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PHYSIOLOGICAL AND GENETIC COMPONENTS OF BLACK ROT RESISTANCE IN DOUBLE HAPLOID LINES OF *Brassica rapa* L.

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Abstract

In some vegetation seasons black rot may damage up to 80 % of cabbage, turnip, rapeseed, mustard crop all over the world including Russia. To prevent the spread of black rot is difficult, and it is almost impossible to fight a pathogen penetrating into a susceptible plant. Among *Brassica rapa* L., the disease is most dangerous for root crops and leaf crops. Data on loci which determine the plant-specific resistance of *B. rapa* plants to black rot is still extremely limited. This study is the first to estimate resistance to four races of *Xanthomonas campestris* pv. *campestris* (Pam.) Dow., the causative agent of black rot in *Brassicaceae*, in the lines of doubled haploids of two *B. rapa* mapping populations, DH38 (♀P175 × ♂P143) and DH30 (♀P115 × ♂P143). Here, we report data on identification and mapping the linkage groups and QTLs associated with physiological resistance to strains PHW231 (race 1), HRI5212 (race 3), HRI1279a (race 4), and B-32 (race 6). For three of these races, OTLs have not been mapped so far. The study revealed lines which were resistant or hypersensitive to the four races of black rot agent. Monogenic non-linked inheritance of resistance to these races prevailed. Significant correlation was found between response to an individual strain and general infection in plants. A total of 13 QTLs which control resistance to four races of the black rot pathogen were identified for DH30 population and 19 QTLs were found for DH38 population. All detected loci did not change their localization during two years of investigation. The most important loci responsible for manifestation of physiology resistance to different races of black rot pathogen in DH30 were mapped in the linage groups A01, A03 and A07, whereas in DH38 these were in A03, A06 and A08. SSR analysis of the lines contrast in resistance to individual races of the pathogen revealed the microsatellite markers linked to the loci which control resistance to several races of black rot agent. So we have found effective molecular descriptors of *B. rapa* black rot resistance to each race separately and to the pathogen as such. The obtained data are of interest in elucidation of basic physiological and genetic mechanisms of gene-to-gene interaction and *B. rapa* resistance to different races of *X. campestris* pv. *campestris*.

Keywords: *Brassica rapa* L., *Xanthomonas campestris* pv. *campestris* (Pam.) Dow., black rot resistance, QTL mapping, SSR markers, molecular screening

Vascular bacteriosis is the most harmful and widespread bacterial disease of plants of the *Brassicaceae* family in the world [1, 2]. In some years, it affects up to 80% of the crops of cabbage, turnip, rape, mustard, including in Russia. It is difficult to prevent the spread of vascular bacteriosis, and the only means is to use disinfected seed and to eliminate potential sources of

infection in the field [3]. Fighting the pathogen that has penetrated a susceptible plant is almost impossible. Vascular bacteriosis in cabbage is caused by pathovar *Xanthomonas campestris* pv. *campestris* (Pam.) Dow. (hereinafter *Xcc*), which includes a large number of races [4]. The pathogen penetrates the plant through hydathodes, stomata and mechanical damage, and colonizes the xylem. Symptoms of vascular bacteriosis include edge chlorosis on the leaves, necrosis and darkening of the leaf veins and conductive tissues inside the stem. In the species *Brassica rapa* L., the disease is most dangerous for turnip root crops and leaf crops [5], including the widespread Chinese cabbage [6].

The *B. rapa* species combines important oilseeds, vegetables and feed crops, and serves as a model object for genetic and molecular research. Despite the high importance of this species for human nutrition, there are few publications on the genetic nature and inheritance of morphological, physiological, immunological and other economically valuable traits in *B. rapa*. For example, the geographical and taxonomic distribution of race-specific resistance to phytopathogenic xantomonads in samples of *B. rapa* and *B. napus* was studied [7]. A high frequency of resistance to race 4 was found among the *B. rapa* subspecies of Central Asian and Japanese origin and in *B. napus*. For the first time, donors were found of the complex resistance of *B. rapa* to all used races of the pathogen and sources of resistance to races 1 and 3 among the samples of *B. rapa* and *B. napus*. QTL (quantitative trait loci) identification using RFLP (restriction fragment polymorphism) and RAPD (random amplified polymorphic DNA) markers showed that the plant response is associated with several additive loci in different linkage groups [8]. The *Xcc* gene of resistance to race 4 in Chinese cabbage (*B. rapa*) is located about 3 cM from the clubroot resistance locus [9]. Inheritance of resistance to three *Xcc* races was studied using hybrids from crossing resistant and susceptible *B. oleracea*, *B. carinata* and *B. napus* lines [10]. A single dominant *Xca4* locus (resistance to race 4) was mapped in the doubled haploid lines that were used to create an RFLP map. The *Xca4* locus was located in the N5 linkage group of the A genome in the *B. napus* species, thereby confirming that resistance was inherited from *B. rapa*. *Xca4* was the first mapped main locus controlling race-specific resistance to *Xcc* in *Brassica* species. British and Spanish scientists [11] studied the inheritance of resistance to two the most common *Xcc* races 1 and 4 in the segregating generation F_2 from crossing a sample of Chinese cabbage B162 with non-specific resistance and a susceptible inbred R-o-18 line. They created a genetic linkage map with a total length of 664 cM based on 223 AFLP (amplified fragment length polymorphism) and 23 microsatellite markers. Resistance to both races correlated. The authors localized a cluster of highly significant resistance QTLs determining 24-64% of the trait variability in A06 linkage group. Two additional minor resistance QTLs to race 4 were found in A02 and A09 linkage groups. Earlier, we studied two mapping populations of *B. rapa* in order to identify QTLs that determine morphological and phenological characteristics [12-15]. QTL positions have been identified (mainly in A02, A03, A06, A07 and A09 linkage groups), where one locus controls several traits, which indicates the important role of this locus in plant development.

