Resistance to Powdery Mildew in Selected Czech Winter Barley Breeding Lines

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Abstract: Powdery mildew resistances in Czech winter barley breeding lines were postulated on the basis of their reaction types to 32 pathotypes of the pathogen with different combinations of virulence genes. The results from testing 38 lines represent a substantial part of found variability in the examined trait with 13 found resistances. The resistances typical of winter barley cultivars dominated in six-row lines whereas the resistances typical of spring barley cultivars were more frequent in two-row lines. None of these resistances was effective enough to all used pathotypes. Desired effectiveness is present neither in the lines with a combination of corresponding resistances nor in the cases when their resistance is controlled by more (up to six) postulated genes. Resistances "Ch" (detected in the line CH 669) and "Lu" (detected in the line LU 1258/A/02) are novel, the former without any practical importance. A need of breeding winter barley cultivars resistant to powdery mildew is discussed since they are required for both conventional and particularly low-input farming systems.

Keywords: *Blumeria graminis* f.sp. *hordei; Erysiphe graminis* f.sp. *hordei;* powdery mildew; *Hordeum vulgare;* winter barley; breeding lines; resistance

Growing cultivars resistant to harmful biotic factors is a low-cost, and simultaneously effective and health-safe way of protection against unfavourable impacts caused by the co-existence of related organisms on both grain quality and quantity and production profitability. Considering general efforts aimed at the decrease in concentrations of undesirable substances in food products, the breeding of cultivars resistant to frequently occurring diseases should be conducted mostly in the crops whose production enters the food chain, and from the economic aspect, particularly in widely grown low-value crops.

On average of 2000–2004, barley was planted in the Czech Republic on the area of 500 000 ha where winter barley accounted for 26%. Powdery mildew is the most common disease on both barley forms (Dreiseitl 2003a; Dreiseitl & Jurečka 2003), however winter barley becomes a critical factor for the pathogen winter survival and reproduction. The

high frequency of virulence for resistance genes present in grown winter barley cultivars (Dreiseitl 2004) facilitates a frequent and heavy infection of this crop (Dreiseitl 2003a). Growing winter barley cultivars that are not resistant to powdery mildew leads toward damaging the crop itself and it even increases the inoculation potential of the pathogen for spring barley cultivars. It results in a more rapid adaptation of the pathogen to resistance genes present in the cultivars (Dreiseitl 2003b) along with lower effectiveness of other treatments to protect barley stands from infection. It is necessary to change such an unfavourable state by improving the resistance in barley cultivars, particularly in winter barley ones.

The objectives of this study were to identify specific resistances to powdery mildew present in current promising Czech breeding lines of winter barley and to evaluate their diversity and effectiveness.

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MATERIAL AND METHODS

Resistance genes were identified using the method of postulation (STATLER 1984; DREISEITL & STEFFENSON 1996) which is also designated an approximate method. It is based on the fact that a specific resistance gene of the host is matched by a virulence gene of the pathogen (the so called gene-for-gene hypothesis, Flor 1955).

A total of 129 winter barley breeding lines from the 2003 and 2004 harvests were tested for Selgen, Co. Ltd. (Selgen is now the only home company successful in breeding winter barley cultivars). Of them, 32 lines were included in the set which was enlarged by another six breeding lines that had been tested for the company in some of the years from 1999 to 2002. Another three lines, standards of corresponding resistances, were added. The lines designated by "CH" letters were developed by the Breeding Station at Chlumec nad Cidlinou and are two-row barleys. The other lines were provided by the Breeding Station at Lužany and are mostly six-row ones.

Tests were carried out using 32 pathotypes of Blumeria graminis f.sp. hordei (Bgh) that are characteristic of different virulence/avirulence gene combinations. Each of pathotypes of the powdery mildew pathogen induces a certain phenotype (reaction type = RT) on a corresponding host line. Four-place identification numbers give information on virulences of the pathotypes for 12 selected differential cultivars that possess various genes for resistance to the disease. The virulences are coded in an octal system (LIMPERT et al. 1994). Each digit implies information on virulence/avirulence for three resistance genes (triplet). The first 10 differential cultivars are listed in an agreed order (Limpert & Dreiseitl 1996). The line P21 possessing the gene Mlat (Kølster et al. 1986) and cv. Borwina possessing the gene Ml(Bw) (Dreiseitl & Jørgensen 2000) are added.

