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FULL RESEARCH PAPER

# Genetics of host-pathogen interactions in the *Pyrenophora teres* f. *teres* (net form) – barley (*Hordeum vulgare)* pathosystem

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**Abstract** The genetics of host-pathogen interactions in the *Hordeum vulgare* – *P. teres* f. *teres* pathosystem was studied in twelve resistant barley accessions, i.e. CI 9825, CI 9819, Diamond, CI 4922, CI 5401, Harbin, c-8755, c-21849, c-8721 c-23874, c-19979, c-15811. $F_2$ analyses of crosses with susceptible genotypes employing various isolates (from Europe, USA, Canada, and Australia) revealed that resistance is mostly isolate-specific and controlled by one or two genes. Segregation in ascospore progeny from two crosses between isolates of different origin revealed that avirulence in *P. teres* is also determined by one or two genes. An epistatic effect of suppressor genes on avirulence genes is proposed for the genetics of virulence to Diamond, Harbin, CI 5401 and c-8721 in the fungal crosses D (181-6 × A80) and F (H-22 × 92-178/9). Segregation in $F_2$ of crosses of three new sources of resistance (c-23874, c-19979, c-15811) to the susceptible cv. Pirkka was studied in laboratory and greenhouse tests by using seven *P. teres* isolates, i.e. 181-6, d8-3, d8-4, d9-1, d9-4, F4 and F74. In addition, virulence to these barley accessions of ascospore progeny from crosses of the same isolates was studied. Based on these studies it was concluded that depending on the isolate used, resistance of c-23874 is determined at least by two genes and in c-19979 and c-15811 by three genes. The results of this parallel analyses of genetics of resistance and genetics of virulence allows the postulation of a gene–for–gene interaction in the *P. teres* – *H. vulgare* pathosystem.

**Keywords** Barley (*Hordeum vulgare*) · Genetics · Net form of net blotch (*Pyrenophora teres* f. *teres*) · Resistance · Virulence

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## Introduction

Net form of net blotch, caused by the ascomycetous fungus, *Pyrenophora teres*. f. *teres* (anamorph *Drechslera teres*. f. *teres*) is one of the most important diseases of barley world-wide. Yield losses in susceptible cultivars can be up to 40–45% (Steffenson, Webster, & Jackson, 1991; Kashemirova, 1995). The most effective means of controlling net blotch is the use of resistant cultivars. One of the first studies on the genetic control of resistance to *P. teres* in barley was conducted in 1955 in the USA (Schaller, 1955).

Since this time, many different sources of race-specific resistance (Mode & Schaller, 1958; Khan & Boyd, 1969; Bockelman, Sharp, & Eslick, 1977; Weiland, Steffenson, Cartwright, & Webster, 1998; Afanasenko, Makarova, & Zubkovich, 1999a) and race non-specific resistance (Arabi, Sarrafi, Barrault, & Albertini, 1990; Harabi, Cherif, & Slama, 1993) have been identified. In addition to these studies on the host, *P. teres* was characterised with respect to virulence on barley (Tekauz, 1990; Afanasenko, 2001). Significant differences in the virulence phenotypes of *P. teres* populations derived from different geographic regions were detected (Afanasenko, Hartleb, Guseva, Minarikova, & Janosheva, 1995).

Several previous genetic studies have revealed that resistance is dependent on the pathogen isolate used (Ho, Tekauz, Choo, & Martin, 1996; Afanasenko et al., 1999a; Afanasenko, Zubkovich, & Makarova, 1999b). For example, in the analysis of 27 barley accessions, one or two genes were determined to be effective against several *P. teres* isolates, but in addition, isolate-specific resistance genes were discovered (Afanasenko, 1996). Weiland et al. (1998) demonstrated the interaction between a dominant major resistance gene in the barley cv. Harbin and its corresponding dominant major avirulence gene in *P. teres*. As current information on the genetics of the barley - *P. teres* interaction is quite limited, the aim of our study was to investigate the genetics of the host-pathogen interaction in the *H. vulgare* – *P. teres* (net form of net blotch) pathosystem using a broad spectrum of genotypes and isolates.

## Materials and methods

### Plant material

The characteristics of a set of twelve resistant and seven susceptible barley accessions selected for this study are shown in Table 1. The barley genotypes resistant to *P. teres* (net form of net blotch) were selected from the collection of the All-Russian Research Institute for Plant Protection because in preliminary studies, they have shown a differential interaction with *P. teres* isolates of various origins. Crosses were made between these twelve resistant accessions and the susceptible cvs Pirkka, Zazerski 85, Vezha, Gastinets, Nadia, Belogorski, and Krasnoufimski 95 chosen because of complete susceptibility to many isolates. The $F_2$ population was derived from five $F_1$ plants for each cross. In four cross combinations (CI 9825 × Pirkka, CI 4922 × Zazerski 85, Harbin × Pirkka and c-8755 × Pirkka) additional studies of $F_3$ seedlings were conducted. The inheritance of resistance in CI 9825 and Diamond (Afanasenko et al., 1999a, b), and CI 9819 (Afanasenko et al., 1999a, b; Afanasenko, Manninen, & Terentieva, 2004; Manninen, Kalendar, Robinson, & Schulman, 2000) to different *P. teres* isolates has been reported previously.

