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FULL RESEARCH PAPER

Virulence and diversity of *Blumeria graminis* f.sp. *hordei* in East China

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Abstract Four hundred and sixty-one isolates of *Blumeria graminis* f.sp. *hordei* were obtained from eight populations occurring on cultivated barley (*Hordeum vulgare*) at four geographically distant locations in China during 2003 and 2004. Their virulence frequency was determined on 30 differential lines. No isolate was virulent on differential lines possessing the resistance genes *Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mla13*, *Mlat*, *Mlg*, *Mla10*, *Mla22*, *Mla23*, *Mlp1*, *Ml*(*N81*) and *Mlmw*. Virulences to the first nine resistance genes are prevalent in Europe and constitute the main part of genetic distance between Chinese and European populations. Conversely, no isolate was avirulent on the differential lines possessing the genes *Mla8* and *Ml(Ch)*. The frequencies of isolates overcoming the genes *Mla2*, *Mla11*, *Mlk1* and *Mlk2* were .4–9.3%, and frequencies of isolates overcoming the genes *Mlh*, *MlLa*, *Ml*(*Bw*), *Mlra*, *Ml*(*Ru2*), *mlw*, *MlGa*, *MlWo* and *Mlnn* ranged from 18.2% to 98.7%. Based on reactions of differential lines possessing the genes *Mlk1*, *Mlh*, *MlLa*, *Ml*(*Bw*), *Mlra* and *Ml*(*Ru2*), pathotypes were identified and diversity parameters calculated. Eleven of 22 detected pathotypes were found in both years and comprised 94.6% of isolates. Generally, the populations from different locations in 1 year were more closely related than populations collected from the same locations in different years. Complete effectiveness of the resistance genes, for which no corresponding virulences were found, will allow Chinese breeders to access many modern European barley cultivars that are fully resistant to powdery mildew in China, including those possessing the non-host resistance gene *mlo*.

Keywords Barley · *Erysiphe graminis* f.sp. *hordei* · *Hordeum vulgare* · Pathogen population · Powdery mildew

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Introduction

Barley for grain production (*Hordeum vulgare*) is an important commodity in China and was grown on 8 m ha annually in 1914–1918, which comprised 24% of the world area. Now barley is planted on only 1.4 m ha, which accounts for about 1% of arable land and is similar to the cultivated area of tea or tobacco. The current demand of barley for feeding farm animals and

direct human use as well as for beer production markedly exceeds its domestic production. Since 2002, China has become the world's biggest producer of beer although home consumption per capita is still low; its further fast growth is expected. To make up for the deficit in domestic production grain is imported, but there is still a shortfall between supply and demand. To deal with the shortage of barley a Chinese National Barley Improvement Centre has been established and international cooperation promoted to solve these problems. In the coastal area of south-east China, barley is grown in paddy fields that are primarily used for rice production; their area is usually less than .1 ha. Local cultivars are mostly grown, and these range from highly susceptible to highly resistant to powdery mildew (Huang, Guo, Chen, Xu, & Chen, 2002). The environmental conditions of this region allow 2–3 crops to be harvested per year.

Powdery mildew of barley, caused by the ascomycete fungus *Blumeria graminis* f.sp. *hordei*, is a wind-borne and obligate biotrophic pathogen, often used as a model in understanding host-parasite interaction (Bushnell, 2002; McDonald & Linde, 2002). This pathogen spreads mostly by conidia, but survives unfavourable conditions through a pseudosexual type of teleomorph, which terminates by production of chasmothecia with numerous asci containing ascospores (Braun, Cook, Inman, & Shin, 2002).

Blumeria graminis f.sp. *hordei* is particularly prevalent under cool conditions when the maximum daily temperature does not exceed 25°C. In south-east China, the pathogen population is often reduced by a long period of high summer temperatures above 30°C as well as the absence of green leaf tissue towards harvest. *Hordeum vulgare* ssp. *spontaneum*, the only species of wild barley that could be a natural source of inoculum of *B. graminis* f.sp. *hordei*, does not occur here.

The objectives of this study were: (i) to characterize the Chinese *B. graminis* f.sp. *hordei* population using standard European differentials and other barley genotypes carrying resistance genes to powdery mildew; (ii) to evaluate and assess diversity in, and genetic distances between these populations by using recent measures of genetic diversity and genetic distances; (iii) to find isolate(s) avirulent to the resistance gene *Mla8*, which is absent in Europe.