At the same time, there is still very little information on the location of loci that determine the race-specific resistance of *B. rapa* plants to vascular bacteriosis. This study is the first identification i) of chromosomal loci that control resistance to the four most common races of the causative agent of vascular bacteriosis in doubled haploid lines of two *B. rapa* mapping populations, and ii) of new resistance donors found in these lines.

Our goal was to identify and map the genetic components involved in *Brassica rapa* in the physiological and genetic mechanisms of gene-gene-gene re-

lationships between a plant and various races of the causative agent of vascular bacteriosis.

Techniques. We studied two mapping populations of doubled haploid lines (DHL) of *B. rapa*, the DH38 (♀P175 × ♂P143) and DH30 (♀P115 × ♂P143) that were obtained at Wageningen University (Netherlands), by the culture of microspores of a single F_1 plant in each crossing combination of three the main phenotypically distinct subspecies of the species: oil yellow sarson (original sample YS-143, k-FIL500, male parent DHL P143), Chinese leafy/stalked cabbage (PC-175, variety Nai Bai Cai, first mother parent DHL P175) and root turnips (VT-115, variety Kairyou Hakata, second maternal parent DHL P115) [15]. The lines of mapping populations DH30 and D38 were genotyped, respectively, with 299 and 294 AFLP and SSR markers. For the SSR analysis, we used 100 markers that were developed in the Multinational Brassica Genome Project (MBGP) (http://www.brassica.info).

For artificial infection, we used strains of four races of *Xanthomonas campestris* pv. *campestris* (Pam.) Dow. (hereafter *Xcc*) — PHW231 (race 1), HRI5212 (race 3), HRI1279a (race 4), B-32 (race 6) (strains were courtesy of Dr J. Vicente and Dr N.W. Schaad). The bacteria were stored at –84 °C; the inoculum (10^6 cells/ml) was obtained from a 2-day culture in the Kings B medium.

The evaluation of 64 duplicated haploid lines of mapping populations (26 population lines of DH30 and 38 population lines of DH38) and their parental forms for *Xcc* resistance took 2 years. The plants were grown in pots of 10 cm in diameter, in a greenhouse at 20/16 °C (day/night) and a 16-hour photoperiod until 3-4 true leaves. Three true leaves on each plant were inoculated in approximately 10 points, pinching half of the leaf near the veins 2-3 mm from its edge, with surgical forceps dipped in the bacterial suspension [16]. Each plant was inoculated with all *Xanthomonas* isolates. After inoculation, the plants were placed in a humid chamber for 24 hours, then in a greenhouse at 24 °C (day/night) for 2 weeks. The first signs of vascular bacteriosis appeared 10 days after inoculation. Accounting was carried out on a 4-point scale: 0 — no signs, 1 — necrosis around the point of inoculation (hypersensitivity reaction, HSR); 2 — necrosis around the inoculation point and chlorosis up to 0.5 cm in diameter; 3 — development of typical V-shaped necrosis. Annually, 3 independent tests were performed at different times, with 2 replications for each sample. Resistant samples were additionally inoculated to confirm the result.

To study the nature of the inheritance of the reaction to the pathogen in the winter greenhouse conditions, we compared 33 combinations of back-crossed plants with contrasting resistance to a particular race. The crossing was repeated 3 times, using forced pollination, with the application of pollen on castrated buds 3-4 days before the blooming. After the autogamic pollination of F_1, F_2 progeny were obtained, in which the splitting was evaluated by resistance to pathogen strains.

The significance of differences between the samples in the proportion of plants resistant to each race was determined by analyzing the variance, using the χ-square test at a statistical significant level of 95% [17]. The interdependence between the response of samples to different races of the pathogen was established by the Pearson correlation analysis [18], using Statistica 6.0 software (StatSoft, Inc., USA).

