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Mapping of the Loci Controlling the Resistance to *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* in two Double Haploid Barley Populations

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Abstract—Net blotch of barley (*Hordeum vulgare* L.), caused by *Pyrenophora teres* f. *teres*, and spot blotch, caused by *Cochliobolus sativus*, are the most widespread and harmful diseases in the geographic range of the crop. Barley breeding for resistance to these diseases should employ large genetic diversity. The 11_11067 SNP marker was revealed on chromosome 6H position 58 cM in the double haploid (DH) population A developed by crossing the Ethiopian accession k-23874, highly resistant to *P. teres* f. *teres*, to the susceptible Pirkka cultivar. It was reliably ($p < 0.05$) associated with resistance to three *P. teres* f. *teres* isolates. In population B (Zernogradsky 813 (MR to *C. sativus*) Ranniy 1 (MR to *P. teres* f. *teres*), 11 QTL controlling resistance to 12 *P. teres* f. *teres* isolates were found on all barley chromosomes and 14 QTL for resistance to 12 *C. sativus* isolates, on all chromosomes except for 4H. For both pathogens, the revealed QTL were shown to be isolate-specific. The majority of the loci detected were mapped in the same intervals between SNP markers where QTL controlling resistance to *P. teres* f. *teres* and *C. sativus* had been found by other scientists. Four novel QTL controlling resistance to *P. teres* f. *teres* were found on chromosomes 1H, 4H, and 5H. Five novel QTL associated with resistance to *C. sativus* were found on chromosomes 2H, 3H, 5H, and 6H in DH population B.

Keywords: barley, disease resistance, double haploid populations, QTL mapping, SNP markers, *Pyrenophora teres* f. *teres*, *Cochliobolus sativus*, molecular markers

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INTRODUCTION

Global crop production is focused on resource-saving and environmentally friendly technologies. The basic component of such technologies is the cultivation of disease-resistant crop varieties. All basic strategies for creating genetically protected crop varieties are based on the presence of genetic diversity of resistance, because the cultivation of varieties with highly effective resistance genes on large areas inevitably leads to the loss of resistance due to microevolutionary processes in the populations of parasites.

The possibilities of breeding for resistance to plant diseases have broadened with the appearance of new technologies of molecular mapping and sequencing, because molecular markers (MMs), a powerful tool for controlling the transmittable trait of resistance, have appeared.

Marker-assisted selection (MAS) of plants for resistance to diseases is now widely used in Europe, United States, Canada, and Australia. Its advantages are obvious, especially when pyramiding resistance genes, involving genes that are expressed only in adult plants, as well as genetic determinants of quantitative resistance of plants to diseases. MMs are of particular importance in creating varieties with long-term resistance, as they allow us to combine resistance genes in one genotype, providing effective protection against a wide range of isolates with different virulence.

The greatest number of MMs in crops is for resistance genes to the pathogens of rust diseases. MMs developed abroad in recent years are used in Russia to determine the genetic diversity of wheat resistance to stem rust (Tyryshkin et al., 2006; Gultyaeva and Volkova, 2009; Gultyaeva et al., 2009; Lapochkina et al., 2009; Vasiliev and Bespalova, 2011; Krupin, 2011).

Table 1. Characteristics of barley accessions used in crossing to obtain populations of DH lines

Accessions, no. in VIR catalog	Origin	Variety/species	Resistance
Population "A"			
k-18530	Finland	Pirkka/pallidum	Universally susceptible variety
k-23874	Ethiopia	WGA-148-3/parallelum	Highly resistant to net blotch, resistant to loose smut and mildew
Population "B"			
k-27737	Russia, Novosibirsk region	Ranniy 1/Nutans	Average resistance to net blotch
k-30453	Russia, Rostov region	Zernogradsky 813/erectum	Average resistance to spot blotch and rhynchosporium

A significant increase in disease severity of crops caused by hemibiotrophic parasites was noted both in Russia and abroad in the last decade. Among them net blotch (pathogen–the fungus *Pyrenophora teres* Drechs f. *teres*) and spot blotch (pathogen–*Cochliobolus sativus* (Ito et Kurib.) Drechsler ex Dastur.), also known as "helminthosporiotic" blotches, are the most harmful for barley in all areas of its cultivation. The epidemics of barley blotches occurs 5 times in 10 years. Yield losses of susceptible barley varieties from these diseases during epidemics range from 20 to 40%. In this respect, the problem of developing a genetic method to protect barley against these diseases is acute.

The high heterogeneity of the *P. teres* f. *teres* populations on the basis of virulence and differences between geographic populations, caused mainly by the influence of genotypes of cultivated varieties on the formation of pathogen populations, indicate the need to use barley donors of resistance that are effective against local populations of the parasite and are adapted to the specific agro-climatic zone (Afanasenko et al., 2009; Afanasenko et al., 2010).

A collection of sources and donors of barley resistance to hemibiotrophic pathogens was created in the Laboratory of Plant i Resistance to Diseases, All-Russian Research Institute for Plant Protection, as a result of many years of work and active cooperation with the Vavilov Institute of Plant Industry and foreign institutions. The collection consists of more than 400 accessions of barley.