### *P. teres* isolates

Isolates for studies on the genetics of resistance of barley were chosen according to their origin

**Table 1** Origin of barley cultivars and accessions

| Name CI or VIR* catalogue number (c) | Subtaxa or subspecies of the species *Hordeum vulgare* | Origin |
|---|---|---|
| Resistant accessions | | |
| CI 9825** | *deficiens* | Ethiopia |
| CI 9819** | *deficiens* | Ethiopia |
| Diamond | *rikotense* | Canada |
| CI 4922 | *pallidum* | USA |
| Harbin** | *pallidum* | Manchuria |
| CI 5401 | *rikotense* | Canada |
| c-8755** | *nudum* | Ethiopia |
| c-21849 | *stendelii* | Ethiopia |
| c-8721 | *stendelii* | Ethiopia |
| c-23874 | *parallelum* | Ethiopia |
| c-19979 | *brunneinudum* | Ethiopia |
| c-15811 | *pallidum* | China |
| Susceptible cultivars | | |
| Pirkka | *pallidum* | Finland |
| Nadia | *nutans* | Germany |
| Krasnoufimski 95 | *nutans* | Russia |
| Zazerski 85 | *nutans* | Belarus |
| Gastsinets | *nutans* | Belarus |
| Vezha | *pallidum* | Belarus |

* = CI – cereal invistigation number; c – number of catalogue of All Russian Plant Industry Institute, named by N.I. Vavilov

** = barley accessions from the sets of differentials (Afanasenko et al., 1995)

(different parts of the world) and their contrasting reaction to the different barley genotypes included in this study. The origin of the *P. teres* isolates is shown in Table 2.

Single-conidia isolates of *P. teres* were grown on CLM medium (modified Chapek's medium with lactose and urea) containing (g $l^{-1}$): 0.5 $KH_2PO_4$, 0.5 $MgSO_4$, 0.5 KCl, 1.2 urea, 20 lactose, and 20 agar. After 10 days at 20–22°C under constant illumination with a daylight lamp (3000 lux) the cultures were flooded with distilled water containing 0.01% Tween 20 surfactant and conidia were dislodged with a sterile spatula. The spore concentration was determined by haemocytometer analyses and adjusted to 5,000 conidia $ml^{-1}$.

## Inoculation and evaluation

*Laboratory test.* The virulence of *P. teres* isolates was assayed on detached barley leaves (Afanasenko et al., 1995). In order to avoid environmental effects, barley plants were grown under constant conditions on water-soaked cotton in enamelled trays for 8–10 days at 20–22°C with an alternating 12 h period of light and darkness. For studying the genetics of resistance, the first leaf of each $F_2$ seedling from the crosses of resistant with susceptible cultivars was cut into 3–4 segments (1.5–2.0 cm) and placed in a vertical line on filter paper soaked in 0.004% benzimidazole in enamelled trays (30 × 20 cm, Fig. 1). Each vertical column of three or four leaf segments represents one plant. Single leaf segments of each $F_2$-plant were inoculated by 0.05 ml conidial suspension (concentration 5000 conidia $ml^{-1}$) of different isolates. This procedure facilitates the simultaneous estimation of the reaction to different isolates for each $F_2$ plant.

The same procedure was used for studies on the genetics of virulence: groups of 10 sections (1.5–2.0 cm) of different leaves of each resistant barley genotype were placed in a vertical line in one tray. A vertical line of 16–18 groups of five leaf segments represented one variety, Eight barley genotypes in each tray were inoculated with 16–18 single ascospore isolates in three replications (each replication in a different tray). After inoculation, trays were covered with glass and maintained for four days at 20–22°C with an alternating 12 h period of light and darkness. Subsequently, leaf segments were rated for reaction to *P. teres* isolates using the 1 to 4 lesion type rating scale developed for laboratory tests (Afanasenko, 1977): 1 = brown pinpoint lesions, no chlorosis (highly resistant); 2 = brown necrotic lesions restricted to the diameter of the inoculum drop, no or slight chlorosis (resistant); 2–3 = necrotic lesions not restricted to the diameter of the inoculum drop partially occupying the leaf surface, no or slight

**Table 2** Origin of *P. teres* isolates used in genetic studies of barley resistance and pathogen virulence

| Isolates | Origin | Source |
|---|---|---|
| L1, 23, 181-6, P1 | Russia, St. Petersburg region | O. Afanasenko |
| Ch1 | Russia, South Ural | U. Kushnirenko |
| 1 | Russia, West Siberia | O. Afanasenko |
| H-22, 8ax | Russia, Far East | O. Afanasenko |
| 9, 30 | Moldova | O. Afanasenko |
| 10 | Ukraine | O. Afanasenko |
| UK 80-12 | United Kingdom | V.W.L. Jordan via B. J. Steffenson |
| 27–36 | Australia | R. Loughman |
| Zh3, zh4, zh12, Km4 | Belorussia | O. Afanasenko |
| 92–128, 92–46, 92-178/9 | Canada | A. Tekauz |
| 84-28-1, 85-53-1 | USA | B. J. Steffenson |
| V12 | Czech Republic | V. Minaricova |
| A80 | Single ascospore isolate from cross 181-6 × 8ax (A) | N. Mironenko |
| F4 | Single ascospore isolate from cross H-22 × 92178/9 (F) | N. Mironenko |
| F74 | Single ascospore isolate from cross H-22 × 92178/9 (F) | N. Mironenko |
| d8-3, d8-4, d9-1, d9-4 | Single ascospore isolates from cross 181-6 × A80 (D) | N. Mironenko |


**Fig. 1** Method for studying the segregation of $F_2$ populations for resistance to different isolates of *P. teres*. Each vertical column represents a single $F_2$ plant tested to different isolates (zh12, 27-36, 85-53-1, UK80-12)

chlorosis (intermediate reaction type, moderately resistant); 3 = brown necrosis developed all over the leaf surface surrounded by chlorotic regions (susceptible); 4 = necrotic lesions occupying the entire surface of the leaf segment with chlorosis (highly susceptible) (Fig. 2). Plants in $F_2$ populations showing an intermediate type of reaction were in general classified as heterozygous. In the virulence tests of the ascospore isolates the intermediate type of reaction was not included.

In each experiment the cv. Pirkka was included as a susceptible control and each 10 th leaf segment of each barley genotype was inoculated with a mixture of water and Tween 20 without conidia. For determining the virulence of the ascospore isolates on each barley cultivar, three replications were used.

## Greenhouse test

In order to support the data of the laboratory tests, the studies on the genetics of the barley – *P. teres* interaction were also conducted on intact plants in the greenhouse. Three hundred and fifty seedlings of the $F_2$ population of the cross c-15811 (resistant) × Pirkka (susceptible) were sprayed at the two-leaf stage with 50 ml of the conidial suspension of the parental isolates F4 and F74.

