Virulence Frequencies to Powdery Mildew Resistance Genes of Winter Barley Cultivars

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Abstract

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The virulence frequencies to powdery mildew resistance genes possessed by winter barley cultivars registered and newly tested in the Czech Republic were studied in 2000, 2002 and 2004. Random samples of the populations originating from winter and spring barley fields were obtained from the air by a mobile version of a jet spore sampler mounted on a car roof. Conidia were sampled by driving across the Czech Republic. Fourteen differentials, carrying 18 out of 20 currently identified resistance genes present in winter barley cultivars, were used. High virulence frequencies (85–100%) to most resistance genes were found. Lower virulence frequencies (14.1–40.1%) were found to only three resistance genes that have not been described yet; their preliminary designations are *Ml(Va)*, *Ml(Dt)* and *Ml(Ca)*. The importance of resistance of winter barley cultivars is discussed with respect to limiting the speed with which the pathogen adapts to genetic resistances possessed by commercial cultivars of both winter and spring barley, and to the necessity of lowering the costs for powdery mildew control in barley.

Keywords: *Blumeria graminis* f.sp. *hordei*; *Erysiphe graminis* f.sp. *hordei*; powdery mildew; *Hordeum vulgare*; barley; winter cultivars; virulence frequencies; resistance genes; pathogen populations

Barley (*Hordeum vulgare* L.), both spring and winter form, is the second most widely grown crop in the Czech Republic, and powdery mildew, caused by the fungus *Blumeria graminis* f.sp. *hordei* = *Bgh* (syn. *Erysiphe graminis* f.sp. *hordei*), is the most common disease on it (Dreiseitl 2003a). *Bgh* is an obligate pathogen of barley that is able to survive only on green organs of the host.

If, hypothetically, only spring barley is grown, *Bgh* would be able to survive the end of the vegetation season by mycelium and conidia, first on late but still green tillers, and subsequently on volunteer plants until young plants emerge in spring. In that case the perfect stage of the pathogen would play, beside a genetic, also a more important epidemiological role.

If, however, winter barley is also grown, the pathogen has a better chance of survival because the vegetation period of later tillers of spring barley coincides with the period of emergence of first plants from volunteer winter barley and also multiply number of host plants, particularly in winter. Growing susceptible cultivars of winter barley facilitates mass reproduction of the pathogen during all its vegetation period.

At least since 1970, every cultivar in the registered assortment of winter barley carries a gene for specific resistance to powdery mildew (Dreiseitl & Jørgensen 2000; Dreiseitl unpublished). In spite of that, under conditions favourable for pathogen development, almost all cultivars of winter barley are heavily infected by powdery

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mildew. That would not be possible without an abundance of corresponding virulences in the pathogen population. The objective of this study was to determine the virulence frequency to resistance genes possessed by the currently registered cultivars of winter barley.

MATERIAL AND METHOD

Populations studied. Three *Bgh* populations (2000, 2002 and 2004) were used for the assessment of the virulence frequencies to powdery mildew resistance genes in winter barley cultivars.

Sampling of isolates. Random samples of *Bgh* conidia originating from cultivated winter and spring barley fields were obtained from the air by means of a mobile version of a jet spore sampler (Schwarzbach 1979) mounted on the roof of a car. The sampling of conidia was done by driving across the Czech Republic from 23 to 27 May 2000 (1500 km), on 23 and 24 May 2002 (820 km), and from 22 to 24 May 2004 (840 km). The collected conidia settled on detached healthy and fully-expanded primary leaves of the susceptible barley cultivar Pallas in 2000 (Kølster *et al*. 1986,) or line B-3213 in 2002 and 2004 (Dreiseitl & Steffenson 1996) that were placed in Petri dishes with 0.7% water agar and 35 ppm benzimidazole and inserted in the bottom part of the spore sampler. After approximately 100 km, the dish was exchanged for one with fresh detached leaves of the susceptible cultivar, taking into account agroecological conditions in the region. During the sampling, dishes with fresh or exposed leaves were kept in a car refrigerator at 10–15°C. After sampling, the exposed leaves were transferred into dishes with fresh agar. To increase the conidia, dishes with leaves were kept under laboratory conditions for 10–11 d (20 ± 3°C under 8 h artificial light at 25 ± 5 μmol/m per s).