Six to eight seeds per winter barley line were sown for each pathotype in a pot (upper diameter/lower diameter/height = 60/40/50mm) filled with peat moss substrate. Plants were cultivated in a greenhouse from November to February at the controlled and checked temperature of $16.5 \pm 2^{\circ}$ C under natural light with additional four-hour lightening with cool-white fluorescent lamps. Ten days after sowing, the plants at the seedling stage (DC11 – fully-expanded primary leaf) were inoculated separately with the *Bgh* pathotypes. The inoculation

was carried out by brushing and shaking plants of a susceptible cultivar (DC12) with well-developed powdery mildew colonies (14 days after their inoculation and cultivation under the identical conditions described above) on and over tested plants. The reaction of plants (RT) was scored nine days after inoculation of the examined accessions. The upper half of the adaxial side of each primary leaf was evaluated using a nine-score 0–4 scale (inclusive intertypes) (TORP *et al.* 1978). All lines were tested in two replications. If any of the corresponding pair of RTs had significantly differed, an additional test(s) was carried out.

The identification of specific resistances is based on the above-described method for experimental determination of reaction spectrum (RS) for each line to the pathotypes used. The reaction spectrum consists of a set of RTs (32 RTs in this case) and is compared with RS of standard barley cultivars that carry known resistance genes (or similarly, with known virulence spectra of the pathotypes used). The results revealed that all obtained RS could be sufficiently characterised using 10 out of the total number of 32 pathotypes. The found resistances are designated by European codes (Boesen *et al.* 1996).

RESULTS

Winter barley lines, used *Bgh* pathotypes, found RS and identified resistances to powdery mildew are given in Table 1. In this work, 38 breeding lines were selected to which three standard lines with the single resistances Ra, Ha and Bw were added because these resistances were often found in the examined set, however always in combinations with other resistances only. Each of the lines is characteristic of a specific RS which is a phenotypic expression of individual resistances or their combinations present.

The reaction spectrum for the line CH 977 consists of susceptible RT4 only, which documents the absence of resistance to powdery mildew. The spectra for the other lines contain at least one resistant RT documenting the presence of at least one resistance to the disease examined. Eleven lines (including the three standards mentioned earlier) possessed one of individual resistances and the other 26 lines combined two to five resistances. A total of 13 resistances were found with the frequency given in brackets following the corresponding codes: HH (12), Ch (5), Ra (23), Dr

Table 1. Lines of winter barley, their resistance to powdery mildew and resistance spectra to 10 selected pathotypes of the pathogen (using nine-score 0–4 scale – inclusive intertypes)