The aim of this study was to identify and map the genetic determinants of resistance in barley to the pathogens of net and spot blotches in double haploid mapping populations derived from the cross of the Ethiopian accession k-23874 and Pirkka variety, as well as varieties Zernogradsky 813 and Ranniy 1 using isolates of pathogens of various origins and to test the hypothesis of isolate-specificity of quantitative trait loci (QTL).

MATERIALS AND METHODS

Parental Genotypes of Double Haploid Populations

F_1 hybrids of the crossed Ranniy 1 and Zernogradsky 813 varieties were used to initiate an anther culture, as well as k-23874 and Pirkka (Table 1). The characteristics of the parental crossing components are given in Table 1.

It should be noted that varieties Zernogradsky 813 and Ranniy 1 showed a differentiating reaction to both pathogens.

Double Haploid Mapping Populations

Double Haploid barley plants were obtained from the anther culture of F_1 hybrid combinations k-23874 × Pirkka and Ranniy 1 × Zernogradsky 813 according to O. Manninen (1997), where the plant phase and nutrient media composition were optimized. The seed progeny of 42 double haploid lines (DH) was obtained in combination k-23874 × Pirkka (population A) and 114 DH in combination Zernogradsky 813 × Ranniy 1 (population B).

Isolates of Pathogens

Barley leaves with the symptoms of blotches were collected on variety test plots in Leningrad, Novgorod, and Pskov Regions and Krasnodar Krai. *P. teres* f. *teres* and *C. sativus* fungi were isolated and mono-conidial isolates were obtained on a modified Czapek medium by standard methods (Afanasenko and Levitin, 1979). Resistance of DH to *P. teres* f. *teres* was determined in two ways—by inoculating vegetative plants in the greenhouse and detached leaves in the laboratory.

Inoculation in the Greenhouse

Resistance of DH lines of both mapping populations to the pathogens of net and spot blotch was assessed under greenhouse conditions of the Agricultural Research Centre of Finland (Agrifood Research Finland, MTT). The plants of each DH line were

seeded one plant per pot, with the soil and peat mixture in quadruplicate. The plants were grown at 18–22°C and a 12-hour photoperiod. The relative humidity in the greenhouse was raised to 100% within 14 days after sowing and plants at the stage of 2–3 leaves were sprayed with a conidial suspension of *P. teres* f. *teres* isolate V278 at a concentration of 50000 conidia/mL, 0.3 mL per plant. The reaction type of each plant and the average values in replications were measured on the 10–14th day post-inoculation according to a modified 10-point Tekauz scale (Tekauz, 1985): reaction types, evaluated on a scale ranging from 1 to 5, were associated with resistant types and those ranging from 5.1–10 were associated with susceptible types.

Plants were inoculated by the fungus *C. sativa* suspension of mono-conidial isolate C_Fin at a concentration of 10000 conidia/mL. Fetch and Steffenson's 9-point scale was used to determine the type of plant response to *C. sativus*: 1–3 points corresponded to resistance, 4–5 corresponded to an intermediate reaction, and 6–9 corresponded to susceptibility (Fetch and Steffenson, 1999). In this case, the presence of chlorosis was the main criterion for determining both the intermediate and susceptible reaction types. The averages of reaction types from the replicates were considered resistant (from 1 to 4.5) or susceptible (from 4.6 to 9).

Inoculation of Detached Leaves of Seedlings

Inoculation of detached leaves of DH seedlings, placed on a filter paper moistened with 0.004% solution of benzimidazole, was carried out by spraying with a suspension of *Cochliobolus sativus* (10000 conidia/mL) or *Pyrenophora teres* f. *teres* (5000 conidia/mL) monoconidial isolates (Afanasenko, 1977). The reaction type of each leaf segment on inoculation with *P. teres* isolates was registered on the 4th day according to the modified Tekauz scale (Tekauz, 1985). Average points ranging from 1 to 5 corresponded to resistant; and from 5.1 to 10, to susceptible. A high correlation between the results of plant infection in the greenhouse and detached leaves of seedlings has been shown in several studies (Mikhailova and Afanasenko, 2005; Tuohy et al., 2006; Afanasenko et al., 2009).

DNA Isolation

DNA was extracted from fresh leaves of three week old seedlings according to the standard procedure with CTAB buffer (Saghai-Maroof et al., 1984). Genotyping with SNP markers was performed in the Laboratory of Genome Technology at The James Hutton Institute (Dundee, United Kingdom) using the BeadXpress (Illumina Inc.) instrument. A panel of 384 SNP markers with known genetic positions on barley chromosomes was used for the genotyping of the population (Rostoks et al., 2006). The SNP markers belong to the first of the barley oligonucleotide pool assays (BOPA1), developed on the basis of Illumina GoldenGate assay technology (Illumina Inc., San Diego, California, United States).