Differential sets. Before using cultivars for a given purpose, seeds of the differential and susceptible cultivars were increased from progenies of single plants. The set of differentials in 2000 was comprised of seven cultivars, and in 2002 and 2004 of eight cultivars, carrying different genes for resistance to powdery mildew (Table 1). In 2000 and 2002, six near-isogenic lines of the cultivar Pallas (Kølster *et al*. 1986) were included. In

Table 1. A set of barley differential cultivars, their genes for resistance to *Blumeria graminis* f.sp. *hordei* and virulence frequencies (%) found in the Czech Republic in 2000, 2002 and 2004

No.	Differential cultivar	*Ml* resistance genes	2000	2002	2004
1	P03	*a6*, *a14*[1]	85.5	84.9	
2	P04B	*a7*, *aNo3*[1,2]	96.7	98.7	
3	P11	*a13*, *aRu3*[1,2]	87.0	92.8	
4	P21	*g*, *(CP)*, *a8* [1,3]	97.6	97.5	
5	P23	*La*, *a8*[1,3]	97.9	96.9	
6	P14	*ra*, *a8*[1,3]	100.0	98.4	
7	Borwina	*(Bw)*, *a8*[4,5]	98.5	–	90.8
8	Pallas	*a8*[3]	–	100.0	100.0
9	Weihenstephan 37/136	*h*[6]	–	99.7	98.5
10	Dura	*ra*, *(Dr2)*[7]	–	–	99.6
11	SG C-669	*(SG)*[5]	–	–	100.0
12	Vanessa	*a7*, *(Va)*[5]	–	–	40.1
13	Duet	*a6*, *a14*, *g*, *(CP)*, *h*, *(Dt)*[5]	–	–	21.8
14	Carola	*a6*, *a14*, *ra*, *(Ca)*[5]	–	–	14.1
	No. of isolates		331	318	262

[1]Kølster *et al*. (1986), [2]Jørgensen (1994), [3]Jensen (1995), [4]Dreiseitl & Jørgensen (2000), [5]Dreiseitl – unpublished, [6]Wiberg (1974), [7]Jensen & Jørgensen (1981)

2000, the cultivar Borwina (Dreiseitl & Jørgensen 2000) and in 2002 the cultivars Pallas (Kølster *et al*. 1986) and Weihenstephan 37/136 (Wiberg 1974) were added. In 2004, the cultivars Borwina, Pallas and Weihenstephan 37/136 were used to which the cultivars Dura (Jensen & Jørgensen 1981), Carola, Vanesa, Duet and a breeding line SG C-669 (Dreiseitl unpublished) were added. About 20 seeds of each differential cultivar were sown in pots (80 mm diameter) filled with a mixture of peat and soil. Plants were grown in a greenhouse at 22 ± 5°C for 10–12 d. Of each differential, leaf segments 25 mm long were removed from the central parts of healthy, fully-expanded primary leaves and placed on the mentioned agar in plastic 90 mm-Petri dishes.

Inoculation. Inoculation was performed in a metal micro-settling tower 200 mm high, with a circular basis of 90 mm diameter, and with a hole of 10 mm diameter in the central part of the upper cap. Each of the colonies that developed during incubation of the collected conidia usually contained 15 to 25 thousand conidia of the new "generation". They were used to inoculate a set of leaf segments of differentials (Table 1) placed in a dish with agar. The conidia of one colony were sucked into a replaceable tip of a varipipette AW 1000 set to a 0.4 ml volume, and then blown into the micro-settling tower using a syringe (10 ml) with a small tube. Inoculum density was ca. 3 conidia per mm^2. After inoculation with the conidia of one colony, the dish with a set of leaf segments of the differentials was removed, and the micro-settling tower, surface of a laboratory desk and the small tube were sterilized with 98% ethyl alcohol, and a new dish was inserted for inoculation with conidia of another colony. Inoculated dishes were kept under laboratory conditions.

Evaluation. Reaction types (RTs) produced by the response of each differential to a corresponding *Bgh* isolate were scored 8 d after inoculation according to the nine-point 0–4 scale including intertypes (Torp *et al*. 1978). Isolates that produced RT 4 or 3–4 on a differential were considered virulent on the corresponding resistance gene(s). In cases where virulence was manifested towards a differential with more than one resistance gene, the virulent isolate has more than one corresponding virulence genes. If an isolate was avirulent, it could be due to the presence of one or more of these avirulence genes. Some or all of the isolates that are avirulent on this differential possessing more resistance genes, may possess corresponding virulence gene(s) which cannot be expressed.

RESULTS

2000. Three hundred and thirty-one *Bgh* isolates were examined. Very high virulence frequencies to all resistance genes possessed by the differential cultivars were found. The lowest virulence frequency (85.5%) was found to resistance genes *Mla6* and *Mla14* present in line P03, and the highest (100%) to resistance gene *Mlra* possessed by line P14.