The QTL analysis of 64 doubled haploid lines of two mapping populations of *B. rapa* DH38 and DH30 was performed as described above [15], using MAPQTL 6.0 [19] software to establish the presence and location of candidate loci in the linkage group (5 cM mapping interval), LOD values (logarithm of odds) ($P = 0.05$) and degrees of variation of QTL-related resistance traits to dif-

ferent races, for each indicator of resistance and each population. The significance of each LOD was established by the permutation method (1000 repetitions). To establish the number and exact location of the identified QTLs on the genetic map, we used interval mapping assuming one QTL in the interval between the linked markers with a certain degree of recombination [12]. In order to efficiently identify linkage groups and establish their number, we used an integrated genetic map of *B. rapa* with a total length of 1068 cM, saturated with molecular AFLP and SSR markers [15]. AFLP and SSR markers, due to their neutrality [12, 14], cover the entire *B. rapa* genome, with the distance between markers of 2.27 cM on average. The genetic values of each of the possible genotypes in the two marker loci were expressed as a function of the values of the respective QTL and determined the frequency of recombination between the QTL and its flanking markers. When translating recombination data into distances in the linkage groups we used Kosambi map function [20].

To verify associations, a marker/trait was genotyped using MBGP's SSR markers, for which we found a link to resistance to certain *Xcc* races: BRMS-014, BRMS-043, BRMS-050, BRMS-051, BRMS-096 (Japan), SSR-87 and SSR-89 (China), Na10D09, Na12E02, Na12H09 and Ra2E12 (United Kingdom) (https://vegmarks.nivot.affrc.go.jp/VegMarks/app/page/marker). DNA for the PCR analysis was extrcted from young green leaves as described [21]. The PCR was performed in a 12.5 μl mixture containing 10× incubation buffer (1.25 μl), 0.25 μl dNTP mixture (10 mM), 0.25 μl each of the recommended primers (10 pmol/μl), 0.1 μl of Taq DNA polymerase (5 U/μl) (QBiogene, Germany) and 20 ng of genomic DNA. Amplification was carried out in a thermal cycler C-1000 (Bio-Rad, USA). PCR mode: 94°C 3 min; 30 cycles — 94°C 30 s, 55°C 30 s, 72°C 30 s; final elongation 75°C 7 min; then 4°C without time limit. Amplification products were separated by electrophoresis in a 1.8% agarose gel, stained with ethidium bromide, and documented using the BioDoc system (Bio-Rad, USA).

Results. The studied lines (Table 1) demonstrated a reaction from resistance to high susceptibility. In general, similar results were obtained in different seasons, although a higher susceptibility of some plants was noted in the spring. Genotypes that exhibit immunity or hypersensitivity to all four races of the pathogen have been distinguished: in DH30 lines 35A, 69B, 98E; in DH38 lines 1g, 32d, 97a, 172a. In the DH30 population, responses to infection with strains B-32 (race 6) and 1279a (race 4) ($r = 0.64$), as well as HRI5212 (race 3) and PHW231 (race 1) ($r = 0.43$) were the most correlated. There was a significant correlation (r from 0.54 to 0.73) between the response to individual strains and the total damage of plants. Three strains turned out to be resistant to all strains of the pathogen. When inoculated with races 3, 4 and 6, the lines demonstrated susceptibility more often. The distribution of lines by response to infection with races 1 and 3 most closely corresponded to the monogenic inheritance of the trait. Segregation by the response to the strain B-32 of the most virulent race 6 (increase in the number of susceptible lines) was the most different from what was expected. In the DH38 population, there was a significant relationship between the response to strains B-32 (race 6) and 1279a (race 4) ($r = 0.52$), PHW231 (race 1) ($r = 0.4$), and between the response to individual strains and the general damage (r from 0.58 to 0.78). Four lines were resistant to all tested pathogen strains. The segregation for the response to PHW231 strain (increase in the number of susceptible lines in the DH38 population) deviated the most from what we expected. The distribution of lines by sensitivity to race 4 most closely corresponded to the monogenic inheritance of the trait (see Table 1).

1. Results of artificial infection of plants mapping populations of doubled haploid lines of *Brassica rapa* with *Xanthomonas campestris* pv. *campestris* (Pam.) Dow.