Genetic distances on the map of population A were not calculated due to the small sampling of recombinant progeny for this cross (42 recombinants); previously published genetic distances between SNP markers on the map of the reference population of barley Steptoe/Morex were used instead (Rostoks et al., 2006). The genetic distances for the mapping population B (114 recombinants) were calculated by the recombination frequency between markers with software MAPMAKER v.2 (Lander et al., 1987) using the Kosambi function (Kosambi, 1944), minimum LOD = 3.0 (logarithm of odds, statistics of likelihood ratio), and a maximum recombination frequency of 50%.

Mapping of QTL for the Trait of Resistance

Mapping of QTL (Quantitative Trait Loci) was carried out with the Windows QTL Cartographer version 2.5 software using the CIM algorithm (Compositive Interval Mapping). The minimum threshold of LOD, significant at 95% ($p = 0.05$), was calculated for each isolate of *P. teres* and *C. sativus* based on the results of 1000 permutations.

RESULTS

Resistance of Parental Crossing Components and DHs

The selection of *P. teres* f. *teres* and *C. sativus* isolates of different origin was conducted to map the genetic determinants of resistance, taking into account the reaction of parental crossing components (Table 2). The reaction types of sample k-23874 and variety Pirkka to three isolates of net blotch pathogen were the same: the reaction of sample k-23874 after inoculation of both intact plants in the greenhouse and leaf segments corresponded to 1–2 points and of the Pirkka variety, to 8 (Table 2, Fig. 1). A uniform segregation of resistance in the DH population A to the three studied isolates was revealed. The correlation between types of reaction in intact plants and leaf segments gave a high correlation coefficient of $r = 0.89$.

The resistance of the Ranniy 1 variety to the selected isolates of the net blotch pathogen was higher than that of the Zernogradskiy 813 variety, but the differences were not as contrasting as in population A (Table 2, Fig. 1). Isolates of *P. teres* f. *teres* and *C. sativus* were used for the phenotyping of DH populations, and contrasting reactions of parental crossing components were observed following inoculation (Table 2).

The distribution of lines in DH population B according to the types of reaction to inoculation with different isolates of *P. teres* f. *teres* and *C. sativus* is shown in Figs. 1 and 2. Transgressive segregation in the mapping population was observed for all 12 isolates of *P. teres* f. *teres* and for 10 of 12 isolates of the *C. sativus*

studied: a class of lines appears that is more resistant than the resistant parent.

Genotyping of DH Lines and QTL Mapping

Of the 384 SNP markers, 108 that were tested were polymorphic for population A and 164 were polymorphic for population B. Polymorphic markers were used to determine the haplotypes of the recombinant progeny derived from the crosses of the parental samples. The genetic maps for the two populations of DH lines A and B were constructed based on the results of SNP genotyping, their length amounted to 1000 cM and 992 cM, respectively. The dimensions of the genetic maps obtained are comparable to those developed previously for crosses Steptoe/Morex (1035 cM), Morex/Barke (1065 cM), and OWB Dominant/OWB Recessive (1215 cM) on the basis of genotyping with BOPA1 and BOPA2 (Close et al., 2009).

Only those isolates whose QTL peaks reached a threshold of confidence ($p = 0.05$) and were mapped in the same position for two or more independent biological replicates of the experiment were taken into account during the QTL analysis, (Fig. 3).

Mapping of a Gene Locus That Determines Strong Resistance to P. teres f. teres in Population A

An interval of 42–81 cM was identified on chromosome 6H as a result of the QTL analysis of V-278 isolate, within which the maximum association (LOD = 3.9) with the mapped feature in four replicates of the experiment was established. The closest SNP marker (11_11067, position 58 cM) on chromosome 6H showed a linkage with resistance ($p < 0.05$, LOD = 3.4) to the other two isolates of *P. teres* f. *teres* (PL5 and PN10). Another locus of resistance to *P. teres* f. *teres* isolates was already described earlier in the same interval of chromosome 6H (75–78 cM) (Grewal et al., 2008).

Mapping of QTL Controlling the Resistance to P. teres f. teres in Population B

QTL controlling the resistance to 12 isolates of *P. teres* f. *teres* in crossing combination B were identified on all 7 chromosomes of barley (Table 3). In three cases, the linkage of the trait for resistance in the same interval between SNP markers was identified for more than one isolate. QTL (closest SNP marker 11_11189) significantly linked with resistance to 6 isolates of *P. teres* f. *teres* from the Krasnodar Krai (PK4, RK5), Pskov Region (PP7, PP5, and PP6), and Novgorod Region (PN18) was located in the same interval on the long arm of chromosome 1H (Table 3). A coincidence of positions of QTL, controlling the resistance to PP1 and PP6 isolates on the long arm of the chromosome 6H and to the PL9 and PN7 isolates on chromosome 7H, was also revealed. The rest of the QTL identified were isolate-specific. Two loci, controlling resistance to each isolate, were identified for each of five isolates (PP6, PP5, pH 7, and PP7 PN18). Eleven QTL, controlling resistance to 12 isolates of *P. teres* f. *teres*, were found in total.