For determining the parental plant response to 36 ascospore isolates, five plants of each parental line (c-15811 and Pirkka) grown in one pot (12 cm diameter) were inoculated with one

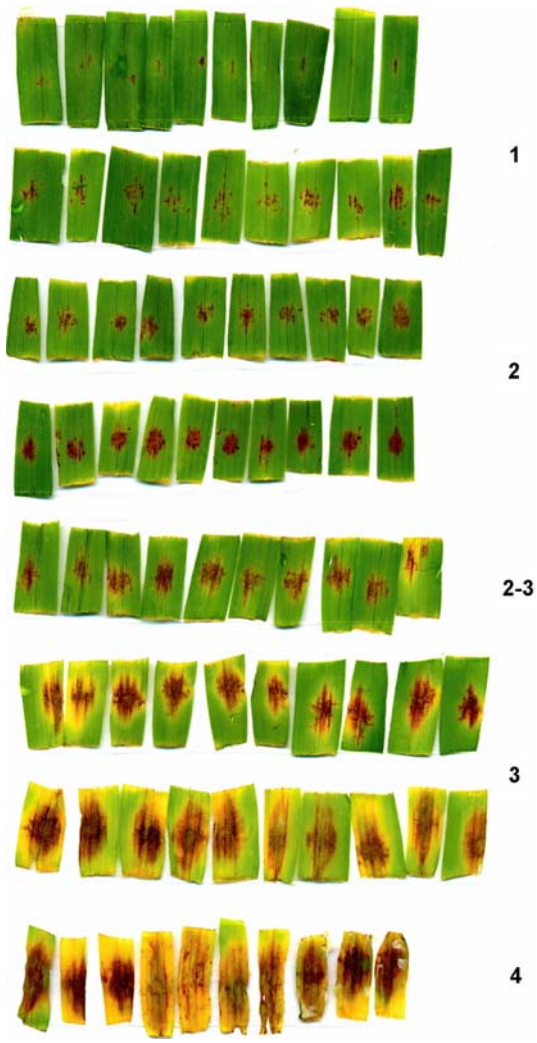


**Fig. 2** Scale (1–4) for the evaluation of the reaction of barley to *Pyrenophora teres* f. *teres*

ascospore isolate. Inoculated plants were incubated in a moist chamber for 40 h at 24°C and then cultivated at 22–24°C with a 12 h photoperiod. The reaction of the barley accessions was recorded 10–12 days after inoculation. The central part of the second leaves was scored using the scale of Tekauz (1985). Infection responses (IRs) 0, 1, 2, 3 were classified as resistant and IRs 7, 8, 9 as susceptible. IRs 4, 5, 6 were rated as intermediate types. In contrast to the laboratory test, IR 0 could be detected in the greenhouse probably because of the limited period (40 h) of 100% relative humidity.

### Crosses of *P. teres* isolates

The method used for crossing *P. teres* isolates was described by McDonald (1963). Briefly, mixtures of conidia and mycelia of isolates with different mating types were paired on autoclaved lemon leaves and placed on moist filter paper in Petri dishes at 15°C and a 12 h photoperiod for two months. The mating type was determined by randomly pairing *P. teres* isolates of different origin. In successful crosses, mature pseudothecia with asci and ascospores were detected after 60–120 days. Only seven out of 120 cross combinations (5.5%) yielded mature pseudothecia. Unfortunately, attempts to generate crosses between *P. teres* isolates used in previous genetic analyses (Afanasenko, 1996; Afanasenko et al., 1999a, b) were not successful. In genetic analysis of virulence, only those crosses were used for which a significant number of ascospore isolates was obtained, i.e. H22 × 92-17879; 181-6 × A80; 181-6 × 8ax. In addition, backcrosses and sibcrosses were used in order to support the hypothesis of segregation in fungal crosses.

### Collection of ascospore isolates

Petri dishes with mature pseudothecia were placed in refrigerators at 2–4°C for 30 min and then covered by a lid with a thin layer of 3% water agar and placed under a lamp at 25–28°C. Within about 30 min ascospores were ejected from asci within the mature pseudothecia onto the lid of the Petri dishes. Individual ascospores were transferred to CLM media. In some cases, octads and tetrads of ascospores were collected from Petri dish lids. Fungal cultures (mixture of conidia and mycelium) were stored on sterile filter paper in plastic packages with silica gel at 10°C.

### Data analysis

A $\chi^2$ test was used to test the goodness of fit of the observed and expected segregation ratios in segregating populations of barley and *P. teres*. The analysis of segregation in the laboratory test allowed us to compare the reaction of each $F_2$ plant to different isolates of *P. teres* within one experiment (see above). This approach offers the opportunity to determine the genes effective against specific isolates and to verify the hypothesis of the presence of genes effective against many isolates. Theoretical ratios of $F_2$ plants resistant (r/r) and susceptible (s/s) to both isolates as well as the number of plants resistant to the first and susceptible to the second isolate (r/s), and susceptible to the first and resistant to the second isolate (s/r) were determined by using a Punnet square table and taking into account the number of effective genes against each isolate (Table 3), resulting in four classes (r/r, s/s, r/s, and s/r) for the $\chi^2$ test.