Line	Autumn and winter				Total points by year	Spring			
	B-32	1279a	5212	231		B-32	1279a	5212	231
DH30 (♀P115 × ♂P143)									
P1 115	0	1.0	2.0	0	3/4	0	2.0	2.0	0
P2 175	0	1.0	0	0	1/6	2.0	2.0	0	2.0
P3 143	0	0	2.0	0	2/2	0	0	2.0	0
6A	2.0	2.0	1.0	2.0	7/8	2.0	2.0	2.0	2.0
$18M_2$	1.0	0	0	0	1/4	2.0	1.0	1.0	0
28A	2.0	1.0	1.0	0	4/7	2.0	2.0	2.0	1.0
35A	1.0	0	0	0	1/4	1.0	1.0	1.0	1.0
38B	1.0	1.0	2.0	2.0	6/6	1.0	1.0	2.0	2.0
44A-V2	3.0	3.0	1.0	1.0	8/8	3.0	3.0	1.0	1.0
67	2.0	0	2.0	2.0	6/6	2.0	0	2.0	2.0
69B-1	1.0	1.0	1.0	0	3/3	1.0	1.0	1.0	0
79C-2	1.0	1.0	2.0	0	4/4	1.0	1.0	2.0	0
97	3.0	3.0	1.0	1.0	8/8	3.0	3.0	1.0	1.0
98E-2	1.0	1.0	1.0	1.0	4/4	1.0	1.0	1.0	1.0
110A-3	2.0	1.0	2.0	0	5/5	2.0	1.0	2.0	0
127c	1.0	1.0	3.0	3.0	8/10	2.0	2.0	3.0	3.0
160A	1.0	2.0	1.0	1.0	5/8	2.0	2.0	2.0	2.0
163A-2	2.0	2.0	1.0	2.0	7/7	2.0	2.0	1.0	2.0
164A-A^h	2.0	2.0	2.0	1.0	7/3	2.0	0	0	1.0
178A	2.0	1.0	0	1.0	4/7	2.0	2.0	1.0	2.0
189-A	2.0	1.0	3.0	3.0	9/10	2.0	2.0	3.0	3.0
206A^h	2.0	2.0	3.0	1.0	8/8	2.0	1.0	2.0	3.0
215c-1	3.0	2.0	2.0	2.0	9/9	3.0	2.0	2.0	2.0
238A	1.0	2.0	1.0	1.0	5/6	1.0	2.0	2.0	1.0
251-1	1.0	2.0	2.0	1.0	6/6	1.0	2.0	2.0	1.0
94	3.0	3.0	1.0	нд	7/8	3.0	3.0	2.0	0
113 i	3.0	3.0	2.0	нд	8/10	3.0	3.0	2.0	2.0
188A-3	1.0	2.0	1.0	нд	4/6	1.0	2.0	1.0	2.0
192H-1^h	3.0	3.0	3.0	нд	9/12	3.0	3.0	3.0	3.0
Resistant:susceptible	11:15	11:15	14:12	14:8		8:18	10:16	10:16	13:13
DH38 (♀P175 × ♂P143)									
1g-2	1.0	0	0	1.0	2/2	1.0	0	0	1.0
13a-VI	1.0	1.0	1.0	2.0	5/5	1.0	1.0	1.0	2.0
15в-3	0	0	2.0	1.0	3/3	0	0	2.0	1.0
23a-VI	2.0	1.0	2.0	2.0	7/7	2.0	1.0	2.0	2.0
25a-VI	2.0	1.0	1.0	2.0	6/6	2.0	1.0	1.0	2.0
32d-1	1.0	1.0	0	0	2/2	1.0	1.0	0	0
36c-3	3.0	3.0	2.0	2.0	10/10	3.0	3.0	2.0	2.0
40c-1^h	3.0	3.0	3.0	3.0	12/12	3.0	3.0	3.0	3.0
44a-1	3.0	2.0	2.0	0	7/7	3.0	2.0	2.0	0
51 f-1	1.0	2.0	2.0	2.0	7/7	1.0	2.0	2.0	2.0
52a-1	3.0	1.0	1.0	3.0	8/8	3.0	1.0	1.0	3.0
55h-1	2.0	1.0	2.0	1.0	6/6	2.0	1.0	2.0	1.0
57d-V3	0	0	1.0	2.0	3/3	0	0	1.0	2.0
58d-1	2.0	3.0	2.0	2.0	9/9	2.0	3.0	2.0	2.0
59a-2^h	2.0	3.0	1.0	2.0	8/6	2.0	0	2.0	2.0
62a-2^h	2.0	3.0	3.0	3.0	11/12	3.0	3.0	3.0	3.0
64-b-V-2^h	3.0	1.0	2.0	2.0	8/6	3.0	0	0	3.0
65a-2	3.0	2.0	1.0	2.0	8/8	3.0	2.0	1.0	2.0
72a-VI	2.0	2.0	1.0	1.0	6/6	2.0	2.0	1.0	1.0
75a-1	1.0	1.0	2.0	2.0	6/6	1.0	1.0	2.0	2.0
76в-1	1.0	2.0	1.0	1.0	5/8	3.0	3.0	1.0	1.0
78a-1	2.0	2.0	3.0	3.0	10/9	2.0	3.0	3.0	1.0
80a-1^h	2.0	2.0	2.0	2.0	8/12	3.0	3.0	3.0	3.0
92в-1	3.0	2.0	1.0	1.0	7/7	3.0	2.0	1.0	1.0
95a-1	3.0	1.0	3.0	2.0	9/9	3.0	1.0	3.0	2.0
97a-3	0	0	1.0	1.0	2/2	0	0	1.0	1.0
103a^h	3.0	3.0	0	2.0	8/7	3.0	0	1.0	3.0
123a	1.0	2.0	2.0	2.0	7/7	1.0	2.0	2.0	2.0
124a-1	1.0	3.0	2.0	2.0	8/8	1.0	3.0	2.0	2.0
127a-1	3.0	2.0	2.0	2.0	9/9	3.0	2.0	2.0	2.0
134a-4	3.0	1.0	2.0	2.0	8/8	3.0	1.0	2.0	2.0
136VI	0	3.0	3.0	3.0	9/8	1.0	1.0	3.0	3.0
142b-2	1.0	1.0	2.0	2.0	6/6	1.0	1.0	2.0	2.0
151a-2	1.0	1.0	1.0	2.0	5/5	1.0	1.0	1.0	2.0
154в	0	0	2.0	2.0	4/4	0	0	2.0	2.0