Table 2. Virulence of *P. teres* f. *teres* and *C. sativus* isolates to the parental crossing components

Isolate	Origin	Variety reaction type, points	
		Pirkka	k-23874
Population "A"			
Pyrenophora teres			
V_278	Finland	8	1–2
P_L5	Leningrad region	8	1–2
P_N10	Novgorod region	8	1–2
Population "B"			
		Zernograd-sky 813	Ranniy 1
Pyrenophora teres			
PL9	Leningrad region	7.3	3
PN3	Novgorod region	7	3
PN7		7	3
PN10		7	3
PN18		7.3	3.2
PN19		7	2.5
PP1	Pskov region	7	2.5
PP5		7	3
PP6		7	3.5
PP7		7	3.2
PK4	Krasnodar Krai	7	3.5
PK5		7	3
Cochliobolus sativus			
CL1	Leningrad region	3	7.2
CL8		3.2	7
CL12		2.5	7
CL18		2	7
CN1	Novgorod region	2	7
CN12		3.6	7
CN18		3	7
CP1	Pskov region	3	7.5
CP2		3	7
CP6		2.5	7
CP8		3	7
CK9	Krasnodar Krai	3	7.2
C_FIN	Finland	2	7

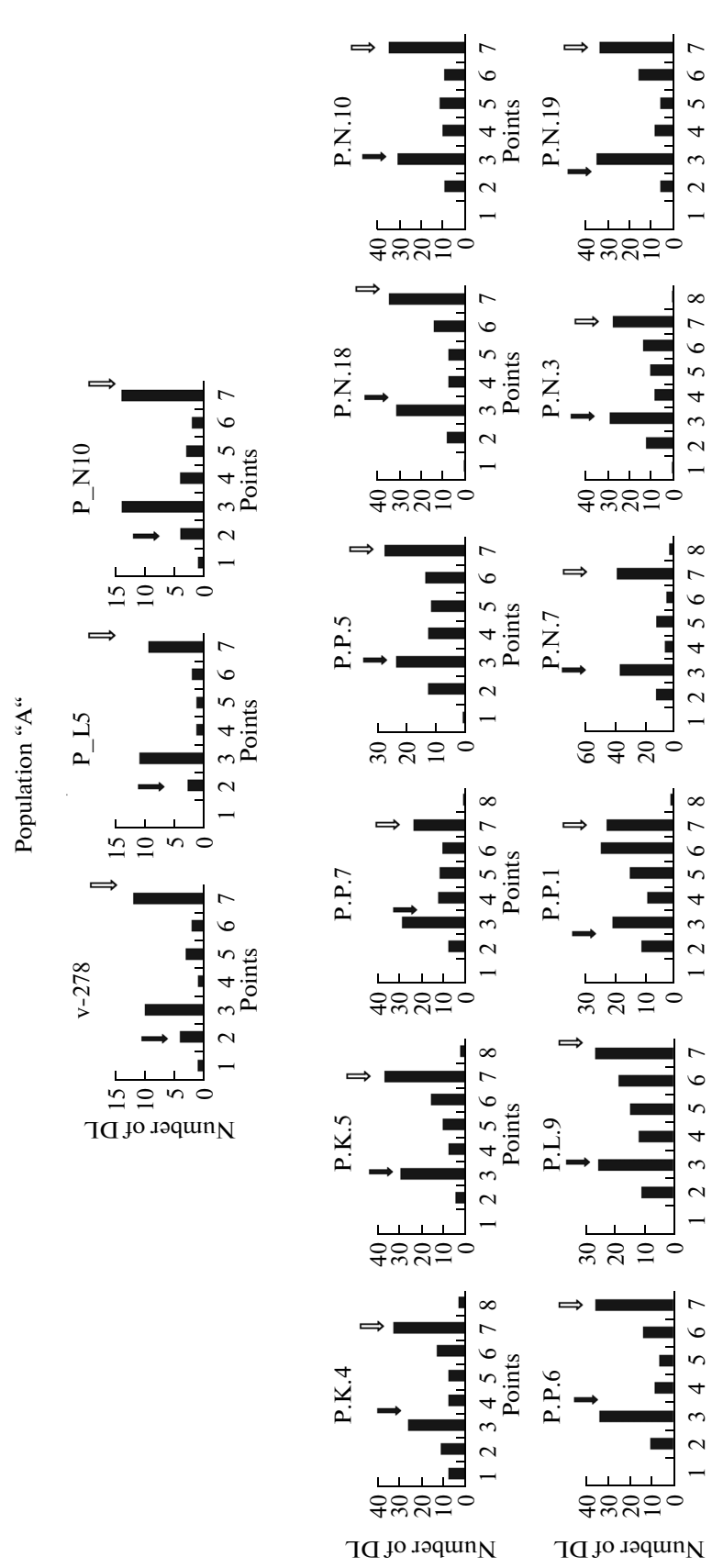

Fig. 1. Distribution of DH lines by reaction type to inoculation with different *P. teres* f. *teres* isolates in mapping populations A and B. The arrows indicate reactions of parental components: white—susceptible parent, black—resistant one.