## Results

### Inheritance of resistance and virulence

#### CI 9825

The mode of inheritance of resistance detected was isolate-specific (Table 4 and 5). Segregation ratios observed indicate that the resistance of barley accession CI 9825 to isolates 84–28-1 and p1 is controlled by one dominant gene, to isolates UK 80-12, Km4, zh23 and ch1 by one dominant or one dominant and one recessive gene, to isolate 85-53-1 by two dominant genes, and to isolate v12 by one dominant and one recessive gene. Due to the fact that comparative studies of the reaction of the same $F_2$ plants to two isolates could only be conducted on a limited scale, results can only be given for some isolates (Table 5). One dominant

**Table 3** Expected frequencies of plants in $F_2$ populations from crosses between resistant and susceptible barley cultivars with different resistance genes and reaction to specific isolates

| Isolates | Observed segregation corresponding to r:s | Presumed genotype of resistance | Expected frequencies of plants resistant (r) and susceptible (s) to isolates 1 and 2 | | | |
|---|---|---|---|---|---|---|
| | | | r/r | s/s | r/s | s/r |
| 1 | 3:1 | AA | 12 | 1 | 0 | 3 |
| 2 | 15:1 | AABB | | | | |
| 1 | 3:1 | AA | 12 | 3 | 0 | 1 |
| 2 | 13:3 | AAbb | | | | |
| 1 | 3:1 | AA | 9 | 1 | 3 | 3 |
| 2 | 3:1 | BB | | | | |
| 1 | 13:3 | AAbb | 49 | 9 | 3 | 3 |
| 2 | 13:3 | AAcc | | | | |
| 1 | 13:3 | AAbb | 43 | 3 | 9 | 9 |
| 2 | 13:3 | CCbb | | | | |
| 1 | 13:3 | AAbb | 36 | 12 | 16 | 0 |
| 2 | 9:7 | AACC | | | | |
| 1 | 13:3 | AAbb | 51 | 3 | 1 | 9 |
| 2 | 15:1 | AACC | | | | |
| 1 | 13:3 | AAbb | 25 | 9 | 27 | 3 |
| 2 | 7:9 | ccbb | | | | |

gene A was effective against isolates 85-53-1, v12 and 84-28-1 (Table 5). Therefore, it is proposed that in this accession at least two dominant and one recessive gene(s) controlled resistance to the isolates investigated.

As mentioned before, crosses of isolates which were used in the genetic analysis of resistance were not successful. Therefore, we can only speculate about the complement of avirulence genes to the resistance genes detected. Data given in Table 6 demonstrate that four genes for avirulence were found in two isolates avirulent to CI 9825 in cross combination F (H-22 × 92-178/9) and two genes in cross combination D (181-6 × A80).

CI 9819

In CI 9819, one dominant or one dominant and one recessive gene, controlled resistance to isolates 27–36, v12, zh3 and zh12 and two dominant genes were found to control resistance against isolates Km4, 85-53-1 and ch1. Using the same pairwise comparison it appears that at least two dominant and one recessive gene(s) are present in this accession. Data on segregation in the meiotic progeny of cross D (Table 6) (58avr:26vir = 3:1) corresponded to two avirulence genes in the first parental isolate; in cross F segregation in the progeny of crosses of two avirulent isolates correspond to four avirulence genes (82avr:5 vir = 15:1).

Diamond

Segregation of resistance to six isolates in the $F_2$ of the crosses Vezha × Diamond and Gastsinets × Diamond were the same corresponding to one dominant and two recessive resistance genes (Table 4). Pairwise comparative studies revealed that beside resistance gene A effective against all isolates, additionally two recessive genes were present, one effective against isolates v12 and L1 and the other against 92–128 (Tables 4; 5). The segregation (54avr:30vir) in the meiotic progeny of cross D (avirulent × virulent) can be interpreted as 5:3 (two avirulence genes and 1 suppressor gene) or 9:7 corresponding to two avirulence and two suppressor genes (Table 6).

CI 4922

In our studies of $F_2$ and $F_3$ populations two genes were found controlling the resistance of CI 4922 to a Russian isolate L1 (Table 4). Segregation data in meiotic progeny of cross D also demonstrated the presence of two avirulence genes to CI 4922. At the same time the segregation data of cross F demonstrated the control of avirulence to this accession by one gene (Table 6).

Harbin

One resistance gene was identified in the Harbin × Pirkka cross to the three investigated isolates (23, 1, 5), and two genes were detected as being effective against isolates 9 (Moldova) and 10 (Ukraine) in the cross Harbin × Nadia (Table 4). Segregation of virulence in two crosses of avirulent and virulent isolates A (26avr:17-vir = 1:1) and D (39avr:25vir = 1:1) correspond to one avirulence gene in Harbin. The data of segregation on virulence to Harbin in cross F (20avr: 70vir = 1:3) can be explained by the assumption of parental isolates carrying one

**Table 4** Results of $F_2$ segregation analyses of crosses between resistant (printed in bold) and susceptible barley genotypes

