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What types of powdery mildew can infect wheat-barley introgression lines?

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Abstract This work is a detailed study of the infection of fungal biotrophic pathogens causing powdery mildew diseases on introgression lines originating from the intergeneric hybridisation between wheat and barley (*Triticum aestivum* L. × *Hordeum vulgare* L.). Powdery mildew fungi are among the most widespread biotrophic pathogens of plants also and infect dicot and monocot species. Most powdery mildew species are strictly host specific. They colonize only a narrow range of species or one particular host species. The intergeneric hybridisation between wheat and barley could result in expansions of host ranges of the barley powdery mildew. Our experiments covered natural infections in the field and artificial infections under greenhouse conditions. *Formae speciales* of powdery mildew were identified on the basis of the sequencing results of ribosomal internal transcribed spacer sequences (rDNA-ITS). We identified *Blumeria graminis* f.sp. *tritici* isolate 14 (HM484334) on the wheat parent and all wheat-barley introgression lines and *B. g.* f. sp. *hordei* isolate MUMH1723 (AB 273556) on the barley parent, respectively. The wheat-barley introgression lines were inoculated with barley powdery mildew under greenhouse conditions. According to our results the added barley chromosomes (or segments) do not cause host range expansion of barley powdery mildew.

Keywords Wheat-barley introgression lines · *Formae speciales* of powdery mildew · Host range expansion

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Powdery mildew caused by *Blumeria graminis* (DC.) Speer, is a major problem in cereal production around the world. This obligate biotrophic fungus can reduce the quality and quantity of cereal yields. It has developed eight distinct *formae speciales* (f.sp.) so far, which show strict host specialization (Inuma et al. 2007); for example, *Triticum* spp. and *Hordeum* spp. are appropriate hosts to *B.graminis* f.sp. *tritici* and f.sp. *hordei*, respectively. The evolution of the ability to exploit a novel host in addition to the host of origin is called host range expansion (Giraud et al. 2010). It is expected to occur rather frequently, as only a few modifications in the effector repertoire of the pathogen can enable it to adapt to a closely related plant species (Schulze-Lefert and Panstruga 2011; Troch et al. 2012). This is especially true for wheat and barley, the two important cereal crops worldwide, where the host-pathogen interactions

are well known. Interestingly only a few studies deal with the host range expansion of cereal powdery mildew, however, intergeneric hybridization between wheat and barley (*Triticum aestivum* L. × *Hordeum vulgare* L.) could result in the host range expansion of barley powdery mildew. The introgression of barley chromosomes or chromosome segments into the wheat genome may result in the transfer of useful traits from barley to wheat, e.g., tolerance to drought and soil salinity, earliness, or various traits for specific nutritional quality. Since the first successful hybridization between wheat and barley (Kruse 1973) only a few wheat-barley addition, substitution and translocation lines have been developed (Islam and Shepherd 1992; Koba et al. 1997; Molnár-Láng et al. 2000a, b; 2012). Scarce information is available on the ability of barley chromosomes to compensate for wheat chromosomes regarding important agronomical characteristics (Hoffmann et al. 2011). But together with beneficial traits sensitivity to specialized pathogens is also transferred?

In this study we investigated whether barley powdery mildew can infect the hybrids originating from wheat-barley crosses. The objectives of this study were to: 1) to identify the powdery mildew symptoms on introgression lines under natural infection on the field; 2) to collect the infected leaves from the field and identify the fungus *formae speciales* by ribosomal internal transcribed spacer sequences (rDNA-ITS) regions; and 3) to test the seedling plants in accordance with the behaviour towards barley powdery mildew infection after artificial infection under greenhouse conditions.

Different wheat-barley addition, substitution, translocation lines as well as parent lines (Table 1) were examined in this study. The experiments included greenhouse and field infections. The field experiment was performed during the 2011-2012 growing seasons at UP Georgikon Faculty, Keszthely. The soil of the experimental site is a lessivated brown forest soil (FAO: Luvic phaosem) with low organic material, medium K- and P content. Row space was 30 cm. Each genotype was sown in a 15 m long row. The 2011– 2012 wheat season was characterized by a spring and summer drought. Irrigation and high plant density helped in making the microclimate more favourable for the development of the disease.

Disease symptoms were visually recorded on the leaves, on different occasions (from booting, Zadoks scale [ZAD] 40 to early maturity, ZAD 80). The plants were in different developmental stages at a given time. We used the Saari and Prescott's severity scale (0–9) to assess wheat foliar diseases (Saari and Prescott 1975). The percentage of the infected leaf area was evaluated for the whole plant and for the flag-leaf.