No.	Breeding or standard line	No. rows	Resistance (European codes)	Pathotypes of Blumeria graminis f.sp. hordei									
				0004	1444	3707	4404	4557	4761	4776	5425	6000	7557
1	CH 977	2	None	4	4	4	4	4	4	4	4	4	4
2	CH 669	2	Ch	4	2	4	4	4	4	4	4	4	4
3	CH 96	2	HH	4	0	4	4	4	4	4	4	4	4
4	$standard^1$	6	Ra	4	4	4	0	4	4	4	4	4	4
5	$standard^1$	6	На	1–2	4	4	4	4	4	4	4	4	1–2
6	$standard^1$	6	Bw	4	4	4	4	4	2–3	4	4	2–3	4
7	SG-L 98/032/A/03	6	Ra Ch	4	2	4	0	4	4	4	4	4	4
8	SG-L 4119/04	6	Ra HH	4	0	4	0	4	4	4	4	4	4
9	SG-L 1258/A/02	6	Ra HH Lu	1–2	0	1–2	0	4	1–2	1–2	4	1–2	1–2
10	SG-L 97/167/04	6	Ra Dr	2–3	4	4	0	2–3	4	4	4	4	4
11	SG-L 4047/B/04	6	Ra Dr HH	2–3	0	4	0	2–3	4	4	4	4	4
12	SG-L 697/A/03	6	На НН	1–2	0	4	4	4	4	4	4	4	1–2
13	SG-L 1229/04	6	Ha Ra HH	1–2	0	4	0	4	4	4	4	4	1–2
14	SG-L 863/A/04	6	Ha Ra Dr HH	0	0	4	0	2–3	4	4	4	4	1–2
15	SG-L 960/A/01	6	Bw Ra	4	4	4	0	4	2–3	4	4	2–3	4
16	SG-L 530/A/03	6	Bw Ra Ch	4	2	4	0	4	2–3	4	4	2–3	4
17	SG-L 1011/E/03	6	Bw Ra HH	4	0	4	0	4	2–3	4	4	2–3	4
18	SG-L 530/D/03	6	Bw Ra Dr	2–3	2–3	4	0	2–3	2–3	4	4	2–3	4
19	SG-L 97/303/04	6	Bw Ra Dr HH	2–3	0	4	0	2–3	2–3	4	4	2–3	4
20	SG-L 4035/04	6	Bw Ha HH	1–2	0	4	4	4	2–3	4	4	2–3	1–2
21	SG-L 1236/C/01	6	Bw Ha Ra	1–2	1–2	4	0	4	2–3	4	4	2–3	1–2
22	SG-L 914/B/04	6	Bw Ha Ra HH	1–2	0	4	0	4	2–3	4	4	2–3	1–2
23	SG-L 1168/A/03	6	Bw Ha Ra Dr HH	0	0	4	0	2–3	2–3	4	4	2–3	1–2
24	CH 148	2	Sp	0	0	0	1–2	4	4	4	4	1–2	4
25	CH 14	2	Ly	0	0	4	0	4	4	4	0	0	4
26	CH 169	2	Ar	0	1–2	4	4	4	4	4	4	0	4
27	SG-L 4128/04	6	Ru	0	0	0	0	4	0	4	0	0	4
28	NSL 99-6738	2	We	0	0	4	0	4	4	0	4	0	4
29	CH 666	2	La	2–3	4	2–3	2–3	4	4	4	2–3	2–3	4
30	CH 138	2	La Ch	2–3	2	2–3	2–3	4	4	4	2–3	2–3	4
31	CH 72	2	La Ha Ra Ch	1–2	2	2–3	0	4	4	4	2–3	2–3	1–2
32	RU 01 111/2	2	We Ha	0	0	4	0	4	4	0	4	0	1–2
33	SG-L 99/013/A/04	6	Ru Dr	0	0	0	0	2–3	0	4	0	0	4
34	RU 01 111/1	2	Sp We	0	0	0	0	4	4	0	4	0	4
35	CH 136	2	Sp La	0	0	0	1–2	4	4	4	2–3	1–2	4
36	SG-L 99/068/04	6	Sp Ha	0	0	0	1–2	4	4	4	4	1–2	1–2
37	RU 01 115/4	2	Sp Ra	0	0	0	0	4	4	4	4	1–2	4
38	RU 01 117/2	2	Sp Ra Ha	0	0	0	0	4	4	4	4	1–2	1–2
39	SG-L 4117/04	6	Sp Ra Dr	0	0	0	0	2–3	4	4	4	1–2	4
40	CH 135	2	Sp Bw Ra	0	0	0	0	4	2–3	4	4	2–3	4
41	SG-L 4032/A/04	6	Sp Bw Ha Ra Dr	0	0	0	0	2–3	2–3	4	4	1–2	1–2

¹Dreiseitl, unpublished

(9), Ha (13), Bw (12), Lu (1), Sp (11), Ly (1), Ar (1), Ru (2), We (3) and La (4). The resistances Dr and Lu are not present separately in any of the known lines or cultivars. The resistances Sp and Ar are controlled each by two tightly linked resistance genes (Sp = Mla6, Mla14; Ar = Mla12, MlaEm2) (Jørgensen 1994) with a different phenotype. In the two resistances, the "main" gene (Mla6 and Mla12, respectively) produces IT0 and the two tightly linked genes IT1-2. The resistances Ly, Ru and We are also controlled by two tightly linked genes (Jørgensen 1994). None of these "additional" resistance genes, however, has been detected in the examined lines by the used pathotypes.

DISCUSSION

Thirteen resistances to powdery mildew have been found in the selected 38 breeding lines of winter barley. The resistance HH is common in cultivars of both spring and winter barley and it is of no practical importance. This is also confirmed by the fact that resistance HH is also present in cvs. Diamant and Tolar (Dreiseitl & Jørgensen 2000), which are used as susceptible controls to powdery mildew. Six resistances (Bw, Ha, Ra, Dr, Ch and Lu) are characteristic of six-row winter barley cultivars. Another six resistances (Sp, Ly, Ar, Ru, We and La) are typical of spring barley cultivars (Brown & Jørgensen 1991; Dreiseitl 2001) and are present mostly in lines of two-row winter barley in the examined set. However, the presence of identical resistances in winter and spring barley is not desirable since it speeds up adaptation of the pathogen to the corresponding resistances due to the all-year-round selection of virulent individuals and their reproduction on the cultivars possessing identical resistances.

