

## Genetic Control of *Pyrenophora teres* Virulence to Three Barley Accessions

N. MIRONENKO, O. FILATOVA and O. AFANASENKO\*

All-Russian Research Institute of Plant Protection; St-Petersburg-Pushkin 196608, Russia

\*E-mail: OAfan@OA4799.spb.edu

### Abstract

Ascospore progeny of cross of *Pyrenophora teres* f. *teres* isolates was evaluated on virulence to three barley genotypes. Monogenic inheritance of virulence (26 a:17 v and 31 a:18 v) was shown to cultivar Harbin and accession c 21272 and supported by results of two fungal backcrosses. The existence of three unlinked avirulence genes to CI 4922 is suggested (37 a:7 v). The model of interaction between barley resistance genes and avirulence genes with postulated fungal genotypes is proposed.

**Keywords:** *Pyrenophora teres*; barley; sexual cross; backcross; avirulence gene; resistance gene

### INTRODUCTION

*Pyrenophora teres* Drechs. f. *teres* Smedeg. (anamorph: *Drechslera teres* (Sacc.) Shoem. f. *teres* Smedeg.) is economically important pathogen, which causes symptoms of net blotch in barley. The sexual stage of fungus was observed in nature on barley debris. There for sexual recombination can be an important mechanism of variability of fungus populations.

Hybridization in culture with producing of fertile perithecia firstly was reported by MCDONALD (1963). The first genetic researches of *P. teres* showed that its traits, such as morphology of colony, mating type, net/spot types symptoms are obeyed the Mendel's laws (MCDONALD 1967; SMEDEGAARD-PETERSEN 1977). The first work devoted to inheritance of *P. teres* f. *teres* virulence to one cultivar was published in 1999 (WEILAND *et al.* 1999).

The aim of this research was to study the inheritance of virulence to three resistant barley accessions.

### MATERIALS AND METHODS

*P. teres* f. *teres* monoconidial isolate 8ax was collected from a barley field in Far East of Russia and isolate 181-6 in St. Petersburg region. The isolates were determined to be of opposite mating type by pairing in culture. Monoascospore isolates A-80 and A-1 from cross 181-6 × 8ax were used for backcrossing.

Ascospores were produced by growing of two compatible isolates for 2–4 months at 15°C in a 16-h photoperiod on the autoclaved lemon leaves supported on filter paper over wet sand in a Petri dishes (MCDONALD 1967). Ascospores were ejected from asci within the mature pseudothecia onto the lid of a Petri dish containing water agar. Single ascospores were transferred to PDA.

For the evaluation of pathotypes of *P. teres* barley cultivar Harbin and barley accessions CI 4922 and c 21272 with known genes of resistance (MODE & SCHALLER 1958; AFANASENKO 1997) were used.

The *P. teres* isolates produced conidia on the CLM medium (g/l): KCl – 0.5; KH<sub>2</sub>PO<sub>4</sub> – 0.5; MgSO<sub>4</sub> – 0.5; CH<sub>4</sub>N<sub>2</sub>O – urea (carbamid) – 1.2; lactose – 20; agar-agar – 20. For inoculation of detached barley leaves (seedlings) placed on the solution of bensimidazole (0.004%) conidial suspension of concentration of 5 000–10 000 conidia per ml was used (AFANASENKO 1977).

### RESULTS AND DISCUSSION

The cross between two isolates of *P. teres* (181-6 × 8ax) was high fertile and produced near 90% viable ascospores. Half of obtained fungal progeny on the CLM medium had abnormal conidia of long curved form with attached conidiophores. It was unexpected because both parental isolates had normal conidia. It

Table 1. Segregation for avirulence to the three barley genotypes in random ascospore progeny from cross of *P. teres* f. *teres* isolates 181-6 × 8ax

Barley genotypes with resistance genes	Virulence of parental isolates		Number of ascospore isolates with phenotypes				Segregation ratio (av):
	181-6	8ax	virulent, normal	virulent, abnormal	avirulent, normal	avirulent, abnormal	
Harbin (Pt2)	v	a	15	2	5	21	26:17 (1:1) $\chi^2 = 1.88$
C 21272 (Pt19, Pt28, Pt29, pt30, pt31)	v	a	16	2	7	24	31:18 (1:1) $\chi^2 = 3.45$
CI 4922 (Pt2 Pt3)	a	a	6	1	16	21	37:7 7:1 $\chi^2 = 0.077$

Table 2. The model of relationships between avirulence genes of *P. teres* and resistance genes of *Hordeum vulgare*