Heavily infected leaves were collected from the field, from all genotypes (except 6H addition line, which genotype was not infected). Eleven chasmotecium were isolated from infected spots of each line. Total genomic DNA was extracted from chasmothecia using the Phire Plant Direct PCR Kit (Thermo Fisher Scientific, USA). For PCR amplifications tissue-direct technique was used requiring very little sample material compared to other alternative methods. The direct PCR (diPCR)

Table 1 The plant material examined in this study. The introgression lines and Mv9 kr1 wheat line were developed at the Department of Plant Genetic Resources and Organic Breeding, Centre for Agricultural research, Hungarian Academy of Sciences

Species	Cultivar or line	Pedigree	References
Wheat	Mv9 kr1	Chinese Spring × Martonvásári 9	Molnár-Láng et al. 1996
Wheat-barley hybrids	2H addition	Mv9 kr1 × Igri	Molnár-Láng et al. 2000a; Szakács and Molnár-Láng 2007
	3H addition	Mv9 kr1 × Igri	
	4H addition	Mv9 kr1 × Igri	
	6H addition	(Chinese Spring × Betzes) × Mv9kr1	Molnár-Láng et al. 2000a
	6HS addition	Mv9 kr1 × Igri	Szakács and Molnár-Láng 2010
	7H addition	Mv9 kr1 × Igri	
	4H (4D) substitution	(Chinese Spring × Betzes) × Mv9kr1	Molnár et al. 2007
	3BL.3HS translocation	(Chinese Spring × Betzes) × Mv9kr1	Molnár-Láng et al. 2000b
	6B-4H translocation	(Chinese Spring × Betzes) × Mv9kr1	
	7D-5HS translocation	Mv9 kr1 × Igri	
Barley	Igri	-	-

Table 2 Referred *Blumeria graminis* isolates in this study with sequences database accession numbers

Host of sampling isolates	Origin host of isolates in database	Database Accession No.	Refences
Triticum aestivum	*Triticum aestivum* (isolate: 14)	HM484334	Walker et al. 2011
Triticum aestivum L. × *Hordeum vulgare* introgression lines	*Triticum aestivum* (isolate: 14)	HM484334	Walker et al. 2011
Hordeum vulgare	*Hordeum distichum* (isolate: MUMH1723)	AB273556	Inuma et al. 2007

amplification was performed with the primers ITS1 and ITS4 described by White et al. (1990). Amplification reactions were performed in 20 μl total volume PCR reaction containing 8.6 μl nuclease-free water, ~20 ng template DNA, 0.2 μM ITS1 and ITS4 primer, 0.2 mM dNTP, 10 μl PCR buffer (1 mM Tris/HCl, pH 8.8 at 25°C, 1.5 mM MgCl2, 50 mMKCl and 0.1 % Triton X-100) and 0.4 μl Phire Hot Start II DNA Polymerase (Thermo Fisher Scientific, USA). PCR was performed using 5 min at 98°C for initial denaturation, 40 cycles of 20 s denaturation at 98°C, 1 min annealing at 53°C, and 2 min extension at 72°C, followed by a final extension for 5 min at 72°C. Amplification products were separated at 1.5 % agarose gels stained with ethidium-bromide. PCR fragments excised from agarose gels were cleaned by removing non-incorporated primers with 10 U exonuclease I and degradation of nucleotides by 1 U thermosensitive alkaline phosphatase (Exo I and FastAP, Fermantas, Lithuania). PCR mixes were incubated at 37°C for 15 min and the reaction was stopped by heating the mixture at 85°C for 15 min. Sequencing was performed on an ABI 3130XL automated sequencer in both directions using the ITS1 and ITS4 primers and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit v.3.0. All sequences were annotated and deposited in GenBank (Benson et al. 2011); accession numbers are given in Table 2.