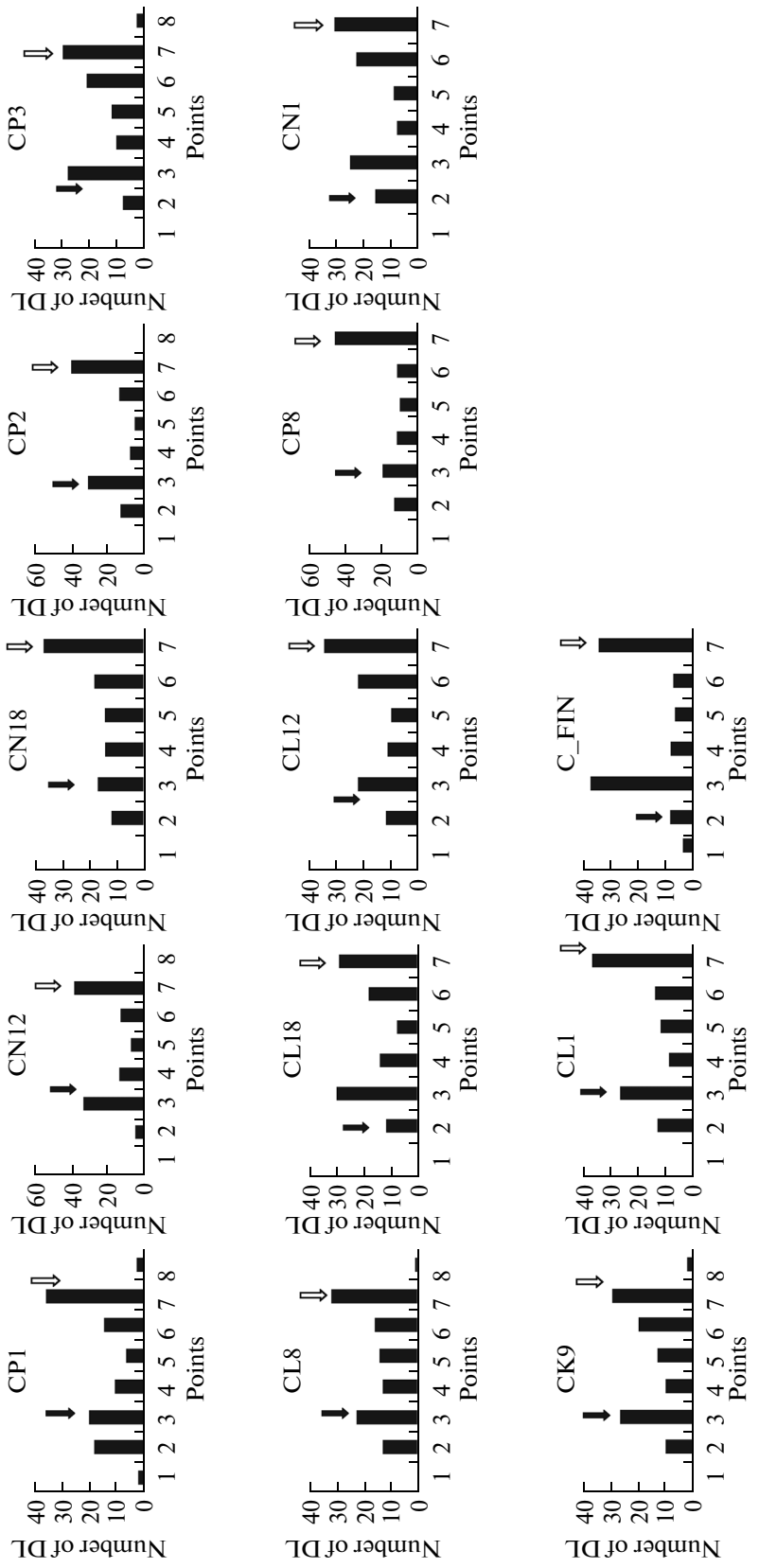

Fig. 2. Distribution of DH lines in mapping population B by reaction type to inoculation with different *C. sativus* isolates. The arrows indicate the reactions of parental components: white—susceptible parent, black—resistant one.