Table 1 continued

160c	0	0	2.0	1.0	3/3	0	0	2.0	1.0
169a-1[h]	2.0	1.0	2.0	2.0	7/6	2.0	0	2.0	2.0
172a	0	0	1.0	0	1/1	0	0	1.0	0
Resistant:susceptible	17:21	20:18	15:22	11:26		16:22	23:25	15:23	12:26

Note. B-32 — strain B-32, race 6; 1279a — strain HRI1279a, race 4; 5212 — strain HRI5212, race 3; 231 — strain PHW231, race 1. Sensitivity rating: 0 — no signs, 1 — hypersensitivity, 2 — weak susceptibility (affected area less than 5 mm or chlorosis), 3 — susceptibility, development of typical symptoms; [h] — lines used for hybridization.

2. Segregation in F_2 after crossing plants with contrasting resistance, selected in the mapping populations of the doubled haploid lines of *Brassica rapa* in artificial infection with *Xanthomonas campestris* pv. *campestris* (Pam.) Dow.

Crossing combination	Number of plants	B-32	1279a	5212	231
DH30 (♀P115 × ♂P143)					
206(r) × 192(s)	56	0:56	42:14[a, *]	0:56	0:56
192(s) × 206(r)[r]	64	0:64	50:14[a, *]	49:15[a, *]	0:64
164(r) × 192(s)	48	0:48	32:16[a]	37:11[a, *]	0:48
192(s) × 164(r)	36	0:36	26:10[a, *]	24:12	0:36
DH38 (♀P175 × ♂P143)					
80(s) × 64(r)	36	0:36	28:8[a, *]	26:10[a, *]	3:33
64(r) × 80(s)	42	1:41	31:11[a, *]	28:14[a]	1:41
62(s) × 59(r)[r]	40	32:8*	26:14[a]	0:40	0:40
59(r) × 62(s)	28	0:28	0:28	0:28	0:28
40(s) × 64(r)	48	0:48	0:48	30:18[a]	0:48
64(r) × 40(s)	64	0:64	49:15[a, *]	50:14[a, *]	0:64
62(s) × 136(r)	20	0:20	0:20	0:20	0:20
136(r) × 62(s)	20	0:20	16:4[a, *]	0:20	0:20
80(s) × 169(r)[r]	24	0:24	20:4[a]	0:24	0:24
169(r) × 80(s)	20	0:20	0:20	0:20	0:20
62(s) × 64(r)	56	0:56	0:56	0:56	0:56
64(r) × 62(s)[r]	60	0:60	0:60	45:15[a]	0:60
62(s) × 103(r)	24	0:24	18:6[a]	0:24	0:24
103(r) × 62(s)	35	0:35	28:7[a, *]	1:34	2:33
169(r) × 62(s)[r]	27	0:27	18:9[a]	0:27	0:27
62(s) × 169(r)	18	0:18	0:18	0:18	0:18
78(s) × 59(r)	28	0:28	0:28	0:28	20:8[a, *]
59(r) × 78(s)[r]	49	0:49	40:9[a]	0:49	35:14[a]
78(s) × 103(r)	56	0:56	40:16[a]	0:56	43:13[a, *]
103(r) × 78(s)	68	0:68	50:18[a, *]	0:68	48:20[a]

Note. B-32 — strain B-32, race 6; 1279a —strain HRI1279a, race 4; 5212 —strain HRI5212, race 3; 231 —strain PHW231, race 1; r — resistant parental line, s — susceptible parental line, [r] — a combination with a reciprocal effect on plant response to infection with a pathogen; [a] — combinations with segregation close to 3:1.
* Segregation corresponding to the expected 3:1 is reliable at 95% accuracy according to the χ-square test.

When studying the nature of the inheritance of the plant sensitivity to the pathogen, we selected for hybridization 20 lines, i.e. 7 susceptible to all strains of the pathogen, 13 resistant to only one race and susceptible to the rest (Table 2). Crossing was easy, resulting in 15-20 seeds per pods. The lines with a reduced self-compatibility, which set 6-7 seeds per pod with autogamous pollination, were rejected. All F_1 plants of both mapping populations showed resistance to at least one out of 4 races of the pathogen. After autogamous pollination, F_1 plants yielded 20 populations of F_2 from the mapping DH38 population lines and 4 F_2 progeny from the DH30 population lines, in which we identified segregation by resistance to the four pathogen strains. The observed ratio of the resistant and susceptible forms in F_2 and the subsequent analysis of hybrids of the selected resistant and susceptible lines showed that the race-specific resistance is mainly determined by a single dominant locus (see Table 2).