Forward and reverse sequence reads for all isolates were assembled with CodonCode Aligner v.3.7.1. (codoncode.org). Discrepancies were manually

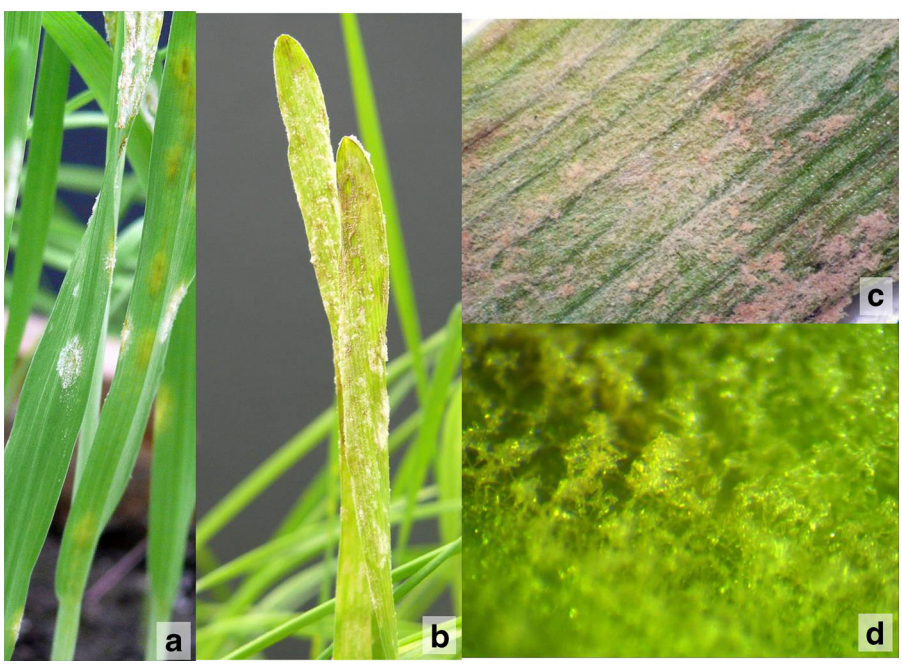

Fig. 1 Symptoms *of Blumeria graminis* f.sp. *hordei* isolate A6 (**a**) and BP (**b**) on leaves of 'Igri' barley parent. Mycelium of isolate BP under stereo microscope, with 15× (**c**) and 70× (**d**) magnification

Fig. 2 Testing of wheat- barley hybrids and their parent lines in isolated glass box in greenhouse with *Blumeria graminis*, f.sp. *hordei* isolates. Both isolates A6 (**a**) and BP (**b**) infected the 'Igri' barley parent (*arrows*), but no symptoms were emerged on the introgression lines and 'Mv9kr1' wheat parent

resolved by editing the traces using the compare option of the advanced assembly function. Single consensus sequences were extracted in FASTA format from the compared assemblies. Multiple sequence assemblages were aligned with MUSCLE (Edgar 2004) as implemented in Geneious v.4.8.5 (geneious.com) using default settings. Ribosomal exons and spacer regions were annotated in the alignments using the fungal reference sequences deposited in the ITS2 database (Koetschan et al. 2010). Obtained sequences were compared with sequences available in GenBank database using the basic local alignment search tool (blastn) with default parameters.

Blumeria graminis f.sp. *hordei* A6 (Wiberg 1974) and BP (Hungarian isolate, El-Zahaby et al. 1995) isolates obtained from the Plant Protection Institute, Centre for Agricultural Research, were used for inoculation. The isolates were formerly tested and propagated on 'Igri' barley parent genotype in an isolated glass box in greenhouse. Ten plants of each genotype were grown in 50×40 cm wooden boxes under isolator with 16 h daily natural and illuminated light conditions at 18 °C. The relative humidity in the isolator box was 80-90 %. The genotypes were completely randomised. Infection was carried out on 10-day-old seedlings in three replications per isolate. Leaves with heavily sporulating

Table 3 Assessment of severity of powdery mildew infection on the introgression lines and their parent cultivars

Genotype	4 May 2012		9 May 2012		18 May 2012		3 June 2012		
	IA, %	S.& P. scale	IA, %	S.& P. scale	IA, %	S.& P. scale	IA, %	S.& P. scale	IA flag-leaf, %
2H add.	30	6	40	6	50	6	50	7	5
3H add.	20	5	30	7	40	7	50	8	40
4H add.	40	7	40	7	60	7	70	8	80
6H add.	<5	1	<5	1	<	1	<5	1	0
6HS add.	10	4	20	6	20	6	30	7	5
7H add.	20	4	20	6	20	6	30	7	5
7D-5HS transl.	10	4	20	6	20	6	30	7	5
6B-4H transl.	10	4	20	6	20	6	30	7	5
3HS.3BL transl.	40	7	40	7	50	7	50	8	40
4H (4D) subst.	10	3	10	3	20	6	20	7	5
Mv9kr1 wheat	40	5	40	6	50	6	60	8	60
Igri barley	10	4	10	4	20	6	30	8	80

IA=percentage of infected area evaluated for the whole plant; S.& P. scale=Saari and Prescott's severity scale, 0 to 9 scale, ranging from no visible symptoms (0) to visible symptoms on spike (9); IA flag-leaf=percentage of infected flag-leaf area

Mv9kr1×Igri: 2H, 3H, 4H, 6HS, 7H addition, 7D-5HS translocation lines;

(Chinese Spring×Betzes)×Mv9kr: 4H (4D) substitution, 3HS.3BL, 6B-4H translocation lines

colonies were shaken in the test box. The presence of powdery mildew on the seedlings was scored on the 10th day after inoculation.