Materials and methods

Location of pathogen populations

Isolates of the pathogen were sampled in commercially-grown crops of barley at four locations on the eastern coastal part of China between about 24° and 34° latitude. The first location was near Putian, ca. 550 km south-west of Hangzhou (second location), the third location was near Jujing, ca. 80 km east of Hangzhou, and the fourth location near Yancheng, ca. 350 km north-east of Hanghzhou. The direct distance between Jujing and Yancheng is about 400 km, between Putian and Jujing about 600 km and between Putian and Yancheng about 900 km.

Sampling populations

Blumeria graminis f.sp. *hordei* isolates were sampled before the peak incidence of powdery mildew development. Eight populations of the pathogen were sampled from 15 different barley fields. The distance between two nearest isolates collected in a field was at least 1 m. Fifty-four to 61 single-colony isolates from leaf samples were sampled within a population at the end of April 2003, and 55–59 isolates at the end of April 2004 (see Table 3 for details).

Multiplication of inoculum

Leaf segments with individual *B. graminis* f.sp. *hordei* colonies that had developed from natural infections were placed in 100 mm plastic Petri dishes with .7% water agar and 35 ppm benzimidazole, then incubated for 1 day at 22 ± 2°C under artificial light (cool-white fluorescent lamps providing 12 h light at 15 ± 5 μmol m^{-2} s^{-1}). Conidia from each colony were shaken onto leaf segments 25 mm long, which were excised from the central part of healthy, fully expanded primary leaves of the line B-3213 with no resistance gene to powdery mildew (Dreiseitl &

Steffenson, 1996) except isolates collected in Putian in 2003 reproduced on susceptible cv. Xiumai 3. Inoculated leaf segments were placed in Petri dishes with water agar, prepared as above, and incubated under similar conditions for 10–11 days.

Differential sets

The set of differentials used in 2003 (Table 1) comprised barley cv. Pallas, 17 near-isogenic Pallas lines (Kølster, Munk, Stølen, & Løhde, 1986), Nepal 81 and Borwina (Dreiseitl & Jørgensen, 2000), which contained different genes for resistance to powdery mildew. Because of their avirulence to all isolates found in 2003, 13 differentials were replaced with 10 others in 2004. The latter were represented by two near-isogenic Pallas lines (P09 and P18), cv. Aibaiyang (Dreiseitl & Yang, 2007) and seven sources of resistance to powdery mildew (Jørgensen, 1994).

Production of differential leaf segments

About 50 seeds of each differential were sown in a pot (130 mm upper diameter) filled with sandy soil. Plants were grown at 22 ± 2°C and natural light for 12–14 days. Leaf segments 25 mm long were cut from the central part of healthy, fully-expanded primary leaves of each differential. The segments were placed in 100 mm plastic Petri dishes on water agar for testing each isolate.

Inoculation of differential leaf segments

Leaves were inoculated in a metal inoculation tower 300 mm high and 105 mm diam. For each isolate, a dish with leaf segments from the differential set was placed at the bottom of the tower. Inoculum of each isolate collected from a leaf segment was shaken onto a square piece (40 × 40 mm) of black paper to control visually the amount of conidia, and blown through a hole

Table 1 A set of barley differential cultivars, their genes for resistance to *Blumeria graminis* f.sp. *hordei* and frequency of corresponding virulences (%) found in Chinese populations in 2003 and 2004

No.	Differential cultivar	Resistance gene(s)	2003	2004
01	P01	*Mla1*, *MlaAl2*	0	–
02	P02	*Mla3*	0	–
03	P03	*Mla6*, *Mla14*	0	–
04	P04B	*Mla7*, *MlaNo3*	0	–
05	P08B	*Mla9*	0	–
06	P10	*Mla12*, *MlaEm2*	0	–
07	P11	*Mla13*, *MlaRu3*	0	–
08	P12	*Mla22*	0	–
09	P13	*Mla23*	0	–
10	P19	*Mlp1*	0	–
11	P20	*Mlat*	0	–
12	P21	*Mlg*, *MlCP*	0	–
13	Nepal 81	*Ml(N81)*	0	–
14	P17	*Mlk1*	7.2 ± 1.7[a]	.4 ± .4
15	P24	*Mlh*	18.2 ± 2.5	62.7 ± 3.2
16	P23	*MlLa*	33.5 ± 3.1	61.3 ± 3.2
17	Borwina	*Ml(Bw)*	84.7 ± 2.3	92.4 ± 1.8
18	P14	*Mlra*	98.3 ± .7	98.7 ± .8
19	P15	*Ml(Ru2)*	98.7 ± .7	98.7 ± .8
20	Pallas	*Mla8*	100.0	100.0
21	P09	*Mla10*, *MlaDu2*	–	0
22	Mian Wali	*Mlmw*	–	0
23	A-222	*Mla11*	–	.9 ± .6
24	Nakaizumi-zairai	*Mlk2*	–	.9 ± .6
25	Black Russian	*Mla2*, *MlaBR2*	–	9.3 ± 1.9
26	West China	*mlw*	–	33.3 ± 3.1
27	Galleon	*MlGa*	–	48.9 ± 3.3
28	Wong	*Ml(Wo)*	–	71.1 ± 3.0
29	P18	*Mlnn*	–	97.3 ± 1.1
30	Aibaiyang	*Ml(Ch)*	–	100.0