Three out of four populations of F_2 DH30 showed monogenic resistance to race 4 (strain HRI1279a) and to race 3 (strain HRI5212), and one showed resistance with a possible deviation from the monogenic (shift toward susceptibility). Interestingly, the parent lines 206 and 164 (see Table 1) were resistant only to race 1. Among the twenty F_2 populations from the crossing of lines of the mapping population DH38, ten had monogenic resistance to race 4 (strain HRI1279a), including resistance with deviation from 3:1 segregation; five had monogenic resistance to race 3 (strain HRI5212); four had monogenic resistance to race 1 (strain PHW231); and one had monogenic resistance to race 6 (strain B-32). Obviously, the resistance to race 4, which is dominant in populations of *B. rapa* [7] species, retained its significance when crossing parental lines (even if the parent has a susceptibility reaction). In 8 out of 12 pairs of parents, the reciprocal effect of crossing was manifested, which gives a possible explanation for

the appearance of stability in the progeny of the two original parental lines that are susceptible to race (when creating F_1). Of these eight pairs, six had reciprocal asymmetry of reaction to infection by race 4; three had reciprocal asymmetry of response to infection with race 3; one pair showed reciprocal asymmetry to race 6; asymmetry to race 1 was not observed.

3. Results of QTL analysis of resistance to *Xanthomonas campestris* pv. *campestris* (Pam.) Dow. in mapping populations of doubled haploid lines *Brassica rapa* in artificial infection

Strain (race)	Linkeage groups	LOD score (minimum-maximum)	Percentage variation of the trait phenotypic variability, % Expl.
	DH30 (♀P115 × ♂P143)		
PHW231 (race 1)	A03, A07	1.09-1.14	19.7-21.1
HRI5212 (race 3)	A01, A03, A06, A07	1.16-2.35	19.9-36.3
HRI1279a (race 4)	A02, A03, A05	0.77-3.08	13.7-44.6
B-32 (race 6)	A01, A03, A04, A05, A07, A09	1.02-1.82	17.8-29.5
	DH38 (♀P175 × ♂P143)		
PHW231 (race 1)	A02, A04, A06, A08	0.75-2.31	9.7-26.9
HRI5212 (race 3)	A01, A05, A10	0.72-1.81	9.3-21.8
HRI1279a (race 4)	A03, A06	1.06-2.93	13.4-32.7
B-32 (race 6)	A01, A03, A04, A06, A08	0.66-3.44	8.5-37.2

QTL analysis (Table 3, Fig. 1) (Fig. 1, see the online version of the article on the website http://www.agrobiology.ru) revealed 13 QTLs controlling resistance to four races of vascular bacteriosis pathogen in DH30. At the same time, 10 loci stably maintained localization in both years of the research (LOD 0.77-3.80; loci with LOD > 1.1 are usually considered). These are loci at the top of linkage groups A01 and A03 which control resistance to race 3 of the pathogen, 2 loci at the bottom of A03, controlling resistance to races 3, 4 and 6, loci in the middle of A05 (they control resistance to race 4, also to race 6 in spring), and 2 QTLs in the lower part of A07 for resistance to race 3, also to race 6 in the autumn and winter and to race 1 in spring). QTL associated with resistance to race 6 was found in the middle of the linkage groups A01 and A04 and in the lower half of A09 (the position of QTL in A09 differed by 7 cM among years). The variability explainable by the found QTLs ranged from 13.7 to 44.6%. The effects of genes in all established loci are additive. The highest and most stable LOD values (in terms of years) were characteristic of three QTLs located in the upper part of A01 and at the bottoms of A03 and A07. Therefore, the most important loci responsible for the manifestation of resistance to different races of the causative agent of vascular bacteriosis in DH30 *B. rapa* are in the linkage groups A01, A03 and A07.

For the DH38 population, 19 QTLs were revealed (see Table 3), and all loci consistently maintained localization during study (LOD 0.66-3.44). Six QTLs controlled resistance to races 1 and 4, 4 — to race 3, and 8 — to race 6. At the same time, four loci of resistance to races 4 and 6 were located in the middle and lower parts of A03, three loci of resistance to races 1, 4, and 6 were located in the middle and lower parts of A06, and three loci of resistance to races 1 and 6 were located in the upper part of A08. That is, the most significant loci of resistance to vascular bacteriosis agent in the DH38 lines are located in linkage groups A03, A06 and A08. At the top of A10, the earlier mapped CAPS marker of the locus of resistance to race 5212-I *FLC1* was found, which is also associated with many important physiological and biochemical characteristics (transition time to the generative phase, ascorbic acid and carotene content) [15].

The QTLs that control resistance to races 3 and 6 in the lines of both mapping populations were located in close positions in A01; to races 4 and 6 — at the bottom of A01, and to race 6 — in the middle of A04. For both mapping populations, the A03 loci were the most important for the genetic control of re-

sistance to *Xcc*. Note that at the bottom of the A03 group we found the previously mapped locus *BrFLC5* which controls the most important features: transition time to flowering, productivity and biochemical composition [15].