Blumeria graminis f.sp. *hordei* isolates A6 and BP infected the 'Igri' barley parent, and they heavily sporulated under artificially infected conditions (Fig. 1). On the contrary, the introgression lines and 'Mv9kr1' wheat parent were not infected by *B.g.* f.sp. *hordei* (Fig. 2).

Powdery mildew symptoms were observed on all genotypes in the field (Table 3; Fig. 3). 'Mv9kr1' wheat parent and 4H addition line were highly susceptible to the disease (infected leaf area 60 % and 70 %, respectively). The highest percentage of infected flag-leaf area was recorded for these genotypes and for 'Igri' barley parent. On the leaves of 6H addition line no symptoms were observed just few mycelium sporadically on the stem and only 5 % of flag-leafs were infected in case of most of the examined lines: 4H (4D) subs.; 6HS add.; 7H add.; 7D-5HS transl.; 6B-4H transl.; 2H add., while in case of the wheat parental genotype 60 % of the flag-leaf area was infected.

Direct PCR amplifications yielded one 562 bp size band (Fig. 4) with no length variation indicating lack of alternative ITS copies amplifying from paralogous loci. Using the primers of White et al. (1990) we detected only fungal ITS sequences and sequencing revealed no traces of plant contamination. We found no variation among the sequenced isolates and natural infection was most probably caused by the same haplotype. The BLAST search carried showed a 100 % match on entry from *Blumeria graminis* f. sp. *tritici* isolate 14 (HM484334) deposited by Walker et al. (2011) and *Blumeria graminis* f. sp. *hordei* isolate MUMH1723 (AB 273556) deposited by Inuma et al. (2007). *Blumeria graminis* f. sp. *tritici*

