used as parental plant materials in different gene deployment strategies aimed at efficiently controlling powdery mildew through gene pyramiding. None of the cultivars assessed in the study showed resistance based on the gene *Mlo*, which is known to provide efficient monogenic, non-race-specific and durable resistance (Hovmøller *et al.* 2000; Jørgensen 1992, 1994). Future

work for barley breeders in Turkey should now focus on pyramiding this gene with the newly described *Ml* genes.

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