| Cross combination | Isolate | Segregation of resistance (R:S) | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| | | Experimental ratio | Theoretical ratio | | |
| **CI 9825** × | | | | | |
| Vezha | UK 80–12 | 52:17 | 3:1 | 0.00 | 0.90–0.95 |
| | | | 13:3 | 1.53 | 0.10–0.25 |
| | Km4 | 66:18 | 3:1 | 0.56 | 0.25–0.50 |
| | | | 13:3 | 0.39 | 0.50–0.75 |
| | 85–53-1 | 89:6 | 15:1 | 0.00 | 0.90–0.95 |
| | v12 | 83:12 | 13:3 | 2.33 | 0.10–0.25 |
| | 84-28-1 | 49:24 | 3:1 | 2.36 | 0.10–0.25 |
| | | | 13:3 | 0.29 | 0.50–0.75 |
| Pirkka | 23 | 117:28 | 3:1 | 2.50 | 0.10–0.25 |
| | | | 13:3 | 0.02 | 0.90 |
| | p1 | 75:27 | 3:1 | 0.12 | 0.50–0.75 |
| | p1 ($F_3$) | 17:3 | 3:1 | 1.06 | 0.25–0.50 |
| Krasnoufimski 95 | ch1 | 104:28 | 3:1 | 1.01 | 0.25–0.50 |
| | | | 13:3 | 0.51 | 0.25–0.50 |
| **CI 9819** × | | | | | |
| Vezha | 27–36 | 85:24 | 3: 1 | 0.52 | 0.25–0.50 |
| | | | 13:3 | 0.72 | 0.25–0.50 |
| | Km4 | 50:5 | 13:3 | 2.40 | 0.10–0.25 |
| | | | 15:1 | 0.76 | 0.25–0.50 |
| | v12 | 39:10 | 3:1 | 0.55 | 0.25–0.50 |
| | | | 13:3 | 0.09 | 0.75–0.90 |
| | zh3 | 97:31 | 3:1 | 0.04 | 0.75–0.90 |
| | | | 13:3 | 2.51 | 0.10–0.25 |
| Gastsinets | UK 80-12 | 87:94 | 7:9 | 1.36 | 0.10–0.25 |
| | zh12 | 139:42 | 3:1 | 0.30 | 0.50–0.75 |
| | | | 13:3 | 1.96 | 0.10–0.25 |
| | 85–53-1 | 174: 6 | 15:1 | 2.61 | 0.10–0.25 |
| | 27–36 | 65:13 | 3:1 | 2.88 | 0.05–0.10 |
| | | | 13:3 | 0.22 | 0.50–0.75 |
| Krasnoufimski 95 | ch1 | 223:23 | 15:1 | 2.19 | 0.10–0.25 |
| **Diamond** × | | | | | |
| Vezha | 92–128 | 134:23 | 13:3 | 1.72 | 0.10–0.25 |
| | v12 | 67:19 | 3:1 | 0.38 | 0.50–0.75 |
| | | | 13:3 | 0.63 | 0.25–0.50 |
| | 85–53-1 | 73:16 | 3:1 | 2.34 | 0.10–0.25 |
| | | | 13:3 | 0.03 | 0.75–0.90 |
| | L1 | 51:14 | 3:1 | 0.41 | 0.50–0.75 |
| | | | 13:3 | 0.33 | 0.50–0.75 |
| Gastsinets | zh3 | 34:14 | 3:1 | 0.44 | 0.50–0.75 |
| | | | 13:3 | 3.42 | 0.05–0.10 |
| | UK 80-12 | 52:21 | 3:1 | 0.54 | 0.25–0.50 |
| **CI 4922** × | | | | | |
| Zazerski 85 | L1 | 140:6 | 15:1 | 1.14 | 0.25–0.50 |
| | L1 (F3) | 27:2 | 15:1 | 0.01 | 0.90–0.95 |
| **Harbin** × | | | | | |
| Pirkka | 23 | 160:56 | 3:1 | 0.09 | 0.75–0.90 |
| | 1 (F3) | 66:14 | 3:1 | 2.40 | 0.10–0.25 |
| | 5 | 117:50 | 3:1 | 2.20 | 0.10–0.25 |
| Nadia | 10 | 108:6 | 15:1 | 0.22 | 0.50–0.75 |
| | 9 | 103:9 | 15:1 | 0.60 | 0.25–0.50 |
| **CI 5401** × | | | | | |
| Zazerski 85 | zh4 | 145:23 | 13:3 | 2.80 | 0.05–0.10 |
| | 92–46 | 97:73 | 9:7 | 0.04 | 0.75–0.90 |

**Table 4** continued

| Cross combination | Isolate | Segregation of resistance (R:S) | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| | | Experimental ratio | Theoretical ratio | | |
| **c-8755** × | | | | | |
| Pirkka | 23 | 152:48 | 3:1 | 0.11 | 0.50–0.75 |
| | ch1 (F3) | 31:9 | 3:1 | 0.13 | 0.50–0.75 |
| **c-21849** × | | | | | |
| Belogorski | P1 (F2 adult plants) | 156:46 | 3:1 | 0.53 | 0.25–0.50 |
| **c-8721** × | | | | | |
| Krasnoufimski 95 | ch1 | 124:43 | 3:1 | 0.05 | 0.75–0.90 |

avirulence gene and one suppressor gene or by the complementation of two avirulence genes.

CI 5401

Resistance of CI 5401 to the Canadian isolate 92–46 is controlled by two complementary genes (97R:73S = 9:7), and by one recessive and one dominant gene to the Belarus isolate zh4 (145R:23S = 13:3) (Table 4). Pairwise comparative studies revealed that resistance gene A is effective against both isolates (Table 5). One hypothesis explaining the segregation in meiotic progeny of cross F (18avr:65vir = 1:3) is the presence of two complementary avirulence genes (Table 6). However, this segregation can also be explained by the presence of one avirulence gene and one suppressor gene. In cross D

**Table 5** Results of $F_2$ segregation analyses based on the inoculation of the same $F_2$ plant with different *P. teres* isolates

| Cross combination | Isolates | Genotype of resistance | Ratio of reaction types of the same plants in $F_2$ population to two isolates in the case of common resistance genes | | | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| | | | r/r | s/s | r/s | s/r | | |
| CI 9825x Vezha | 85-53-1 | AABB | 80 | 3 | 3 | 8 | 4.43 | 0.10–.25 |
| | v12 | AAdd | 51 | 3 | 1 | 9 | | |
| | 85-53-1 | AABB | 47 | 5 | 0 | 19 | 3.25 | 0.25–.50 |
| | 84-28-1 | AA | 12 | 1 | 0 | 3 | | |
| | To all isolates | AABBdd | | | | | | |
| Vezha × CI 9819 | Km4 | AABB | 43 | 3 | 0 | 4 | 3.88 | 0.25–.50 |
| | 27–36 | AA | 12 | 1 | 0 | 3 | | |
| | Km4 | AABB | 43 | 3 | 0 | 4 | 3.96 | 0.25–.50 |
| | 27–36 | AAcc | 51 | 3 | 1 | 9 | | |
| Gastsinets × CI 9819 | Zh12 | BBcc | 38 | 11 | 4 | 36 | 1.39 | 0.50–.75 |
| | UK80-12 | DDcc | 25 | 9 | 27 | 3 | | |
| | Zh12 | BBcc | 56 | 2 | 11 | 10 | 1.09 | 0.75–.90 |
| | 27–36 | AAcc | 43 | 3 | 9 | 9 | | |
| | Zh12 | BBcc | 72 | 1 | 2 | 14 | 2.87 | 0.25–.50 |
| | 85-53-1 | AABB | 51 | 3 | 1 | 9 | | |
| | To all isolates | AABBDDcc | | | | | | |
| Diamond × Vezha | 85-53-1 | AA | 64 | 14 | 0 | 4 | 0.47 | 0.75–.90 |
| | v12 | AAbb | 12 | 3 | 0 | 1 | | |
| Vezha × Diamond | L1 | AAbb | 38 | 4 | 5 | 4 | 5.30 | 0.10–.25 |
| | 92–128 | AAcc | 49 | 9 | 3 | 3 | | |
| | To all isolates | AAbbcc | | | | | | |
| Zazerski 85 × CI 5401 | Zh4 | AAbb | 100 | 23 | 47 | 0 | 3.14 | 0.25–.50 |
| | 92-46 | AACC | 36 | 12 | 16 | 0 | | |
| | To all isolates | AACCbb | | | | | | |