2002. Three hundred and eighteen pathogen isolates were examined. Very high virulence frequencies were found, ranging from 84.9 to 100%. The lowest mentioned virulence frequency was determined again to resistance genes *Mla6* and *Mla14,* and the highest to gene *Mla8* carried by the newly included cultivar Pallas. Similar high virulence frequency (99.7%) was also found to gene *Mlh* that is present in the newly included cultivar Weihenstephan 37/136, and to which only one isolate was avirulent. In 2002, six differential cultivars were identical to those used in 2000. The determined virulence frequencies to corresponding resistance genes were similar. The largest difference was observed in virulence frequencies to genes *Mla13* and *MlaRu3*, i.e. 5.8% higher than in 2000.

2004. Two hundred and sixty-two isolates were examined. The virulence frequencies showed a substantially wider range (from 14.1 to 100%) due to newly included differential cultivars. All isolates were characteristic for their virulence to resistance genes *Mla8* (Pallas) and *Ml(SG)* (virulence frequencies on both 100%), the latter gene is possessed by breeding line SG C-669. Lower virulence frequencies were found to the resistance genes carried by cultivars Vanessa [*Mla7*, *Ml(Va)*], Duet [*Mla6*, *Mla14*, *Mlg*, *Ml(CP)*, *Mlh*, *Ml(Dt)*] and particularly by Carola [*Ml(Ca)*] (Table 1). Compared to 2000, there was a considerable decrease (7.7%) in virulence frequencies to the resistance genes present in cultivar Borwina [*Ml(Bw)*, *Mla8*].

DISCUSSION

To limit the speed with which a pathogen adapts to resistance genes incorporated in commercial cultivars of economically significant crops, it is necessary to use different resistances for spring and winter cultivars, in our case barley. The lack

of suitable sources for resistance, however, has led breeders of winter barley, in particular of two-rowed cultivars, to either a random or targeted use of the resistances possessed by spring barley cultivars.

The virulence frequency was assessed on 14 differential cultivars in 2000, 2002 and 2004. These cultivars possessed 18 out of 20 currently identified resistance genes present in winter barley cultivars that were grown or tested in the Czech Republic (Dreiseitl unpublished). Seven resistance genes (*Mla6*, *Mla14*, *Mla7*, *Mlg*, *Ml(CP)*, *Mlra* and *Mlh*) were always carried by two of the differential cultivars. The gene *Mla8* was present in five differential cultivars (Table 1). The resistance genes possessed by differential cultivars Nos 1–5 are typical for spring barley cultivars (Brown & Jørgensen 1991; Dreiseitl & Jørgensen 2000). However, they are also present in some cultivars of winter barley registered in the Czech Republic. By contrast, the resistance genes present in differential cultivars No-s 6, 7, 9 and 10 are typical for winter barley cultivars. Each of the differential cultivars No-s 12–14 possesses more than one resistance gene, some of which are typical for spring and others for winter cultivars. The resistance genes possessed by differential cultivars Nos 8 and 11 occur in cultivars of both barley forms (Dreiseitl unpublished).

The presence of virulence genes in the pathogen population is an essential condition for infection of cultivars possessing specific resistance gene(s) to the given disease. Under conditions favourable for the development of the pathogen, infection on most winter barley cultivars is high. Therefore, high frequencies of corresponding virulences (= virulence genes) could be expected in the pathogen population. The current results confirmed this assumption. A virulence frequency of 100% to the resistance genes *Mla8* and *Ml(SG)*, present in both spring and winter barley cultivars, was found. Considering that these genes occur rarely in barley cultivars, the two virulences seem (due to the assumed long-term direct selection on earlier barley cultivars) to be genetically fixed in the pathogen population. This is also confirmed by the fact that no isolate avirulent on resistance gene *Mla8* has been found in the European population of this pathogen. The resistance gene, preliminarily designated *Ml(SG)*, has obviously not been identified yet for the same reason. The virulence frequencies to resistance genes *Mlra*, *Ml(Dr2)* and *Mlh*, which are typical for winter barley cultivars, were near the limit (100%). In 2000, the assessed virulence frequency to gene *Ml(Bw)* was also near this limit. Four years later, however, the frequency of *VBw* was 7.7% lower. That can be connected with a decrease in the planted area of winter barley cultivars possessing the corresponding resistance gene *Ml(Bw)*. Furthermore, high virulence frequencies were found to the genes typical for spring barley cultivars and present in differential cultivars Nos 1–5. This was undoubtedly caused by direct selection of the pathogen population mostly on spring barley cultivars possessing corresponding resistance genes (Dreiseitl 2003b).