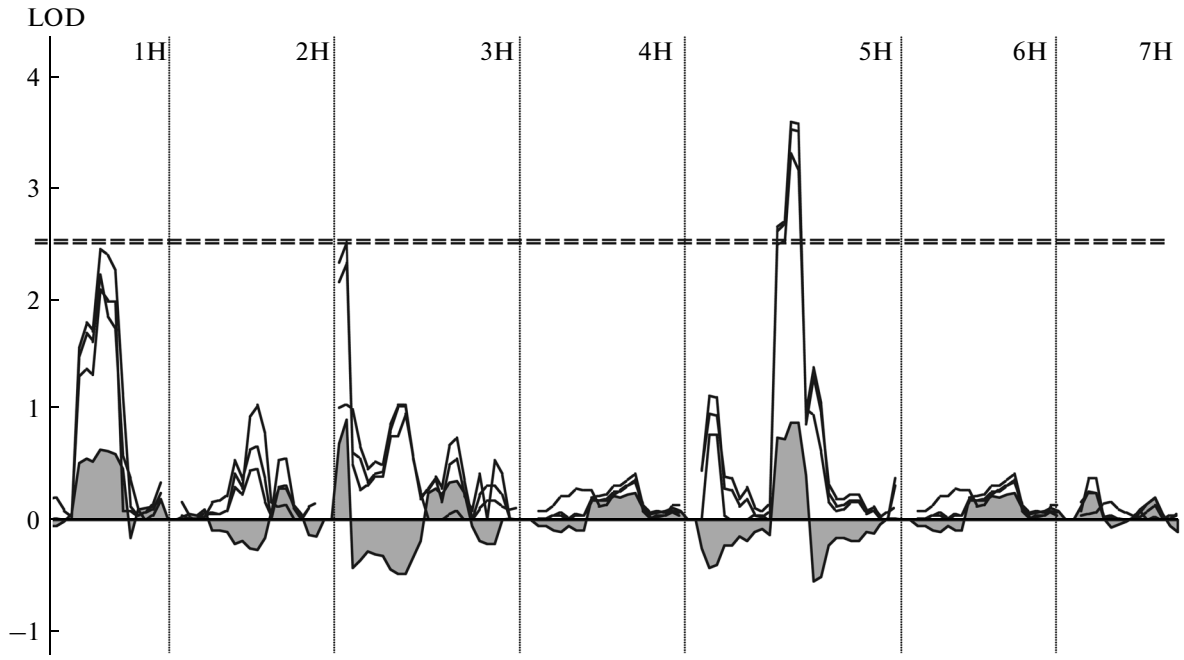

Fig. 3. Mapping of QTL, controlling the reaction of DH lines of the population obtained from crossing Zernogradsky 813/Ranniy 1 to infection with PN3 isolate of *Pyrenophora teres* in four replications of the experiment.

1H–7H—seven barley chromosomes. Horizontal–threshold of a confidence level LOD ($p = 0.05$). Solid color–additive effect of parental alleles: above zero–higher score according to the Tekauz scale corresponds to the parental alleles of variety Ranniy 1; below zero–higher score corresponds to the alleles of the Zernogradsky 813 variety.

Mapping of QTL, Controlling Resistance to C. sativus in Population B

The linkage of SNP markers to resistance in the DH of combination B to 12 isolates of *C. sativus* was determined for all chromosomes of barley, except for 4H (Table 4). The overlapping intervals between SNP markers near the centromere of chromosome 1H, associated with resistance to both isolates CL 18 and CP 8, apparently show that the same locus determines resistance to these isolates. Overlapping intervals of SNP markers, co-segregated with resistance to CN12 and CL1isolates, were identified on the short arm of chromosome 2H; and to CP6 and CN1 isolates, on the long arm of chromosome 6H. The same reactions of intact plants (C_FIN isolate) and leaf segments (CN15 isolate) allowed us to identify a single QTL controlling resistance to these isolates on the long arm of the chromosome 7H, indicating the suitability of using the laboratory method of detached leaf inoculation. Two loci determining resistance to isolates of the fungus were found in five of the twelve cases. One isolate-specific QTL was identified for each of the remaining 7 *C. sativus* isolates. Thus, we identified 14 QTL that control resistance to 12 isolates of *C. sativus*.

DISCUSSION

The high heterogeneity of the *P. teres* f. *teres* population on the basis of virulence is known: to date, from 45 to 119 of fungal pathotypes were revealed, depending on the number of differentiator varieties used (Khan and Tekauz, 1982; Steffenson and Webster, 1992; Serenius, 2006; Afanasenko et al., 2009). Various pathotypes were identified also in the populations of a wider specialized pathogen *C. sativus*: 3 pathotypes were identified in three differentiator varieties among the 36 studied isolates from North Dakota (United States) (Valjevec-Gratian, Steffenson, 1997); studying 34 Australian isolates on 20 differentiator varieties revealed 6 pathotypes (Meldrum et al., 2004). The heterogeneity of the populations of pathogens on the basis of virulence was the justification for using multiple isolates to identify the genetic determinants of resistance.

A wide array of cultivated barley varieties, fast rotation on varietal test plots, and the introduction of inoculum with untreated seeds from different regions of Russia and abroad contributed to the formation of highly heterogeneous pathogen populations. That is why in order to create a collection of isolates of pathogens, the collection of inoculum was carried out on a variety of test plots in the Northwest region of the Russian Federation and Krasnodar Krai.

More than 20 DH populations were created in the world for mapping of genes for resistance to barley diseases (Manninen et al., 2006; Grewal et al., 2008; Roy et al., 2010; Cakir et al., 2003), 14 of them created in Australia (Cakir et al., 2003; Rahman et al., 2003;

Table 3. QTL, associated with resistance to *P. teres* f. *teres* isolates mapped in DH population B obtained by crossing varieties Zernogradsky 813 and Ranniy 1