The resistance, designated "Ch" in this study, was found for the first time in the line CH 669 (SG-C 669 in official trials) in 2002. It is, however, effective to pathotype 1444 only among the 32 ones used. Another novel resistance "Lu" found for the first time in 2003 in the line LU 1258/A/02 (SG-L 1258/A/02) is combined with two other known resistances.

The 38 breeding lines involved in this contribution represent a substantial part of the variability in the examined resistance. The number of 13 detected resistances, which are controlled by at least 15 genes, allowed to develop a large scale of lines with various complexity and combinations of

these resistances. However, none of the 129 tested lines displays full resistance to the studied disease. As it is apparent from both field evaluations and virulence frequencies in the pathogen population (Dreisettl 2004), the influence of found genetic resistances on the resistance of corresponding lines in the field is low and without sufficient practical effect. It is also valid even for lines with the highest complexity of the studied trait, in which five resistances were found that are controlled by at least six genes (line SG-L 4032/A/04 and others that are not presented here). At such a state (considering the studied trait), there are no preconditions for improving the assortment of winter barley cultivars using products of home (Czech) breeding.

Among the known barley resistances, Mlo is highly effective against powdery mildew and has been found in a Czech competitive line of winter barley (Dreiseitl & Jørgensen 2000). However, this resistance has been exploited in spring barley cultivars for a long time. Therefore it should not be concurrently used in winter barley cultivars because potential continuous adaptation of the pathogen would be extremely risky to breakdown this unique resistance.

General conditions for successful breeding of any crop for resistance to a target disease include, among others, clear precise identification of the pathogen and a sufficient amount of available effective resistance sources. Blumeria graminis f.sp. hordei is an incommutable causal agent of powdery mildew. There is also a number of original and effective resistance sources (Dreiseitl & Bockelman 2003; Dreiseitl & Dinoor 2004), some of which can be selected for purposeful breeding of resistant winter barley cultivars only. Current technical equipment enables us to detect the combination of fully effective genes. The use of two or more such resistance genes in a cultivar should limit the speed of the pathogen adaptation (DREISEITL 2003b) and thus extend the effectiveness of cultivar resistance to powdery mildew in winter barley.

The cultivar resistance, whether it is used in farmer's fields by growing pure cultivars, cultivar mixtures or in any other way, is one of the key factors toward profitable conventional farming, and particularly a large scale of low-input systems that increasingly contribute to food production in developed countries. Such a trend is also apparent in the Czech Republic, therefore winter barley cultivars resistant to powdery mildew will surely meet an increasing concern among growers.

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Souhrn

Dreiseitl A. (2005): **Odolnost k padlí travnímu ve vybraných českých šlechtitelských liniích ječmene ozimého.** Czech J. Genet. Plant Breed., **41**: 45–50.

Byla prostudována odolnost šlechtitelských linií ječmene ozimého k padlí travnímu. Ve vybraných 38 liniích, reprezentují podstatnou část zjištěné variability studovaného znaku, bylo metodou postulace a při použití 32 vybraných patotypů daného patogena zjištěno celkem 13 odolností, a to zpravidla v různých kombinacích. Odolnosti "Ch" (nově zjištěná v linii CH 669) a "Lu" (nově zjištěná v linii LU 1258/A/02), jsou nové. První z nich je však je bez praktického významu. V šestiřadých liniích převládaly odolnosti typické pro odrůdy ječmene ozimého,

v dvouřadých liniích naopak odolnosti typické pro odrůdy ječmene jarního. Žádná ze zjištěných odolností nebyla dostatečně účinná k současné populaci patogena. Potřebnou účinností se nevyznačují ani linie s kombinací příslušných odolností, a to ani v případech, kdy je jejich odolnost podmíněna více (až šesti) zjištěnými geny. Diskutováno je vyšlechtění odrůd ječmene ozimého odolných k padlí travnímu, neboť ty jsou požadovány jak pro konvenční, tak zvláště pro low-input systémy hospodaření.

Klíčová slova: Blumeria graminis f.sp. hordei; Erysiphe graminis f.sp. hordei; padlí travní; Hordeum vulgare; ječmen ozimý; šlechtitelské linie; odolnost

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