**Table 6** Results of the genetic analyses of virulence of *P. teres* to different barley genotypes

| Barley accessions | Fungal cross combination | Segregation on virulence (avirulent:virulent) | | $\chi^2$ | *P* value | Proposed number of avirulence genes (Avr) and suppressor genes (Su) |
|---|---|---|---|---|---|---|
| | | Experimental | Theoretical | | | |
| CI 9825 | D (a × a) | 41:19 | 3:1 | 1.42 | 0.10–0.25 | 2 Avr |
| | F (a × a) | 82:2 | 15:1 | 2.43 | 0.10–0.25 | 4 Avr |
| | FS2 (a × a) | 20:7 | 3:1 | 0.01 | 0.90–0.95 | 2 Avr |
| CI 9819 | D (a × v) | 58:26 | 3:1 | 1.59 | 0.10–0.25 | 2 Avr |
| | F (a × a) | 82:5 | 15:1 | 0.03 | 0.75–0.90 | 4 Avr |
| Diamond | D (a × v) | 54:30 | 5:3 | 0.11 | 0.50–0.75 | 2 Avr + 1 Su |
| | | | 9:7 | 2.20 | 0.10–0.25 | 2 Avr + 2 Su |
| | F (v × a) | 32:34 | 1:1 | 0.06 | 0.75–0.90 | 1 Avr |
| CI 4922 | D (v × a) | 56:23 | 3:1 | 0.71 | 0.75–0.90 | 2 Avr |
| | F (a × v) | 40:25 | 1:1 | 3.46 | 0.05–0.10 | 1 Avr |
| Harbin | A (v × a) | 26:17 | 1:1 | 1.88 | 0.10–0.25 | 1 Avr |
| | D (v × a) | 39:25 | 1:1 | 3.06 | 0.05–0.10 | 1 Avr |
| | F (v × a) | 20:70 | 1:3 | 0.38 | 0.25–0.50 | 1 Avr + 1 Su; 2 Avr – complementation |
| CI 5401 | D (a × v) | 69:15 | 3:1 | 2.28 | 0.10–0.25 | 2 Avr |
| | | | 13:3 | 0.04 | 0.75–0.90 | 3 Avr + 1 Su |
| | F (v × a) | 18:65 | 1:3 | 0.48 | 0.25–0.50 | 1 Avr + 1 Su; 2 Avr – complementation |
| c-8755 | D (a × a) | 79:5 | 15:1 | 0.36 | 0.50–0.75 | 4 Avr |
| | | | 7:1 | 3.29 | 0.05–0.10 | 3 Avr |
| | F (a × a) | 78:10 | 7:1 | 0.09 | 0.75–0.90 | 3 Avr |
| c-21849 | D (a × a) | 63:21 | 3:1 | 0.00 | 0.95 | 2 Avr |
| | F (a × a) | 87:2 | 15:1 | 2.43 | 0.10–0.25 | 4 Avr |
| c-8721 | D (v × a) | 48:36 | 9:7 | 0.03 | 0.75–0.90 | 2 Avr + 2 Su |
| | F (v × a) | 40:39 | 1:1 | 0.01 | 0.90–0.95 | 1 Avr |

the observed segregation (69avr:15vir = 3:1) suggests two avirulence genes or (13:3) three avirulence genes and one suppressor gene.

c-8755, c-21849, and c-8721

In the segregation analysis of resistance of barley accessions c-8755, c-21849 and c-8721, we were not able to compare the types of reaction of the same plants to different isolates. Resistance of these barley accessions was found to be monogenic to all investigated isolates (Table 4). However, out of the segregation in ascospore populations only the segregation in cross F (40avr:39vir) to c 8721 corresponded to a one gene ratio for avirulence. In other crosses two, three and four avirulence genes were found (Table 6).

Genetics of host-pathogen interaction

In order to study the genetics of the host-pathogen interaction, genetic analyses on resistance of barley and virulence of *P. teres* were conducted using the same isolates and the same barley accessions. Segregation in $F_2$ from crosses of three new sources of resistance with the susceptible cv. Pirkka was studied by using five *P. teres* isolates, and also ascospore progeny from crosses of the same isolates. Results are presented in Table 7.

Segregation of resistant and susceptible plants for c-23874 to all investigated isolates corresponded to a one gene ratio. Also the segregation in the meiotic progeny of the fungus corresponded to a two gene ratio for avirulence – one in each isolate, because both isolates are avirulent to c-23874. Obviously, the same gene controls resistance to isolates d8-3, d8-4 (from one octade) and d9-1 as indicated by the segregation detected in $F_2$. The same holds true for d9-4 and 181-6. However, segregation in cross d9-1 × d9-4 demonstrated that two different genes determine avirulence in these isolates. Also different genes controlled avirulence in crosses 181-6 × d8-3, 181-6 × d8-4 and 181-6 × d9-4.