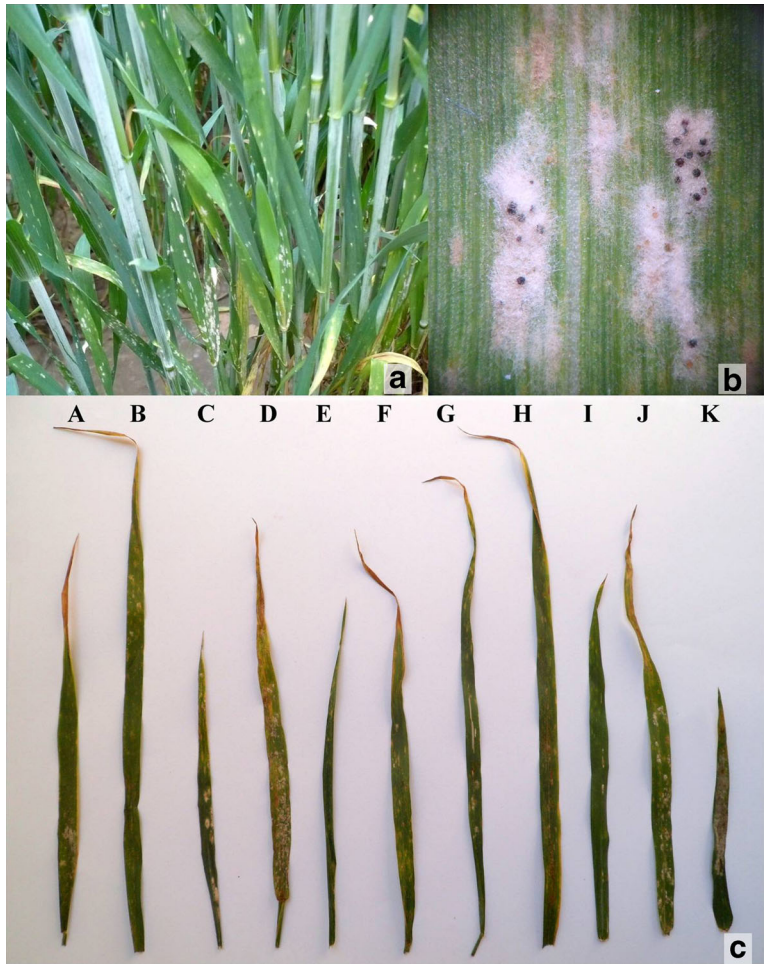

Fig. 3 Symptoms of *Blumeria graminis* f.sp. *tritici* isolate on leaves of '4H addition lines on the field (**a**). Mycelia and conidia (**b**) of *Bgt.* (stereo microscope photo, enlargement: 15×). Disease symptoms on flag-leaves (**c**). A= 4H (4D) substitution; B=2H, C= 3H, D=4H, E=7H, F=6HS addition; G=3BL.3HS, H=6B-4H, I=7D-5HS translocation; J= Mv9kr1; K=Igri

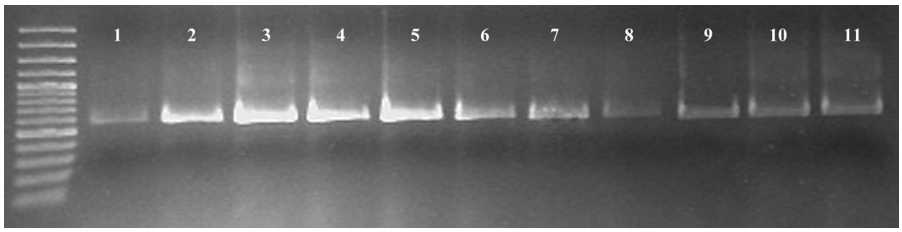

Fig. 4 PCR amplification patterns of the nuclear ribosomal internal transcribed spacer (rDNA ITS) region among isolates, showing one clear 562 bp size fragment excised and used for sequencing; indicating that the identification of isolates was based on orthologous ribotypes. Samples: 1=7D-5HS transl.; 2=3H add.; 3=4H (4D) subs.; 4=2H add.; 5=7H add.,; 6=6HS add.; 7=Igri barley; 8=4H add.; 9=Mv9kr1 wheat; 10=3HS.3BL transl.; 11=6B-4H transl

pathotype was identified exclusively on the wheat parent and wheat-barley hybrids, while *Blumeria graminis* f. sp. *hordei* pathotype was collected only from Igri barley parent.

The present study reveals that *Blumeria graminis* f.sp. *hordei* is currently unable to infect wheat-barley introgression lines. However, our research demonstrated that *B. g.* f.sp. *tritici* is able to infect these lines. Wheat-barley introgression lines had different susceptibility to *B. g.* f.sp. *tritici* isolate 14, nonetheless 'Mv9kr1' wheat parent showed strong susceptibility to this disease. Based on our results we identified *B. g.* f. sp. *hordei* isolate MUMH1723 (AB 273556) on the barley parent (Table 3). This isolate described by Inuma et al. (2007) was collected in Iran.

According to our results the added barley chromosomes (segments) by themselves do not affect host range expansion of barley powdery mildew. A lot of studies discuss the powdery mildew-host interaction, however only a few deal with powdery mildew host range expansion that can occur as a consequence of interspecies hybridization. The most important intergeneric hybrid is triticale (× Triticosecale Wittmack) with rising production area and importance. With the expansion of the triticale production area, powdery mildew emerged on this new host expanded from wheat to triticale multiple times at different locations in Europe (Walker et al. 2011; Troch et al. 2012; Klocke et al. 2013). Only a part of *B. graminis* f.sp. *tritici* isolates collected by Troch et al. (2012), were able to infect both the triticale (the new host) and the wheat cultivars (the previous host), whereas some isolates showed pathogenicity exclusively on triticale cultivars. The studied wheat-barley introgression lines have valuable agronomic traits, such as tolerance for drought, earliness, and high root- shoot ratio (Hoffmann et al. 2011). These advantageous traits can result in the spread of certain barley chromosomes (segments) in breeding material – as was the case with 1BL.1RS translocation (Hoffmann 2008; Kőszegi et al. 2000; Singh et al. 1998), which may result in a growing selection pressure. That is why we need to investigate the possibility of host range expansion.

According to our results the added barley chromosomes (or segments) did not cause host range expansion of barley powdery mildew up to now, on the contrary: wheat powdery mildew infection was reduced in most of the examined genotypes. These results raise hopes that the introgression of barley chromosomes into the breeding material can lead to a reduction of powdery mildew susceptibility of newly bred wheat genotypes.

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