Chromosome	Isolate	Number of replications	The interval between SNP markers, cM	The closest SNP marker	LOD	R^2	Previously identified QTL (cM). Literary source
1H	PK4	3	75–79	11_11189	3.9	0.13	New
	PP7	2			3.3	0.14	
	PN18	2			4.6	0.18	
	PP5	4			5.0	0.20	
	PP6	4	50–86	11_11189	4.0	0.15	(52.4–56.8 cM) Grewal et al., 2012
	PK5	3			3.0	0.17	
	PP5	4	96–107	11_20844	6.5	0.20	New
2H	PN10	2	51–75	11_10909	3.0	0.13	(50–51 cM) Grewal et al., 2008; (62.7 cM) Cakir et al., 2011; (75–79 cM) Konig et al., 2014
3H	PL9	4	112–150	11_20920	3.1	0.08	(115–119 cM) Grewal et al., 2008
4H	PN19	2	52–59	11_11207	2.7	0.10	(50–54 cM) Grewal et al., 2008
	PK5	3	3–16	11_11345	2.8	0.11	New
5H	PP7	3	108–163	11_10845	3.4	0.12	(109 cM) Grewal et al., 2008 (112.1–120.5 cM) Grewal et al., 2012 (125–129 cM) Konig et al., 2014
	PN3	4	56–92	11_21480	3.6	0.09	New
6H	PP1	4	94–126	11_20531	3.0	0.21	(95.1–96.8 cM) (60–65 cM) Konig et al., 2014
	PP6	4			3.3	0.17	
7H	PL9	3	61–75	11_11098	4.0	0.11	

R^2 is the % of the variation of the trait associated with QTL.

Gupta et al., 2010; Hickey et al., 2011). The advantages of using DH mapping populations compared to segregating crossing progenies[OM3] for QTL mapping includes the homozygosity of the studied traits and unrestricted number of replications of experiments to determine the resistance to one or several isolates of the pathogen.

We have initiated work on creating DH mapping populations of barley for the purpose of mapping QTL and the main genes controlling resistance to pathogens of helminthosporiotic blotches. This paper shows the results of mapping barley resistance to pathogens of net and spot blotches in two DH populations.

To assess the response of the DH lines to inoculation with isolates of these pathogens, intact plants in the greenhouse and detached leaves were inoculated, with their metabolism was supported by benzimidazole. The same loci controlling resistance of both intact plants and leaf segments to three isolates of *P. teres* f. *teres* and two isolates of *C. sativus* were identified, which supports using the less time-consuming laboratory method in this kind of research. Inoculation of detached leaves in mapping loci of resistance to *P. teres* f. *teres* was also used by König et al. (2014).

Parental crossing components of population A were the Ethiopian accession k-23874, highly resistant to most *P. teres* f. *teres* isolates (reaction type 1–2 points), and the universally susceptible Pirkka variety. The qualitative difference in the reaction of parents allowed us to reveal the main gene in the region of the centromere of the short arm of chromosome 6H. SNP marker 11_11067 (position 58 cM) was linked to resistance to the V-278 isolate of *P. teres* f. *teres* in all four experimental replicates and to two other isolates of the fungus.

A gene has been localized earlier on this region of chromosome 6H that determines resistance to *P. teres* f. *teres* (Grewal et al., 2008). The highly efficient *Rpt5* gene for resistance to net blotch was identified also in the Ethiopian line CI 9819 near the centromere of 6H (Manninen et al., 2006). A gene was also localized by other researchers in the same area of the same chromosome that determines resistance to *P. teres* f. *teres* in Steptoe (Steffenson et al., 1996), TR 306 (Spaner et al., 1998), Kaputar (Cakir et al., 2003), Chevron

Table 4. QTL of resistance to *C. sativus* isolates, mapped in DH population B obtained by crossing the Zernogradsky 813 and Ranniy 1 varieties

Chromosome	Isolate	Number of replications	The interval between SNP markers, cM	The closest SNP marker	LOD	R^2	Previously identified QTL (cM). Reference
1H	CL18	4	57–96	11_10433	3.5	0.13	(90.7 cM) Roy et al., 2010
	CP8	4	40–60	11_10764	4.1	0.16	(59.7 cM) Roy et al., 2010; (41.0 cM) Zhou et al., 2013; (50, 45.5, 49.7 cM) Gutierrez et al., 2013
	CK9	4	7–21	11_21377	3.5	0.15	(7.8 cM) Roy et al., 2010
2H	CL1	4	21–45	11_21261	5.5	0.18	New
	CN12	3	59–70	11_11015	3.0	0.11	New
	CL1	4			3.5	0.20	
3H	CL8	3	75–122	11_10312	3.2	0.14	New
	CN1	4	59–86	11_20628	2.5	0.17	(66.2 cM) Roy et al., 2010
	CL12	4	80–92	11_20850	3.3	0.13	New
	CP2	3			3.0	0.19	
5H	CL18	4	149–181	11_11216	2.7	0.13	(105.9 cM) Roy et al., 2010 (151.4 cM) Gutierrez et al., 2013
	CP8	4	57–92	11_21480	3.3	0.18	(82.9 cM) Roy et al., 2010 (80.6 cM) Gutierrez et al., 2013
6H	CP6	4	87–119	11_20531	3.5	0.15	New
	CN1	4			3.5	0.19	
	CP1	4	64–76	11_10153	3.6	0.12	New
	C_ FIN	4	14–58	11_10920	5.0	0.16	(31.7 cM) Zhou et al., 2013
7H	CN15	4			4.4	0.11	(151.4 cM) Gutierrez et al., 2013; Roy et al., 2010
	CK9	4	76–120	11_21229	3.0	0.22	New