**Table 7** Genetics of host-pathogen interaction in the pathosystem *P. teres* – *H. vulgare*

| Genetics of resistance of three barley accessions to different *P. teres* isolates | | | | | | Genetics of *P. teres* virulence to three barley accessions | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Barley cross combination | Isolate | Segregation ratio (R:S) | | $\chi 2$ | *P* value | Fungal cross combination | Barley accession | Segregation ratio (R:S) | | $\chi 2$ | *P* value |
| | | Observed | Expected | | | | | Observed | Expected | | |
| c-23874 × Pirkka | d 8-3 | 61:29 | 3:1 | 2.40 | 0.10–0.25 | d9-1 × d9-4 | c-23874 | 21:6 | 3:1 | 0.10 | 0.75 |
| | d 8-4 | 60:30 | 3:1 | 3.30 | 0.05–0.10 | 181-6 × d8-4 | | 15:5 | 3:1 | 0.00 | 0.90–0.95 |
| | d 9-1 | 60:30 | 3:1 | 3.30 | 0.05–0.10 | 181-6 × d8-3 | | 11:4 | 3:1 | 0.01 | 0.90–0.95 |
| | d 9-4 | 73:17 | 3:1 | 1.78 | 0.10–0.25 | | | | | | |
| | 181-6 | 75:15 | 3:1 | 3.30 | 0.05–0.10 | | | | | | |
| c-19979 × Pirkka | d 8-3 | 50:30 | 3:1 | 1.21 | 0.25–0.50 | d9-1 × d9-4 | c-19979 | 23:4 | 3:1 | 1.49 | 0.10–0.25 |
| | d 8-4 | 63:17 | 3:1 | 2.20 | 0.10–0.25 | 181-6 × d9-4 | | 18:2 | 7:1 | 0.11 | 0.50–0.75 |
| | | | 13:3 | 0.32 | 0.50–0.75 | | | | | | |
| | d 9-1 | 66:14 | 3:1 | 1.55 | 0.10–0.25 | 181-6 × d8-3 | | 13:2 | 7:1 | 0.00 | 0.90–0.95 |
| | d 9-4 | 56:24 | 3:1 | 1.20 | 0.25–0.50 | | | | | | |
| | 181-6 | 59:21 | 13:3 | 0.01 | 0.90–0.95 | | | | | | |
| c-15811 × Pirkka | d 8-3 | 169:64 | 3:1 | 0.94 | 0.25–0.50 | d9-1 × d9-4 | c-15811 | 23:4 | 3:1 | 1.42 | 0.10–0.25 |
| | | | | | | | | | 7:1 | 0.12 | 0.50–0.75 |
| | d 9-1 | 183:50 | 3:1 | 1.55 | 0.10–0.25 | 181-6 × d9-4 | | 16:4 | 3:1 | 0.26 | 0.50–0.75 |
| | | | 13:3 | 1.20 | 0.25–0.50 | | | | 7:1 | 0.22 | 0.50–0.75 |
| | d 9-4 | 178:55 | 3:1 | 3.64 | 0.05–0.10 | 181-6 × d8-3 | | 11:4 | 3:1 | 0.02 | 0.90 |
| | | | 13:3 | 0.26 | 0.50–0.75 | | | | 7:1 | 2.76 | 0.05–0.10 |
| | 181-6 | 200:33 | 13:3 | 3.26 | 0.05–0.10 | | | | | | |
| c-15811 × Pirkka Greenhouse test | F4 | 233:90 | 3:1 | 1.35 | 0.10–0.25 | F4 × F74 | c-15811 | 36:0 | | | |
| | F74 | 266:71 | 3:1 | 2.77 | | | | | | | |

Therefore, in spite of monogenic segregation of resistance, additional information on the genetics of avirulence allows us to postulate that at least two genes control resistance in c-23874.

Comparative studies on segregation of resistance in the $F_2$ population of cross combinations c-19979 × Pirkka and c-15811 × Pirkka and segregation of fungal virulence demonstrated that at least three resistance genes are present in c-19979 and c-15811: one dominant and one recessive to isolate 181-6 and another dominant gene to isolates d9-4 and d8-3. The segregation of 7:1 (three avirulence genes) in crosses 181-6 × d9-4 and 181-6 × d8-3 support the hypothesis of two resistance genes in c-19979 and c-15811 to isolate 181-6 and one gene to isolates d9-4 and d8-3 and two avirulence and one avirulence gene(s), respectively, in these isolates.

In $F_2$ of the cross c-15811 × Pirkka infection responses (IR) were scored using the 10 point scale of Tekauz (1985) allowing the assessment as a qualitative and quantitative trait. IR on c-15811 after inoculation with the isolate F4 was 2.2, and 0.95 with the isolate F74. The susceptible cv. Pirkka IR was evaluated at 7.3 for isolate F4, and 7.0 for F74. In the segregating $F_2$ population a prevalence of genotypes with the same IR as the resistant parent was observed. The number of plants with intermediate types of reactions did not correspond to a Mendelian model of 1:2:1 or 12:3:1. However, the ratio of resistant and susceptible plants (without intermediate plants) fits to the expected 3:1 segregation for both isolates. All ascospore isolates from the F4 × F74 cross were avirulent to barley accessions c-15811 suggesting that these isolates have common avirulence gene(s).

## Discussion

The results of segregation analyses in populations from crosses of resistant and susceptible barley genotypes strongly depend on the genotype of the *P. teres* isolates used. Similar data showing the presence of several genes controlling resistance to various isolates of *P. teres* were obtained by Ho et al. (1996). They found that resistance of barley accession CI 9831 to isolate WRS102 is controlled by three recessive genes, to isolate WRS858 by one recessive gene, and to WRS857 by one dominant or two complementary genes. Gupta, Loughman, Lance, Jones (2002) have shown that the resistance of barley accession WA 4794 to pathotypes 97NB 1, NB81 and NB52B is determined by two dominant genes and to pathotype NB50 by one gene, and that the resistance of the cv. Pompadour to pathotypes NB50 and NB52B is due to two dominant genes and to 97NB1 and NB81 to one gene. Results suggesting the presence of several resistance genes in the host and virulence genes in the pathogen also have been obtained by Valjavec-Gratian and Steffenson (1997) in the *Cochliobolus sativus* – barley pathosystem.

