Identity of Barley Powdery Mildew Resistances Bw and Ru2

Antonín DREISEITL

Agrotest Fyto Ltd., Kroměříž, Czech Republic

Abstract: A large number of resistances to powdery mildew (*Blumeria graminis* f.sp. *hordei*) and their combinations are known in barley (*Hordeum vulgare* L.). A similarity of resistance spectra between cultivars carrying the resistance Bw, designated for the winter barley cultivar Borwina, and the near-isogenic spring barley line P15, which carries the resistance Ru2, derived from the landrace Rupee, was found. The objective of this study was to test the difference between resistances Bw and Ru2. Six cultivars were tested, four with Bw and two with Ru2. Testing with 40 isolates showed identical reaction spectra between both groups. Testing of the cultivar Kompolti 4 (Bw) and line P15 (Ru2) with 300 isolates confirmed this result. Thus, the resistances currently designated Bw and Ru2 can be regarded as identical, and are determined by the gene *Ml(Ru2)*. Both Bw and Ru2 should be designated by the earlier code, Ru2.

Keywords: *Blumeria graminis* f.sp. *hordei*; *Hordeum vulgare*; pathogen isolates; resistance gene postulation

In the Czech Republic powdery mildew of barley (*Hordeum vulgare* L.) caused by the fungus *Blumeria graminis* (DC.) E. O. Speer, f.sp. *hordei* emend. É.J. Marchal (anamorph *Oidium monilioides* Link), hereafter referred to as *Bgh*, is a common disease on barley (Dreiseitl 2011a). A large number of resistances and numerous cultivars with various combinations of these resistances are known in barley (Jørgensen 1994; Kintzios *et al*. 1995; Schonfeld *et al*. 1996; Czembor 2002) and some others were recently described (Dreiseitl 2011b, c, d, e). Some of these resistances have been used to develop near-isogenic lines of spring barley using the cv. Pallas as the recurrent parent (Kølster *et al*. 1986). These lines differ in their powdery mildew resistance genes.

Line P15 carries the gene *Ml(Ru2)*, which is derived from the Indian barley landrace Rupee. Rupee carries four resistance genes, out of which the gene in P15 was identified as the second (Kølster *et al*. 1986; Jørgensen 1994). Out of 699 barley cultivars, Brown and Jørgensen (1991) reported the resistance controlled by *Ml(Ru2)* in two cultivars only, Rupee and P15, which was derived from Rupee (Kølster *et al*. 1986). This resistance is designated here Ru2 in accordance with the recommended convention (Boesen *et al*. 1996).

In 1983, the German six-rowed winter barley cv. Borwina was registered in the Czech Republic. It carries a resistance, designated Bw, that differs from all others currently found in winter cultivars (Dreiseitl 1993; Boesen *et al*. 1996). The gene *Ml(Bw)* has not yet been localized in the barley genome, but Bw has become frequent in German cultivars, and in six-rowed cultivars of winter barley developed in the Czech Republic (Dreiseitl 2007).

In a recent examination of resistances in the cultivars included in the Kromeriz Agricultural Research Institute barley gene bank, a similarity of resistance spectra between cultivars carrying Bw and line P15 was noticed. Therefore, the objectives of this study were (*i*) to test the resistance in winter barley cultivars with resistance Bw and in spring barley cultivars with resistance Ru2; (*ii*) to compare these resistance spectra; and (*iii*) to confirm or refute the identity of resistances Bw and Ru2.

MATERIAL AND METHODS

Six cultivars were tested (Table 1). Four have resistance Bw (three have Bw only, and cv. Borwina also has Lo (Dreiseitl 2011e)). The other two have resistance Ru2: P15 (which also has HH) and the American cv. Mollybloom (which also has Ch). In a subsequent experiment, line P15 (Ru2) and cv. Kompolti 4 (Bw) were used as differential cultivars.

Forty reference isolates of *Bgh* held in the pathogen gene bank at the Agricultural Research Institute Kromeriz were used for resistance tests. The designation of the isolates is derived from their virulence patterns in relation to 12 *Ml* resistance genes in coded triplets (Limpert & Müller 1994) in the order *a1*, *a3*, *a6*; *a7*, *a9*, *a12*; *a13*, *k1*, *La*; *g*, *at*, *(Ru2)*. Additional 300 isolates were subsequently tested; these belonged, on the basis of reactions to 12 differential cultivars, to 144 different pathotypes.

About 20 seeds of each cultivar were sown into each of two pots (80 mm diameter) filled with a gardening peat substrate and placed in a mildew-proof greenhouse under natural daylight in October. Leaf segments 20 mm long were cut from the central part of healthy, fully-expanded primary leaves (DC11). For testing with an isolate, three leaf segments of each cultivar were placed with the adaxial side facing up in a Petri dish on water agar (0.8 %) with benzimidazole (40 mg/l) – a leaf senescence inhibitor. For each isolate, a Petri dish with leaf segments was placed at the bottom of a metal inoculation tower and inoculated with ca. 8 conidia/mm^2. The dishes with inoculated leaf segments were incubated at 18 ± 2°C under artificial light (cool-white fluorescent lamps providing 12 h light at 30 ± 5 μmol/m^2/s).