[a] Standard error of binomial distribution

of 15 mm diam in the upper part of the inoculation tower. Inoculum density was ca. 8 conidia mm^{-2}. The dishes with inoculated leaf segments were incubated in a chamber at 17 ± 1°C under artificial light (cool-white fluorescent lamps providing 12 h light at 30 ± 5 µmol m^{-2} s^{-1}).

Virulence determination

Reaction type on each differential and *B. graminis* f.sp. *hordei* isolate combination was scored 8 days after inoculation on a 0–4 scale (Torp, Jensen, & Jørgensen, 1978). Isolates that produced reaction type 4 or 3–4 were considered virulent on the corresponding resistance gene(s). Virulence frequencies found in the 2 years using eight individual populations are presented in Tables 2 and 3. All tests for virulence of the Chinese populations were carried out at the National Barley Improvement Centre, Zhejiang Academy of Agricultural Sciences, Hangzhou.

Pathotype designation

The isolates were assigned numerical designations based on their virulence to matching resistance genes in six differentials (Nos. 14–19 in Table 1). The differential set was divided into two triplets and each of the two digits indicates virulence or avirulence on the three differentials of the respective triplet. If a virulence to the corresponding resistance gene is detected, the first differential line has the value 1 (2^0), the second line has the value 2 (2^1), and the third line has the value 4 (2^2). Therefore, each digit can have a value from 0 (no virulence on any of the three differential lines) up to 7 (=1 + 2 + 4, virulent on each of the three differential lines). The resulting number based on six differentials defines the virulences of the isolates and consequently their classification as pathotypes (Gilmour, 1973; Limpert et al., 1994) (Table 4).

Data analysis

Parameters for comparison of all populations were calculated on the basis of virulence patterns of isolates on the set of the six differentials in the given order (Table 3). Descriptive parameters of populations (virulence frequency, virulence complexity, number of pathotypes, abundance, richness and evenness) were calculated for the isolates with the HaGiS programme (Hermann, Löwer, & Schachtel, 1999). Abundance generally signifies the frequency of the predominant pathotype (%); richness defines the number of pathotypes as a proportion of the number of isolates tested; and evenness (Sheldon, 1969) reflects the variation in frequencies of individual pathotypes, where a more even distribution of pathotypes results in higher evenness (Table 6). The relative

Table 2 A set of 13 barley differential cultivars, their genes for resistance to *Blumeria graminis* f.sp. *hordei* and frequency of corresponding virulences (%) found in eight Chinese populations in 2003 and 2004

Differential cultivar	Resistance gene(s)	Pu-03	Pu-04	Ha-03	Ha-04	Ju-03	Ju-04	Ya-03	Ya-04
P17	*Mlk1*	4.9 ± 2.8[a]	1.8 ± 1.8	16.9 ± 4.9	0.0	6.5 ± 3.1	0.0	0.0	0.0
P24	*Mlh*	4.9 ± 2.8	81.8 ± 5.2	1.7 ± 1.7	38.2 ± 6.6	62.9 ± 6.1	76.8 ± 5.6	0.0	54.2 ± 6.5
P23	*MlLa*	36.1 ± 6.1	61.8 ± 6.6	8.5 ± 3.6	25.5 ± 5.9	38.7 ± 6.2	98.2 ± 1.8	51.8 ± 6.8	59.3 ± 6.4
Borwina	*Ml(Bw)*	90.2 ± 3.8	90.9 ± 3.9	94.9 ± 2.9	92.7 ± 3.5	75.8 ± 5.4	100.0	77.8 ± 5.6	86.4 ± 4.5
P14	*Mlra*	100.0	100.0	100.0	100.0	100.0	100.0	92.6 ± 3.6	94.9 ± 2.9
P15	*Ml(Ru2)*	100.0	100.0	98.3 ± 1.7	100.0	98.4 ± 1.6	100.0	98.1 ± 1.9	94.9 ± 2.9
A-222	*Mla11*	–	0.0	–	0.0	–	3.6 ± 2.5	–	0.0
Nakaizumi-zai	*Mlk2*	–	0.0	–	3.6 ± 2.5	–	0.0	–	0.0
Black Russian	*Mla2*, *MlaBR2*	–	5.5 ± 3.1	–	1.8 ± 1.8	–	26.8 ± 5.9	–	3.4 ± 1.9
West China	*mlw*	–	61.8 ± 6.6	–	0.0	–	64.3 ± 6.4	–	8.5 ± 3.6
Galleon	*MlGa*	–	61.8 ± 6.6	–	60.0 ± 6.6	–	0.0	–	72.9 ± 5.8
Wong	*Ml(Wo)*	–	56.4 ± 6.7	–	80.0 ± 5.4	–	71.4 ± 6.0	–	76.3 ± 5.5
P18	*Mlnn*	–	94.5 ± 3.1	–	98.2 ± 1.8	–	100.0	–	96.6 ± 1.9