Genetic analyses of resistance to *P. teres* as a quantitative trait conducted on doubled haploids from the cross Rolfi (susceptible) × CI 9819, and six different types of markers (REMAP, IRAP, ISSR, SSR, RAPD and RFLP) have revealed that one dominant gene effective against eight isolates from USA, England, Finland, and Canada on chromosome 6H is responsible for the resistance of CI 9819. This locus explains 60–88% of the phenotypic variance. Also genes effective against specific isolates were determined on chromosomes 1H, 2H, 3H, 5H and 7H (Manninen et al., 2000). Mendelian analyses, using the same isolates, also revealed additional genes in CI 9819 that were effective against certain isolates (Afanasenko et al., 2004). As the cause of the detected minor effect, QTLs are effective against some isolates only; it may therefore be assumed that they control partial resistance in an isolate-specific manner. The biggest contradiction between the results of qualitative and quantitative genetic analysis of resistance is that only one major gene (QTL) was determined on chromosome 6H against all investigated isolates, while in the Mendelian analyses a digenic mode of inheritance of resistance to four isolates and a monogenic control of resistance for additional isolates has been observed (Tables 4 and 5). Khan and Boyd (1969) also found resistance in CI 9819 controlled by two genes. Bockelman, Sharp, and Eslick (1977) used $F_2$ progeny between Betzes primary trisomics and CI 9819 and concluded from their results that one resistance gene, *Rpt1b*,

is located on chromosome 3H and the other, *Rpt2c*, probably on 1H. These contradictions could be due to different isolates used. As shown above, the segregation detected in $F_2$ populations is strongly dependent on the isolate used. For example, in the combination CI 9819 × Gastsinets a monogenic segregation was detected for zh12 and 27–36, and a digenic to isolates 85-53-1 and UK 80-12 (Table 4). Besides this, the genetic background of the susceptible parent may influence segregation ratios. For example, in the combination Harbin × Pirkka a monogenic segregation was observed for seven isolates, but in the combination Harbin × Nadia a digenic segregation was observed for two isolates. In studies of three DH populations (Steptoe/Morex, Harrington/Morex, and Dicktoo/Morex) for resistance to *Cochliobolus sativus* it was shown that QTL detection is dependent on the genetic background of the susceptible parent (Bilgic, Steffenson, & Hayes, 2005).

In addition, these contradictions may be due to the classification of intermediate types of reaction which in studies on genetics of resistance and virulence is always observed (Khan, & Boyd, 1969; Ho et al., 1996; Jonsson et al., 1999; Afanasenko et al., 1999a). In Mendelian analysis of *P. teres* resistance the incomplete dominance of resistance is often observed. Out of 44 investigated $F_1$ plants from reciprocal crosses of 15 resistant and two susceptible barley accessions, in 41 cases incomplete dominance of resistance to isolate zh3, in 34 cases to isolate P1, in 22 cases to isolate v12, and in 16 to isolate v2 was observed (Afanasenko et al., 1999a). Therefore, plants in $F_2$ populations from resistant × susceptible crosses showing an intermediate type of reaction were in general classified as heterozygous. In the case of complete dominance (c-15811), intermediate types of reactions may probably be due to the presence of additional genes in the resistant accession encoding incomplete resistance with weak phenotypic display. Therefore, we propose in c-15811 the presence of one major resistance gene and probably minor genes, as was shown for CI 9819 (Manninen et al., 2000). Unfortunately, this conclusion cannot be supported as there was no segregation of virulence between isolates F4 and F74.

From the studies on the inheritance of virulence to the same barley accession, a strong influence of the genotype of the parental isolates was determined. Depending on the cross combination, one gene controlled avirulence to c-8721, 21849, 23874, 19979, 15811, CI 5401, 9819, 4922, Harbin and Diamond, two genes to c-8721, 21849, 8755, 19979, 15811, CI 5401, 4922, 9825 and Harbin, three genes to c-8755, and four genes to c-21849, CI 9825, 9819. In spite of the fact that different isolates were used in the genetic analysis of resistance to *P. teres* of nine barley accessions and the studies on virulence of different isolates, the results obtained testify to the existence of a balanced polymorphism of the genetic systems of resistance and virulence.

The proof of a gene-for-gene relationship requires the characterization of both the genetics of avirulence and the genetics of resistance (Flor, 1971). The results of parallel analysis of genetics of resistance and genetics of virulence presented in Table 7 allow the postulation of a gene–for–gene interaction in the *P. teres* – *H. vulgare* pathosystem. The number of resistance genes in three barley accessions to different isolates corresponds to the number of virulence genes in these isolates. Segregation for virulence in crosses of *P. teres* isolates used in genetic studies of resistance reveal that even when segregation ratios in $F_2$ populations from crosses of susceptible and resistance barley genotypes fit to the presence of one dominant gene, resistance against some isolates is controlled by additional genes. For example, the result of segregation in $F_2$ in the cross of c-15811 and Pirkka to five *P. teres* isolates demonstrated a monogenic control of resistance to each isolate, since segregation in crosses between some of these isolates showed that different genes controlled avirulence and accordingly resistance to some isolates.

Obviously, the epistatic effect of suppressors gene on the avirulence genes, which was observed in fungal crosses D and F, may also be an important mechanism for the appearance of new virulent races. Such an epistatic effect was also shown in the pathosystem rice—*Magnaporthe grisea* (Leung, Borromeo, Bernardo, & Notteghem, 1988; Lau, Chao, & Ellingboe, 1993). These authors proposed the hypothesis of the presence

of suppressors gene for each avirulence gene. In *Melampsora lini*, mutation in one non-specific suppressor gene inactivates five avirulence genes leading to the appearance of new virulence genes (Jones, 1988).

During the current genetic studies, several isolates from the ascospore progeny were characterized for their virulence to barley genotypes with known and unknown genes for resistance. In these studies isolates with rare virulence as a result of sexual recombination between two avirulent isolates were found in the ascospore progenies (Table 6). This indicates the possibility for a rapid creation of new races by sexual recombination on plant residuals in field conditions (where perithecia are formed). Besides this, the presence of isolates with one or two virulence and avirulence genes to specific resistance genes gives the opportunity for a detailed estimation of the genetic diversity of resistance.

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