Eight days after inoculation, reaction types (RTs) on the adaxial side of leaf segments were scored. A nine-point scale (0–4, including intertypes) was used for scoring RTs, indicating the pathogen growth (Torp *et al.* 1978). Each cultivar was tested in two replications (independently prepared experimental series). If there were notable differences between replications in RTs, additional tests were carried out. The set of 40 RTs that each cultivar developed in response to the 40 reference isolates provided the basis for a resistance spectrum (RS) for each cultivar.

Table 1. Six barley cultivars and their powdery mildew resistance

Cultivar	Resistance spectrum	Resistance code	Reference
Spring barley			
P15	3	Ru2, HH	Kølster *et al.* (1986); Dreiseitl unpublished
Mollybloom	2	Ru2, Ch	Dreiseitl and Steffenson (1996); Dreiseitl unpublished
Winter barley			
Borwina	4	Bw, Lo	Dreiseitl (1993, 2007, 2011e)
F 12872/08 L1	1	Bw	Dreiseitl (2011f)
Kompolti 4	1	Bw	Dreiseitl (2007)
Zhhlaoluomang	1	Bw	Dreiseitl and Yang (2007)

Table 2. The three resistance spectra found in six barley cultivars inoculated with 10 selected isolates of the barley powdery mildew pathogen (*Blumeria graminis* f.sp. *hordei*)

Resistance spectrum	Resistance code	Isolate of *Blumeria graminis* f.sp. *hordei*									
		0004	1044	2567	3707	4523	4711	4761	5511	7467	7777
1	Ru2	4	4	4	4	2–3	2–3	2–3	2–3	4	4
2	Ru2 Ch	4	2	4	4	2–3	2–3	2–3	2–3	4	4
3	Ru2 HH	4	0	4	4	2–3	2–3	2–3	2–3	4	4
4	Ru2 Lo	4	0	4	4	2–3	2–3	2–3	2–3	0	4

RESULTS

Among the two spring barley and four winter barley cultivars, four RSs were detected. RS 1 was characterized by RT 2–3 in response to 24 isolates and RT 4 to 16 isolates. RSs 2 and 3 were nearly identical to RS 1, except for RT 2 in RS 2 and RT 0 in RS 3 instead of RT 4 to isolate 1044 and RS 4 was characterized by RT 0 in response to isolate 7467. In this contribution ten RTs were used to characterize the four RSs (Table 2); three cultivars showed RS 1, one showed RS 2, one showed RS 3, and another one showed RS 4 (Table 1).

DISCUSSION

Testing with the 40 reference isolates showed identical RSs between cultivars with resistances Ru2 and Bw. Small differences in four RSs were due to the presence of "additional" resistance HH in line P15, Lo in cv. Borwina, and resistance Ch in cv. Mollybloom. Among the 40 pathotypes used, HH and Ch are effective against pathotype 1044 only and Lo is effective against pathotypes 1044 and 7467. However, practical importance of these three additional resistances is negligible.

In the subsequent experiment with 300 isolates of 144 pathotypes, line P15 (Ru2) and cv. Kompolti 4 (Bw) showed identical responses in all cases. Thus, the resistances currently designated Bw and Ru2 can be regarded as identical, and are determined by the gene *Ml(Ru2)*. Therefore, this resistance should be designated as Ru2, which was published earlier (Kølster *et al*. 1986), even though it is often present in winter barley cultivars long identified as Bw.

The source of the resistance in cv. Borwina has not been identified from its pedigree ((Valja ×Vogelsanger Gold) × Hohenthurm 7246). Dreiseitl and Yang (2007) studied the resistance in 147 Chinese cultivars. It was a big surprise to find that 47% of them, including cv. Zhhlaoluomang tested also here, showed identical resistance to that of cv. Borwina. The corresponding virulence is also very frequent in east Asia, as documented by a recent study of the pathogen population (Dreiseitl & Wang 2007), which found this virulence in more than 84% of the isolates examined (including isolate 0004, used here), and by the virulence of an old Japanese isolate, 1044 (also known as Race I; Hiura & Heta 1955), which helped us in distinguishing the four RSs (Table 2). Therefore, the resistance Bw could have had roots in east Asia (Dreiseitl 2007). However, Ru2 is likely to be present in cv. Borwina owing to a not fully successful effort to use the resistance of Rupee (Moseman & Jørgensen 1973), and especially the gene *Mla13* (= Ru1).

The most frequent expression of Ru2 is reaction type 2–3. However, the RT is "only" a phenotype of the given resistance influenced by environmental conditions. The phenotype assessment depends also on the evaluator's subjective reading. Therefore, during testing of resistances with RT 2–3 or RT 3, inaccuracies are common, necessitating multiple replications in order to eliminate them and to obtain the necessary agreement in spectra between individual replications. Similar inaccuracies also occur when tests of isolates are repeated infrequently. Therefore, to determine the virulence of each isolate to such resistance genes, it is useful to use at least two cultivars with identical resistance, and if different results are obtained, these cultivars should be inoculated again.

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Corresponding author:

Doc. Ing. Antonín Dreiseitl, CSc., Agrotest fyto s.r.o., Havlíčkova 2787, 767 01 Kroměříž, Česká republika
e-mail: dreiseitl.antonin@vukrom.cz