Locations: Pu, Putian; Ha, Hangzhou; Ju, Jujing; Ya, Yancheng

[a] Standard error of binomial distribution

Table 3 Origins of 461 *Blumeria graminis* f.sp. *hordei* isolates of eight populations sampled from commercial cultivars at four Chinese locations in 2003 and 2004, and the distribution and frequency of 22 pathotypes

Year	Location	Province	Host cultivar	Number of isolates from:			Pathotypes and number of corresponding isolates (in parentheses) found in the field
				Field	Location	Year	
2003	Putian	Fujian	Mongmei 8477/84	61	61	236	06 (4), 07 (34), 17 (1), 46 (1), 47 (17), 57 (1), 66 (1), 67 (1), 77 (1)
	Hangzhou	Zhejiang	Zau 3	59	59		03 (1), 06 (3), 07 (41), 17 (8), 27 (1), 47 (3), 57 (2)
	Jujing	Zhejiang	Fua 30	2	62		07 (2)
			Xiumai 3	3			07 (1), 67 (2)
			Zau 6	57			06 (3), 07 (9), 23 (1), 26 (7), 27 (13), 36 (1), 37 (1), 46 (2), 47 (4), 57 (2), 66 (2), 67 (12)
	Yancheng	Jiangshu	Daner	54	54		05 (2), 06 (6), 07 (18), 42 (1), 44 (1), 45 (1), 46 (4), 47 (21)
2004	Putian	Fujian	Ji-87–8	2	55	225	27 (1), 67 (1)
			93–214	9			06 (1), 07 (2), 27 (2), 47 (1), 67 (3)
			Menker	11			07 (2), 27 (3), 46 (1), 67 (5)
			Damai	33			06 (2), 07 (1), 26 (1), 27 (6), 67 (22), 77 (1)
	Hangzhou	Zhejiang	02–13	23	55		07 (7), 27 (6), 47 (1), 67 (9)
			98-26	32			06 (2), 07 (22), 27 (4), 47 (2), 66 (2)
	Jujing	Zhejiang	Hua 30	56	56		27 (1), 47 (13), 67 (42)
	Yancheng	Jiangshu	Zhepi 7	29	59		06 (2), 07 (5), 23 (1), 25 (1), 27 (4), 43 (1), 47 (6), 65 (1), 66 (1), 67 (7)
			Yanzixian 1	30			03 (1), 06 (3), 07 (3), 25 (1), 27 (3), 46 (2), 47 (4), 67 (13)
Total				461	461	461	

Table 4 Twenty-two pathotypes of *Blumeria graminis* f.sp. *hordei* found in eight Chinese populations in 2003 and 2004, and their virulence complexity

Pathotype	Complexity(virulence number per isolate)	Number of isolates										
		In each population								Per year		Total
		Pu-03	Pu-04	Ha-03	Ha-04	Ju-03	Ju-04	Ya-03	Ya-04	2003	2004	
07	3	34	5	41	29	12		18	8	105	42	147
67	5	1	31		9	14	42		20	15	102	117
47	4	17	1	3	3	4	13	21	10	45	27	72
27	4		12	1	10	13	1		7	14	30	44
06	2	4	3	3	2	3		6	5	16	10	26
46	3	1	1			2		4	2	7	3	10
17	4	1		8						9		9
26	3		1			7				7	1	8
66	4	1			2	2			1	3	3	6
57	5	1		2		2				5		5
05	2							2		2		2
03	2			1					1	1	1	2
23	3					1			1	1	1	2
77	6	1	1							1	1	2
25	3								2		2	2
36	4					1				1		1
37	5					1				1		1
42	2							1		1		1
44	2							1		1		1
45	3							1		1		1
43	3								1		1	1
65	4								1		1	1

Locations: Pu, Putian; Ha, Hangzhou; Ju, Jujing; Ya, Yancheng

virulence complexity was determined as virulence complexity per differential (Kosman, 2003), and its average value per isolate was also calculated.