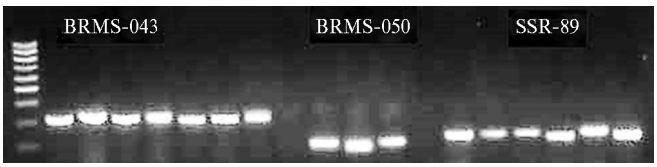

Fig. 2. An example of PCR analysis of the susceptible (+) and resistant (–) to *Xanthomonas campestris* pv. *campestris* (Pam.) Dow. lines of mapping populations DH30 (♀P115 × ♂P143) and DH38 (♀P175 × ♂P143) of *Brassica rapa* with markers BRM-S043, BRMS-050 and SSR-89: 1 — DH30/18 (–), 2 — DH30/97 (+), 3 — DH30/35 (–), 4 — DH38/1 (–), 5 — DH38/36 (+), 6 — DH38/62 (+), 7 — DH38/160 (–); 9 — DH30/17 (+), 10 — DH30/178 (–), 11 — DH30/192 (+); 13 — DH30/35 (–), 14 — DH30/192 (+), 15 — DH30/238 (–), 16 — DH30/18 (–), 17 — DH30/160 (–), 18 — DH30/206 (+);the leftmost lane is a 100 bp molecular weight marker (10 fragments from 100 to 1000 bp) (SibEnzyme, Russia). The expected amplicon size for BRMS-043 is 318 bp, for BRMS-050 164 bp, for SSR-89 199 bp.

SSR analysis of lines of both mapping populations contrasting in resistance (Fig. 2) showed that the molecular marker BRMS-043 associated with resistance to race 4 in the DH30 population and to three races in the DH38 population is present in the expected position (318 bp) in resistant DH38 lines and in a position close to the expected in stable DH30 lines (216 bp). The amplicon size is 321 bp in the susceptible line 97 of the DH30 population, and 312 bp in the susceptible lines 36 and 62 of the DH38 population. The BRMS-050 marker associated with resistance to race 3 in DH30 population is present in the expected position (164 bp) in the stable line 178, while in the susceptible lines 127 and 192 the length of the identified fragment is 180 bp. The SSR-89 marker associated with the locus of resistance to race 3 and (with a minor LOD) to race 6 in the DH30 population is detected in the expected position (199 bp) in the resistant lines 18 and 35. The susceptible lines 192 and 206 (damage score 3), as well as the line 238 (previously assessed as stable) have a 204 bp fragment, and the previously stable line 160 has a 210 bp fragment. However, it should be noted that resistant lines 18 and 35 show no signs of damage, and the lines with controversial results show a hypersensitivity reaction. The BRMS-051 marker of the locus of resistance to race 5212 in the DH30 population is in the expected position of 262 bp in the stable line 18 and 5 bp above in the stable line 69 with a hypersensitivity reaction; in the susceptible line 206, the fragment size is 150 bp. The susceptible line 44 of the DH38 population carries Na12E02 marker associated with the locus of resistance to races 4 and 6 (the expected fragment length is 132 bp). Stable line 160 and line 52 with a hypersensitivity to race 4 and susceptibility to race 6 do not have this marker.

In summary, we found microsatellite markers of *B. rapa* loci of resistance to several races of the causative agent of vascular bacteriosis. In most cases, resistant genotypes with no signs of damage carry the marker in the expected position. It should be noted that the influence of the maternal cytoplasm on the manifestation of a stable phenotype, which we first identified in *B. rapa* in this work, most likely can significantly affect the localization and effect of the identified chromosomal QTLs. However, there is no recognized method for taking into account the interaction of nuclear and cytoplasmic genes in mapping plant resistance.

Vascular bacteriosis is especially harmful for cabbage *Brassica oleracea* L. [1], although it affects almost all species of the *Brassica* genus, including weed cruciferous and ornamental plants, and leads to significant annual economic losses. However, little is known about the genetic components and physiological

mechanisms of resistance to this disease in plants. Since phytopathogenic bacteria interact with plants according to the gene-to-gene principle, they are a convenient object for molecular genetic studies of relationships in the pathogen—plant system. In practice, knowledge of such mechanisms is important for the creation of resistant varieties which is the most economical and environmentally friendly method to combat the disease.

Obtaining resistant genotypes is complicated by the existence of at least nine races of the pathogen. The widespread races 1 and 4 are the most dangerous for the species *B. oleracea* [22, 23], therefore resistance to these two races is the minimum necessary condition for controlling the spread of vascular bacteriosis in cabbage plants. In studies of the *B. oleracea* samples, Dr J.D. Taylor and the team [3] found that resistance to races 1 and 4 was very low or absent, while resistance to less common races 2, 3 and 6 was relatively common. In contrast, race-specific resistance to races 1 and 4 is widely represented in other species of the genus *Brassica*, i.e. in *B. rapa*, *B. carinata* [3, 9], *B. napus* and *B. nigra* [24-26]. However, genetic control and mechanisms of resistance to different races of *Xanthomonas* in these species are still poorly understood. Moreover, not a single study has been conducted to identify and establish the chromosomal localization of quantitative trait loci that determine resistance to individual *Xcc* races in such an economically valuable species as *B. rapa*, the object of our research.