Postulated genotypes of fungal parents and ascospore progeny	Phenotype of pathogene		Postulated genotypes of fungal parents and ascospore progeny	Phenotype of pathogene	
	Harbin (Pt2)	CI 4922 (Pt2Pt3 + PtX*)		Harbin (Pt2)	CI 4922 (Pt2Pt3 + PtX*)
<b>Cross “A”</b>			n Apt2 apt3 aptX		
Parent 181-6 (MAT1-2 )	v	a	Progeny:		
N apt2 Apt3 aptX			N apt2 Apt3 aptX	v	a
Parent: 8 ax (MAT1-1)	a	a	N apt2 apt3 aptX	v	v
n Apt2 apt3 AptX			n Apt2 Apt3 aptX	a	a
Progeny:			n Apt2 apt3 aptX	a	a
n Apt2 Apt3 AptX	a	a	Expected ratios	1 a:1 v	3 a:1 v
n Apt2 Apt3 aptX	a	a	Real segregation	39:25	56:23
n Apt2 apt3 AptX	a	a	$\chi^2$ ( $P = 0.05$ )	3.06	0.71
n Apt2 apt3 aptX	a	a	<b>Backcross “B”</b>		
N apt2 Apt3 AptX	v	a	Parent: 181-6 (MAT1-2)	v	a
N apt2 Apt3 aptX	v	a	N apt2 Apt3 aptX		
N apt2 apt3 AptX	v	a	Parent: A1 (MAT1-1)**	a	a
N apt2 apt3 aptX	v	v	N Apt2 Apt3 aptX		
Expected ratio	1 a:1 v	7 a:1 v	Progeny:		
Real segregation	26:17	37:7	N apt2 Apt3 aptX	v	a
$\chi^2$ ( $P = 0.05$ )	1.88	0.47	N Apt2 Apt3 aptX	a	a
<b>Backcross “D”</b>			Expected ratios	1 a:1 v	1 a:0 v
Parent: 181-6 (MAT1-2)	v	a	Real segregation	10:10	20:0
N apt2 Apt3 aptX			$\chi^2$ ( $P = 0.05$ )	0.0	0.0
Parent: A80 (MAT1-1)	a	a			

\* this hypothetical resistance gene is postulated to explain the segregation of virulence data (v – virulent and a – avirulent) on the barley genotypes; \*\*recombinant genotype

appears that one of the parental genes, controlled the form of conidia, was mutated. Alleles of this gene were arbitrarily called “N” (normal conidial form) and “n” (abnormal conidial form). This trait (form of conidia) segregated as monogenic one (Table 1).

The results of segregation of fungus progeny on virulence to three barley genotypes are presented in the Table 1. The segregation ratio (avirulence:virulence) on two barley genotypes Harbin and c 21272 was near 1:1, suggesting that a single genes control virulence. Tests for gene identity will be done in future. Data of segregation suggested, that gene of conidial form “n” is linked with avirulence gene Apt2. The pattern of segregation on virulence to the third barley accession CI 4922 was more complicated. The segregation 37 avirulent to 6 virulent corresponds to model of segregation 7:1, suitable for three unlinked avirulence genes.

The hypothesis for a single gene segregation of avirulence on cv. Harbin was supported by analysis of two backcrosses “D” (A80 × 181-6) and “B” (A1 × 181-6) (Table 2). Fungal genotypes were postulated according obtained data of segregation and Flor’s gene-for-gene model of host-parasite interactions. One avirulence gene to Harbin and CI 4922 was designed as Apt2 order to show the complimentary of this gene to resistance gene *Pt2* common for both cultivars. Second avirulence gene was designed as Apt3, hypothetically complementary to *Pt3* gene in CI 4922. To explain segregation of virulence to barley accession CI 4922, the existence of additional resistance gene in this barley

genotype – *PtX*, complemented to hypothetical gene of avirulence in *P. teres* AptX was proposed.

### References

- AFANASENKO O. (1977): Laboratory method of estimation of the resistance to net blotch in barley cultivars. S.-kh. Biol., **12**: 297–299.
- AFANASENKO O.S. (1997): Diversity of “*Helminthosporium*” fungi populations causing barley blights and genetic control of the resistance to *Pyrenophora teres* Drechs. [Doctoral (Biol.) Dissertation.] St. Petersburg.
- MCDONALD W.C. (1963): Heterothallism in *Pyrenophora teres*. Phytopathology, **53**: 771–773.
- MCDONALD W.C. (1967): Variability and the inheritance of morphological mutants in *Pyrenophora teres*. Phytopathology, **57**: 747–755.
- MODE C.Y., SCHALLER C.W. (1958): Two additional factors for host resistance to net blotch in barley. Agric. J., **50**: 15–18.
- SMEDEGAARD-PETERSEN V. (1977): Inheritance of genetic factors for symptoms and pathogenicity in hybrid of *Pyrenophora teres* and *Pyrenophora graminea*. Phytopath. Z., **89**: 193–202.
- WEILAND J.J., STEFFENSON B.J., CARTWRIGHT R.D., WEBSTER R.K. (1999): Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on “Harbin” barley. Phytopathology, **89**: 176–181.