Diversities within populations (Table 6) and distances between populations (Table 7) were measured by Kosman indices (Kosman, 1996; Kosman & Leonard, 2007) based on both pathotype and virulence structure. Calculations of the Kosman diversity (KW) and distance (KB) are complex and the explanations can be found in Kosman (1996), where these parameters were denoted as *Ko* and *K*, respectively. All diversity and distance parameters were calculated using the bootstrap method across isolates (Efron & Tibshirani, 1993). Two hundred fictional samples for each population were created by the re-sampling process. Each fictional sample consisted of 100 isolates, which were drawn independently with a replacement from an original population. The values of all diversity indices and distances were calculated for each fictional sample and pair of fictional samples, respectively. The mean of the 200 values obtained for each index/distance was considered as the corresponding index of diversity within a population or distance between populations. Statistical significance of differences between these means was evaluated using the Student *t*-test at $\alpha = .05$. All diversity parameters were computed using the KOIND package (Kosman, 2002; Schachtel & Kosman, 2002). Mantel test correlations between the Kosman genetic distances (*KB*) and the geographical distances between locations were calculated using the MXCOMP programme of NTSYSpc package, version 2.1 (Exeter Software, Setauket, NY).

Results

Virulence frequency

2003 A differential set of 20 cultivars was used to test isolates (Table 1). No virulences were found

Table 5 Virulence spectra of 22 pathotypes of *Blumeria graminis* f.sp. *hordei* found in East China in 2003 and 2004

Pathotype	Virulence (+) of the pathotypes to resistance genes:					
	Mlk1	*Mlh*	*MlLa*	*Ml(Bw)*	*Mlra*	*Ml(Ru2)*
03				+	+	
05				+		+
06					+	+
07				+	+	+
17	+			+	+	+
23		+		+	+	
25		+		+		+
26		+			+	+
27		+		+	+	+
36	+	+			+	+
37	+	+		+	+	+
42			+		+	
43			+	+	+	
44			+			+
45			+	+		+
46			+		+	+
47			+	+	+	+
57	+		+	+	+	+
65		+	+	+		+
66		+	+		+	+
67		+	+	+	+	+
77	+	+	+	+	+	+

against the single resistance genes *Mla3*, *Mla9*, *Mla22*, *Mla23*, *Mlp1*, *Mlat* and *Ml(N81)* and against the combined resistance genes *Mla1*, *MlaAl2*; *Mla6*, *Mla14*; *Mla7*, *MlaNo3*; *Mla12*, *MlaEm2*; *Mla13*, *MlaRu3*; and *Mlg*, *MlCP*. In contrast, all isolates contained the virulence *a8* against the resistance gene *Mla8*. The virulence frequencies *k1*, *h*, *La*, *Bw*, *ra* and *Ru2* ranged from 7.2% to 98.7%.

2004 Thirteen of the differentials that did not differentiate the 2003 populations were replaced with 10 differentials with different resistance genes. Seven of the differentials were the same as used in 2003, making a total of 17 differentials (Table 1). No virulence was found against the single resistance gene *Mlmw* present in Mian Wali, and against the combined resistance genes *Mla10*, *MlaDu2* present in P09. In contrast, all isolates contained the virulences *a8* and *Ch* against resistance genes *Mla8* and *Ml*(*Ch*) in Pallas and Aibaiyang, respectively. The virulence frequencies detected on the same six differentials that were used in 2003, ranged from .4% to 98.7%, and the virulence frequencies detected on the new differentials A-222, Nakaizumi-zairai, Black Russian, West China, Galleon, Wong and P18 ranged from 0.9% to 97.3%.

2003 and 2004 In both years, the differentials P17, P24, P23, Borwina, P14 and P15 were used. Virulence frequencies *ra* and *Ru2* were practically identical in both years; virulence frequency *Bw* was similar whereas virulence frequencies *k1*, *h*

Table 6 Comparison of Chinese populations of *Blumeria graminis* f.sp. *hordei* found in 2003 and 2004 based on six differential lines