(Ma et al., 2004), ND11231*12 (Emebiri et al., 2005), and Pompadour (Gupta et al., 2010) varieties. Many researchers believe that there is a cluster of multiple genes for resistance to *P. teres* f. *teres* in this region of chromosome 6H or suggest multiple allelism of a single gene (Manninen et al., 2006; Abu-Qamar et al., 2008; Gupta et al., 2011). In addition, the following QTL were also identified in the same 6H centromeric region in barley: QTL *Rphq3* controlling resistance to *Puccinia hordei* (Marcel et al., 2007); $QTL_{Triton}Rrs6H_{271\_}$ to *Rynchosporium secalis* (Wagner et al., 2008); and *rym 15* to viruses BaMMV/BaYMV (Le Gouis et al., 2004). The clustering and multiple allelism of genes for resistance to both obligate and hemibiotrophic parasites are common phenomena caused by intra- or intermolecular exchanges of DNA segments that have direct or inverted repeat sequences that contribute to an unequal crossing over. Duplication and subsequent mutational changes have occurred in the ancestral gene during coevolution of the host and pathogen (Dyakov et al., 2001).

The parents of DH population B differ in their resistance to two pathogens. Variety Ranniy 1 was characterized by juvenile resistance to *P. teres* f. *teres*; and variety Zernogradsky 813 to *C. sativus*. At the same time, differences in the reaction types of parents to each pathogen were not as significant as in population A. The presence of *P. teres* f. *teres* isolates, avirulent to both varieties, indicates that the variety Zernogradskiy 813 has also a certain specific resistance to the pathogen. This is apparently due to transgressive segregation in the DH population B, identified in the majority of the *P. teres* f. *teres* isolates studied. The isolate specificity of the identified QTL was shown for both pathogens. In total 11 QTL controlling the resistance to 12 isolates of *P. teres* f. *teres* were found on all barley chromosomes; and 14 QTL controlling the resistance to 12 isolates of *C. sativus* on all chromosomes, except on 4H.

The novelty of QTL identified for barley was determined in accordance with published data on QTL for resistance to net and spot blotches. As can be seen from Tables 3 and 4, the majority of loci identified in the interval in which QTL controlling resistance to *P. teres* f. *teres* and *C. sativus* have already been detected. Four new isolate-specific QTL, controlling resistance to *P. teres* f. *teres*, were identified in the DH population B: on chromosome 1H in the range of 75–79 cM and 96–107 cM, on chromosome 4H within the interval of 3–16 cM, and on chromosome 5H within the range of 56–92 cM (Table 3). New QTL, associated with resistance to *C. sativus*, were identified on chromosome 2H in the intervals of 21–45 cM and 59–70 cM; on chromosome 3H, in the interval of 75–122 cM; on chromosome 5H, in the interval of 80–92 cM; and on chromosome 6H, in the interval of 87–119 cM (Table 4).

The isolate specificity of minor genes for resistance of barley to *P. teres* f. *teres* has been shown previously (Ho et al., 1996; Afanasenko et al., 1999; Manninen et al., 2006; Gupta et al., 2010). Apparently the nature of the relationship in the pathosystem "host with partial (incomplete) resistance–pathogen" is determined by the type of "small gene-for-small gene." The same conclusion was reached by researchers who have studied the localization of the genetic determinants of resistance in *Pyrenophora graminea* species, closely related to barley (Arru et al., 2003).

The number of detected QTL significantly exceeded the number of major resistance genes in all the studied pathosystems. There are only two genes determining strong resistance to the pathogen of spot blotch: *Rcs5*, localized on chromosome 7H (Steffenson et al., 1996) and *QRcs1* localized on chromosome 1H (Grewal et al., 2012), as well as 12 QTL on all barley chromosomes, except for 4H and 6H (Roy et al., 2010; Grewal et al., 2012). The use of molecular markers for genetic determinants of resistance in breeding greatly simplifies the process of combining several genes and QTL in the same genotype. In this respect, it has become particularly important to identify the combinations of genes for resistance to different pathogen races and to different types of pathogens that are the most useful for breeding. Epistasis has been demonstrated by combining some loci controlling resistance, including resistance to *P. teres* f. *teres* (Gupta et al., 2010). On the other hand, we have identified a combination of genes for resistance, which has no corresponding isolates, with an appropriate combination of complementary virulence genes in the natural populations of the net blotch pathogen (Afanasenko and Novozhilov, 2009).

The presence of sources and donors for barley resistance to helminthosporiotic blotches in the Laboratory of Plant Resistance to Diseases of the All-Russia Research Institute for Plant Protection, characterized by their efficiency in different agro-climatic conditions, offers the prospect of creating DH populations and identifying the genetic diversity of resistance by genetic mapping. Research conducted in this direction will contribute not only to the implementation of the genetic potential of resistance to protect crops from diseases but also to the accumulation of fundamental knowledge on the structural and functional organization of the genetic determinants of plant resistance to diseases.

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