Bacteria are known to use effector proteins delivered to a plant cell by a Type 3 Secretion System (T3SS) to suppress constitutional stability stimulated by the microbe-associated metabolite profile (MAMPs). The variability of the presence, expression and amino acid sequence of the T3SS effectors depends on the race of the pathogen and reflects its host plant specificity [2]. The corresponding plant resistance genes respond to such inhibition of expression of resistance. Bacterial genes responsible for interaction with the plant are able to influence the general physiological and genetic processes in bacteria. Unfortunately, the mechanisms of interaction between the physiological and genetic systems of plants and phytopathogenic bacteria have not yet been largely revealed. This is due, primarily, to the lack of information about the natural polymorphism in the population of phytobacteria and a simple model system for analyzing such interaction, which obstructs detection of the chromosomal localization of genes and/or loci that determine quantitative resistance to diseases.

In *B. napus*, PI199947, later identified as *B. carinata* [23, 27], the dominant race-specific resistance to the pathogen of vascular bacteriosis was described, which is widespread among plants with the B genome [3, 6]. Race-specific resistance is usually associated with a hypersensitivity reaction (HSR) at the point of entry of an incompatible *Xcc* race through hydatods, but often only a partial HSR is observed [23]. Identification of QTL using RFLP and RAPD markers has shown that the plant response is associated with several additive loci in different linkage groups [8]. Earlier, Russian scientists [9] found that the resistance gene to race 4 in Chinese cabbage (*B. rapa*) is about 3 cM from the cruciferous keel resistance locus. The resistance gene *Rxc4* in *B. napus* has also been mapped [10].

In the genome-wide sequencing of the *B. oleracea* genome, three minor and one main QTLs were detected which are determining in the progeny resistance to one race *Xcc* [28]. In the forms of parental inbred lines, the authors identified 674521 SNP, without, however, indicating neither the inbreeding generation, nor the number of individual plants from each of the parental forms whose DNA were isolated to establish an SNP. It did not take into account that all individuals of the family obtained from one F_2 individual were genetically identical to each other after *n* generations (with the exception of the remaining

heterozygous in generation n –1), and that the DNA for full genome sequencing was not isolated from the same plant. Obviously, because of this, the share of dCAPS markers created by the authors and suitable for analysis was only 70%, and only half of them could be used to saturate the existing *B. oleracea* basemap. In addition, to create a genetic map and QTL mapping, Korean researchers [27] used the F_2 population with an average interval between markers of 3.88 cM, which is 1.61 cM higher than the average distance between the markers of the mapping populations of *B. rapa* haploid doubled lines in our studies. Since the Korean team used the cumulative DNA of the F_3 plants for mapping, without indicating that the F_3 plants were obtained as a result of individual self-pollination in F_2, in this case the expected segregation for one locus is not 1:2:1, but 3:2:3 because a heterozygous locus in F_2 has only one chance out of two to be fixed in F_3. The lines of doubled haploids are completely devoid of these shortcomings and can also be attributed to the so-called "immortal" populations, since they do not need to be re-created and each time saturated with molecular markers during QTL mapping, especially if it is carried out in different years and/or different ecological and geographical areas [12]. It should also be noted that we are not aware of any work in which SNP stability issues would be considered taking into account the frequency of both natural and artificial mutagenesis, recombinogenesis (primarily at the level of a single nucleotide) and the degree of degeneracy of the genetic code in the coding and non-coding regions of the genome. In our opinion, this would allow for more precisely limits of applicability of this type of molecular markers. Finally, the mapping of chromosome loci which determine the specific resistance of plants to each of the four races of *Xcc* has never been described until this work.

We found chromosomal loci of resistance to races 1 and 4 of the pathogen in the linkage group A06 of the mapping population DH38 lines and additionally to race 4 in the A02 group of DH30, which confirms the results of the QTL mapping of resistance to races 1 and 4 of *X. campestris* in the F_2 population from crossing high-inbred lines of yellow sarson R-o-18 and self-pollinated sample of Chinese cabbage B162 [11]. To date, this is the only study known to us which identifies the exact number and location of the QTLs that determine the resistance to two *Xanthomonas* races. In our studies, QTL resistance to four *Xanthomonas* races has been identified and mapped, for three of which QTL mapping has not previously been carried out. In addition, we established the effect of each QTL and the percentage of phenotypic variability caused by each of the identified QTLs for each race of the pathogen. Hybridological analysis established predominantly monogenic inheritance of the resistance. We also revealed the block structure of individual parts of the genome and the block character of inheritance. It has been shown that a single genomic block (QTL) may be responsible for resistance to different races of the pathogen. Molecular markers genetically linked to localized QTLs have been identified. DHL screening of *B. rapa* was carried out, and the species effective molecular genetic descriptors for resistance to vascular bacteriosis (for each race separately and for the pathogen in general) were identified.

In summary, we have for the first time identified and localized linkage groups and chromosome loci involved in the formation of the physiological stability of *Brassica rapa* to four races of *Xanthomonas campestris* pv. *campestris*. The lines of doubled haploids that are resistant to all the studied races of the pathogen of vascular bacteriosis are revealed. These lines can be included in the scientific and breeding programs as sources of the resistance. The obtained data is important for understanding the mechanism of *B. rapa* race specific resistance and, in the future, it can serve as a basis for an accurate quantitative assessment

of the effects of avr/pth genes and identified QTLs, as well as improvement of the methodology of similar work with other traits.

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