Parameter	Pu-03	Pu-04	Ha-03	Ha-04	Ju-03	Ju-04	Ya-03	Ya-04	2003	2004	Total
Number of isolates	61	55	59	55	62	56	54	59	236	225	461
Number of pathotypes	9	8	7	6	12	3	8	12	19	14	22
Number of pathotypes represented at least two isolates	3	4	5	6	9	2	5	7	11	8	15
Abundance = frequency of the predominant pathotype (%)	55.7	56.4	69.5	52.7	22.6	75.0	38.9	33.9	44.5	45.3	31.9
Average virulence complexity per isolate, *Ci*	3.36	4.36	3.20	3.56	4.08	4.75	3.20	3.90	3.41	4.14	3.77
Average of relative virulence complexity per isolate, *RCi*	.560	.727	.534	.594	.680	.792	.534	.650	.568	.690	.628
Richness (number of pathotypes/ number of isolates)	.148	.145	.119	.109	.194	.054	.148	.203	.081	.062	.048
Evenness (Sheldon index)	.576	.636	.555	.750	.838	.570	.728	.795	.644	.620	.626
Kosman's index of diversity, *KW*[a]	.190	.218	.103	.231	.348	.091	.253	.364	.256	.284	

Locations: Pu, Putian; Ha, Hangzhou; Ju, Jujing; Ya, Yancheng

[a] All *KW* diversity values for local populations in 2003 and 2004 are significantly different

and *La* differed markedly in both years. The isolates avirulent on *Mlra* were found at Yancheng only and in both years (Table 2). The isolates avirulent on *Ml*(*Ru2*) were not detected at Putian and were found at Hangzhou and Jujing in 2003 as well as at Yancheng in both years.

Eight populations In 2003, virulence *k1* was found in three of four populations (Table 2) at frequencies of 4.9–16.9%; in 2004 this virulence was only found at Putian (1.8%). At Yancheng, virulence *k1* was found neither in 2003 nor in 2004. Virulence *h* was detected at frequencies of 1.7–81.8% in all populations, except Yancheng in 2003, but it was found with a frequency of 54.2% at Yancheng in 2004. Virulence *La* was found in all the populations with the largest annual frequency difference between Jujing 2003 (38.7%) and 2004 (98.2%). Similarly, another three virulences, *Bw*, *ra* and *Ru2*, were detected in all the populations, and always at high frequencies. Out of seven virulences examined in 2004, two virulences were found at identical frequencies of 3.6%, but at only one of the four investigated locations, *Va11* at Jujing and *Vk2* at Hangzhou. Virulence *Ga* was not found at Jujing, whereas it was found at the other three locations with frequencies ranging from 60.0% to 72.9%. The other three virulences were found at all locations where the combined virulence *a2*, *aBR2* was detected at lower frequencies of 1.8–26.8%, while the virulences *Wo* and *nn* were found with frequencies of 56.4–80.0 and 94.5–100%, respectively.

Diversity parameters

Pathotypes found

A total of 22 pathotypes out of 64 possible pathotypes were found in both years, 19 in 2003 and 14 in 2004 (Table 5). One half, i.e. 11 pathotypes, was found in only 1 year; eight pathotypes in 2003 and three pathotypes in 2004. The pathotypes that were found in only 1 year were present at a low frequency and comprised only 25 isolates (=5.4%). In contrast, the six most frequent pathotypes were detected in both years and contained 86% and 95% of isolates in 2003 and 2004, respectively. In 2003, pathotype 07 (105 isolates) was the most abundant and the pathotype 67 (15 isolates) ranked fourth. In 2004, pathotype 67 was the most abundant (102 isolates), and the pathotype 07 was the second most abundant (42 isolates). In general, the most abundant pathotype 07 was found in all populations except at Jujing 2004. All eight populations contained pathotype 47 only (third most abundant). The highest numbers of pathotypes (12) were found in the populations Jujing 2003 and Yancheng 2004, whereas only three pathotypes were found in Jujing 2004, where pathotype 27 was represented by a single isolate. In each year, one isolate of pathotype 77 was found (both at Putian) with the highest possible virulence complexity (6). Conversely, five pathotypes showed the lowest detected virulence complexity (2). Some of those pathotypes were found in all populations, except at Jujing 2004.

Descriptive parameters of populations

In both years, 461 isolates were examined (Table 6). Isolate numbers from particular locations and for both years can be considered balanced. Pathotype numbers detected in individual populations ranged from 6 to 12, except at Jujing 2004, where only three pathotypes were found. A number of pathotypes with frequencies higher than one ranged from 2 (Jujing 2004) to 9 (Jujing 2003). Nine pathotypes were found in the population from Putian 2003, but only three had frequencies higher than one. The lowest abundance (relative frequency of the predominant pathotype) was recorded from Jujing 2003 (22.6%) and the highest one from Jujing 2004 (75.0%). Average virulence complexity per isolate ranged from 3.20 (Hangzhou 2003 and Yancheng 2003) to 4.75 (Jujing 2004), an average of 3.77 for all isolates. For the same number of differentials (6), similar averages of relative virulence complexity were calculated. Highest richness was found in the population Yancheng 2004, and the lowest in Jujing 2004. Total richness of all isolates was only .048. Highest evenness (.838) was found in the population Jujing 2003, and the lowest (.555) in the population Hangzhou 2003, which was even lower than that from Jujing 2004 (.570).