The found virulence frequencies to resistance genes present in the mentioned 11 differential cultivars was 85–100%. This level is generally similar to other results achieved during a study of the European pathogen population (Hovmøller *et al*. 2000). However, less attention has been given to determine the virulence frequencies to typical resistance genes in winter barley than to virulence frequencies to resistance genes present in spring barley cultivars. Markedly lower virulence frequencies (14.1–40.1%) were found to the resistance genes present in differential cultivars Nos 12–14 (Table 1). A comparison of these frequencies with those of the preceding 11 differentials suggests that these significantly lower virulence frequencies are conditioned particularly by the three preliminary designated resistance genes. Two of them [*Ml(Va)* and *Ml(Dt)*] are present in the differential cultivars Duet and Vanessa. Six resistance genes for powdery mildew have been identified in the cultivar Duet (Dreiseitl unpublished). However, the first five resistance genes (Table 1) contributed very little to the relatively low virulence frequency (21.8%); it was caused mostly by the effect of gene *Ml(Dt)*. The gene *Ml(Ca)* is carried by one (cv. Carola) of two current winter barley cultivars classified as "resistant". Unfortunately, the last three mentioned and relatively low (within the set of differential cultivars) virulence frequencies are still too high to allow the use of at least one of the three corresponding resistance genes in the breeding of new winter barley cultivars for resistance to powdery mildew. It can also be assumed that the effectiveness of these genes, if carried by widely grown cultivars, will decrease very fast due to direct selection of virulent pathotypes in the pathogen population.

The infection potential of the pathogen induced on susceptible cultivars of winter barley results in

losses of grain yield and quality. In addition, it produces conditions for severe infection of susceptible spring barley cultivars (Dreiseitl & Jurečka 2003) and speeds up the adaptation of the pathogen to resistances possessed by all grown barley cultivars (Dreiseitl 2003b). Besides the accelerated breakdown of genetic resistances, the high infection potential of the pathogen reduces the effectiveness of protection by fungicide in barley. Thus, growing susceptible cultivars of winter barley decreases the effectiveness of inputs for the protection of barley against powdery mildew infection, namely the costs for breeding resistant cultivars as well as the costs for protection of barley by fungicide. Therefore, to increase the level of resistance in new cultivars of winter barley is highly topical.

In the past, resistance to powdery mildew in winter barley was neglected for several reasons. The area planted with winter barley was approximately 1% of the total barley area during 1971–1977 (Dreiseitl & Jurečka 2003). Moreover, winter barley is infected by a broader disease spectrum so that breeders have to solve more problems. Though powdery mildew is the most common disease on both spring and winter barley, its incidence on winter barley is lower (Dreiseitl 2003a) and is supposed to be less harmful because of a later disease outbreak. New and effective sources for resistance have been sought for breeding cultivars of spring barley and corresponding genes have been transferred into pre-breeding lines using conventional breeding methods. Such a service has not been provided yet for breeding winter barley cultivars.

The grain of winter barley is used mostly as feed for farm animals. The buying price of feedgrain produced under conventional farming systems is lower than the price of spring malting barley, therefore the economics of growing this crop is more stressed. On one hand, growers cannot afford lower grain yields caused by powdery mildew infection, while on the other they can hardly use costly fungicidal control of this disease. Thus, winter barley cultivars resistant to powdery mildew should be interesting not only for conventional agriculture, but particularly for diverse lower-input farming systems.

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Souhrn

Dreiseitl A. (2004): **Frekvence virulencí ke genům odolnosti k padlí travnímu u odrůd ječmene ozimého**. Plant Protect. Sci., **40**: 135–140.

V letech 2000, 2002 a 2004 byly zjišťovány frekvence virulencí ke genům odolnosti obsaženým v registrovaných a nově zkoušených odrůdách ječmene ozimého. Náhodné vzorky populací pocházející z pěstovaných ozimých i jarních odrůd ječmene byly získány ze vzduchu nad územím České republiky pomocí mobilní verze lapače spor. Bylo použito celkem 14 diferenciačních odrůd, které obsahují 18 z 20 dosud identifikovaných genů odolnosti obsažených v odrůdách ječmene ozimého. Byly zjištěny vysoké frekvence virulencí (85–100 %) k většině genů odolnosti. Nižší frekvence virulencí (14,1–40,1 %) byly zjištěny jen ke třem dosud nepopsaným genům odolnosti [předběžně označeným *Ml(Va)*, *Ml(Dt)* a *Ml(Ca)*]. Je diskutována důležitost odolnosti odrůd ječmene ozimého s ohledem na omezení rychlosti adaptace patogena ke genetickým odolnostem obsaženým v komerčních odrůdách ječmene ozimého i jarního, jakož i s ohledem na nutnost zvýšení efektivnosti vynakládaných prostředků na ochranu ječmene před napadením padlím travním.

Klíčová slova: *Blumeria graminis* f.sp. *hordei*; *Erysiphe graminis* f.sp. *hordei*; padlí travní; *Hordeum vulgare*; ječmen; ozimé odrůdy; frekvence virulencí; geny odolnosti; populace patogena

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