Table 7 Distance (*KB*, Kosman, 1996) between Chinese populations of *Blumeria graminis* f.sp. *hordei* in 2003 and 2004

Populations	Pu-04	Ha-03	Ha-04	Ju-03	Ju-04	Ya-03	Ya-04	2004
Pu-03	.191[h]	.092	.099[a]	.143[d]	.245	.077	.156[f]	
Pu-04		.268	.154[f]	.118[b]	.088	.200	.096	
Ha-03			.130[c]	.209	.313	.150[e]	.236	
Ha-04				.110	.194[h]	.150[e]	.118[b]	
Ju-03					.168[g]	.166[g]	.100[a]	
Ju-04						.257	.146[d]	
Ya-03							.132[c]	
2003								.153

Locations: Pu, Putian; Ha, Hangzhou; Ju, Jujing; Ya, Yancheng

All *KB* distance values between local populations in 2003 and 2004 are significantly different with the exception of eight identical pairs of distances, denoted a–h

Statistical characteristics in populations and between them

Populations Jujing 2004 (.091) and Hangzhou 2003 (.103) had the lowest diversity (Kosman index of diversity–*KW*), whereas Yancheng 2004 (.364) and Jujing 2003 (.348) had the highest population diversity (Table 6). The lowest Kosman distance (*KB*; Table 7) was found between populations in 2003 from the most distant locations—Putian and Yancheng (.077), whereas the largest distance (.313) was found between populations from the closest locations over several years (between Hangzhou 2003 and Jujing 2004). The lowest average distance between the given population and the other seven populations was shown by the population Hangzhou 2004 and the highest one by Jujing 2004. The least average distance was between populations from various locations over individual years (2004 = .133 and 2003 = .140), a slightly larger distance between populations from individual years at same locations (.155) and the largest distance between populations from various years and various locations (.188).

Significant negative correlations (Mantel test) were found between the Kosman genetic distances (KB) and the geographical distances between locations (–.903 and –.783 for 2003 and 2004 populations, respectively). On the other hand, the genetic distances between populations in 2003 and 2004 were not correlated.

Discussion

The Chinese population of *B. graminis* f.sp. *hordei* had not been studied previously and there were no available data on its virulence for selecting differentials. In 2003, 14 out of the 20 differential varieties with known resistance genes could not differentiate the population since no virulence was found on 13 of them and no avirulence was detected on cv. Pallas. In 2004, Pallas was retained for finding an isolate avirulent on *Mla8*. The other 13 non-differentiating cultivars were replaced by 10 differentials with other resistance genes to obtain more information on the virulence in the population. Three of these 10 cultivars did not differentiate within the population since there was no virulence on the resistance genes in P09 and in cv. Mian Wali, and no avirulence was detected in Aibaiyang (Dreiseitl & Yang, 2007), which carries the recently identified resistance gene *Ml*(*Ch*) (Dreiseitl, 2005a).

Significant changes in the local populations were found. The most interesting changes were in the frequency of virulence *h*, which was very low in 2003 in Putian and Hangzhou and absent in Yancheng, but frequently found in these populations in 2004, and a decrease in the number of pathotypes at Jujing from 12 in 2003 to three in 2004. The genetic resistance of current Chinese cultivars is not known, but since almost all cultivars grown are of Chinese origin, breeding for powdery mildew resistance started only recently, and regarding the fact that older Chinese varieties do not possess resistance genes (Dreiseitl & Yang,

2007) that could directly (directional selection) or indirectly (hitch-hiking) affect individual populations, then it is unlikely that the changes could be explained through selection of the local population responding to resistance genes in a particular cultivar. It is more likely that changes in local populations are based on possible founder effects followed by genetic drift of populations in individual fields, i.e. the changes would be favoured by a combination of a low cultivated area of the host and a marked seasonal reduction of local pathogen populations resulting from unfavourable environmental conditions such as long periods of high temperatures. The large genetic distance and changes between adjacent populations might also be influenced by these effects. Such changes in the genetic structure of local populations often occur and have also been recorded within locations in naturally-growing wild barley (Dreiseitl, Dinoor, & Kosman, 2006).

For studying aerial populations of *B. graminis* f.sp. *hordei*, a spore-sampler can be used to collect pathogen isolates by trapping conidia in the air (Hovmøller, Munk, & Østergård, 1995; Schwarzbach, 1979). A mobile spore-sampler is particularly useful for monitoring the aerial population on a larger scale since isolates can be collected randomly and continuously over great distances (Dreiseitl, 2004b; Wolfe et al., 1992), but it cannot monitor changes in local populations of individual fields. However, these local populations constitute the aerial population in a given area although their individual genetic structures can differ greatly from the average structure of the aerial population.

Populations of air-borne pathogens, of which *B. graminis* f.sp. *hordei* is typical, are carried in the air flow, i.e. in Europe usually from the west to east. During migration, the pathogen encounters many host cultivars, including those possessing specific resistance genes (Dreiseitl, 2002). The chance of surviving a passively transmitted pathogen population increases through its adaptation (Dreiseitl, 2004a; McDonald & Linde, 2002) when the spectrum of virulences against corresponding resistances is gradually enlarged, i.e. the population complexity increases by means of selection of individuals that can reproduce on cultivars possessing various host resistances.

Limpert et al. (2002) assumed that the virulence complexity of air-borne (nomadic) pathogenic populations should increase on their way across the Eurasian continent depending on the resistance of host cultivars and the distance travelled. The virulence of *B. graminis* f.sp. *hordei* is well-known in Europe (Dreiseitl, 2004b; Hovmøller et al., 2000; Wolfe et al., 1992), and it is likely that the European population has not yet reached East China. This can be deduced from the records of differences in virulence frequencies against the resistance genes, such as *Mlg*, *Mla6*, *Mla7*, *Mla12*, which were commonly used by European breeders (Brown & Jørgensen, 1991; Dreiseitl & Jørgensen, 2000; Dreiseitl, 2006), and where the frequency of corresponding virulences now exceeds 50%. For instance, the German barley cv. Union (*Mlg*) was registered in 1955 and was soon followed by other cultivars with *Mlg*. Therefore, the European population has contained a high frequency of the corresponding virulence for several decades (Brückner, 1963; Hovmøller et al., 2000). In our present study we have not found any virulence on these and other European resistance genes. We can only speculate whether the period of about 40 years is enough for the population to spread from Europe to East China, or whether the distance and the absence of the host in large areas between the two regions, is too great for aerial dispersal of the pathogen (Brown & Hovmøller, 2002).

In East China the European resistance genes were generally fully effective, although the high virulence frequency on the resistance gene *Ml*(*Bw*) (88.5%) was surprising. *Ml*(*Bw*) was first identified in the German winter barley cv. Borwina, from which its designation is derived (Dreiseitl, 1993; Dreiseitl & Jørgensen, 2000). Recently, Dreiseitl and Yang (2007) studied powdery mildew resistance in 147 older Chinese barley cultivars and, surprisingly, found *Ml*(*Bw*) in 47% of them. It explains the high virulence frequency against this gene in the Chinese population and, moreover suggests that *Ml*(*Bw*) in cv. Borwina might originate from this part of the world. It can also explain the presence of the virulence *Bw* in the old Japanese isolate known as

Race I (Hiura & Heta, 1955), which is still used by some laboratories, particularly for detecting the resistance gene *Mla8*.

The gene *Mla8* is present in many barley cultivars (Brown & Jørgensen, 1991; Dreiseitl, 2005b; Dreiseitl & Yang, 2007), but it does not contribute to their resistance since no isolate avirulent on this gene has been found except in Japan. Nevertheless, *Mla8* should be used in screening tests for postulating resistance genes in barley accessions. Such information is valuable for confirming the pedigrees of barley cultivars or their progenies and localizing powdery mildew resistance genes. In many cases, however, it is necessary to use an isolate with different virulence/avirulence combinations from Race I. Considering the geographical proximity of East China and Japan, we were expecting to find new isolates avirulent on *Mla8*, but none of the 461 isolates was avirulent on *Mla8*.

Complete effectiveness of the resistance genes in 13 differential cultivars, for which no corresponding virulences were found, allows Chinese breeders access to a wide range of modern European barley cultivars, which are fully resistant to powdery mildew in China, including those possessing the non-host resistance gene *mlo* (Humphry, Consonni, & Panstruga, 2006). Current knowledge and technology will enable these resistance sources to be used for pyramiding powdery mildew resistance genes and for introducing more durable resistance into new Chinese barley cultivars or cultivar mixtures. This can be accomplished without time-consuming searches for new resistance genes, their localization in the host genome and their transfer from wild ancestors into modern cultivars (Řepková et al., 2006).

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