Loci that determine long-term resistance to blast of Russian rice varieties

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Abstract. In all rice-growing countries, including Russia, among the economically important, dangerous and harmful diseases of rice, the main role is played blast (pathogen *Pyricularia oryzae* Cavara). The problem of resistance of rice plants to disease is one of the main problems in modern breeding in most countries. The most effective way to protect rice from blast is using resistant varieties created with marker assistant selection (MAS). The article presents data on chromosomal regions that provide long-term resistance of varieties to pathogen in Russia. The groups of stable and unstable samples significantly differed in the presence of polymorphic loci on the fifth, sixth, eighth, ninth and second chromosomes, which reduces the complexity of evaluating selection material due to the primary screening of gene plasms by variability of resistance loci in the identified chromosomal regions.

To date, more than 100 genes that determine resistance to blast (Pi) in rice have been localized, but so far the data on the effectiveness of the isolated loci in the formation of the trait are contradictory [1-3]. Complicating the task is the absence of a sufficient number of differentiating varieties of the japonica subspecies [4-6]. It was previously established that even 5-7 pathogen resistance genes do not guarantee adaptability to biotic stress [7-9]. Difficulties are also created by the rapid process of mutating a pathogen and changing the racial composition of its populations, which has been significantly accelerated in recent years due to the use of new fungicides [10-12]. Our task was to isolate chromosomal regions that provide long-term resistance of varieties to pathogen in Russia.

To solve this problem, long-term data (2010-2015) on the resistance to blast of 26 domestic varieties were used. Varieties were divided into groups with different resistance to stress (Table 1).

Rice varieties were labeled using sixty molecular SSR markers, both neutral and locus-related to adaptiviity. Analysis of variance allowed us to establish a relationship between pathogen resistance and variety variability for individual SSR markers. Since the gene plasma in the study is genetically heterogeneous, loci with the most significant contribution

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to phenotype could be identified in the study. The influence of variety genotype on the manifestation of the trait was not taken into account. But, even with such assumptions, we identified three chromosomal regions that reliably (at a significance level of 0.05) contribute to the formation of trait in domestic varieties on fifth, sixth, and eighth chromosomes.

Table 1. Resistance to blast of Russian rice varieties, % of damage

Variety	Year						Average value, RBI,%	Resistance group
	2010	2011	2012	2013	2014	2015		
Snejinka	5.6	22.8	10.2	6.5	5.5	7.3	9.6	1
Sprint	26.7	20.0	–	–	–	–	23.3	1
Yujniy	17.2	34.4	15.6	45.6	15.5	–	25.6	1
Viola	36.7	25.6	–	–	–	–	31.1	1
Atlant	28.4	28.9	–	31.1	31.1	40.0	31.9	1
Anait	33.4	38.9	27.8	–	35.6	32.2	33.5	1
Narcicc	–	–	–	–	–	36.7	36.7	1
Guarant	38.9	33.3	34.4	41.1	38.6	-	37.2	2
Khazar	26.2	35.6	31.1	53.3	31.1	50.0	37.8	2
Phlagman	46.1	45.6	25.6	43.3	34.5	36.7	38.6	2
Jupiter	–	38.9	–	–	–	–	38.9	2
Leader	47.2	40.6	30.0	39.4	–	–	39.3	2
Jantar	45	48.9	40.0	33.3	36.6	–	40.7	2
Regul	45.6	45.6	34.4	50.2	32.4	–	41.6	2
Liman	43.9	37.8	–	44.5	43.7	–	42.4	2
Izumryd	55.6	37.8	42.2	47.8	47.8		46.2	2
Rapan	61.1	46.1	45.6	48.9	42.5	35.5	46.6	2
Serpentine	38.9	58.9	-–	–	–	–	48.9	2
Kurchanka	47.8	49.5	50.0	48.9	50.6	–	49.3	2
Khankaiskiy	–	–	37.8	61.1	–	–	49.4	2
Charm	48.4	52.2	64.4	30	62.2	50.1	51.2	3
Amethyst	–	–	50.7	56.7	51.8	–	53.0	3
Novator	52.2	46.7	53.3	65.6	53.3	–	54.2	3
Pavlovskiy	–	55.6	–	–	–	–	55.6	3
Phontan	60.1	–	–	–	–	–	60.1	3
Dalnevostochniy	–	–	–	–	–	76.7	76.7	3
RBI * - the intensity of the development of the disease								

To isolate DNA from rice seedlings and leaves, the STAB method with modifications was used. Polymerase chain reaction (PCR) and analysis of the obtained amplification product were carried out according to the methodology of the International Rice Institute [12]. The following PCR parameters were used in the experiment: initial denaturation for five minutes at 94 ^{0}C, thirty-five cycles: 60 sec. - denaturation 94 ^{0}C, 60 sec. - annealing of primers at 55 ^{0}C, 120 sec - synthesis of 72 ^{0}C; elongation - 420 sec. 72 ^{0}C. The volume of the PCR reaction is 10 µl: DNA - 2 µl), 1 µl (1 mm) of deoxynucleotide triphosphates; 3.7 µl H_2O; 1 µl of PCR buffer solution, 0.5 µl of each primer, 1 µl of Taq polymerase. Electrophoresis was carried out using a polyacrylamide gel at a voltage of 100 V. Data processing was carried out in the Statistica 10 program; the group selection method was

used for marking [13-14]. The studied markers were distributed across all 12 chromosomes of rice.

When dividing varieties into 2 groups, stable (group 1) and unstable (group 2 and 3), two loci were identified reliably with marker RM289 and RM5371 (Figure 1).

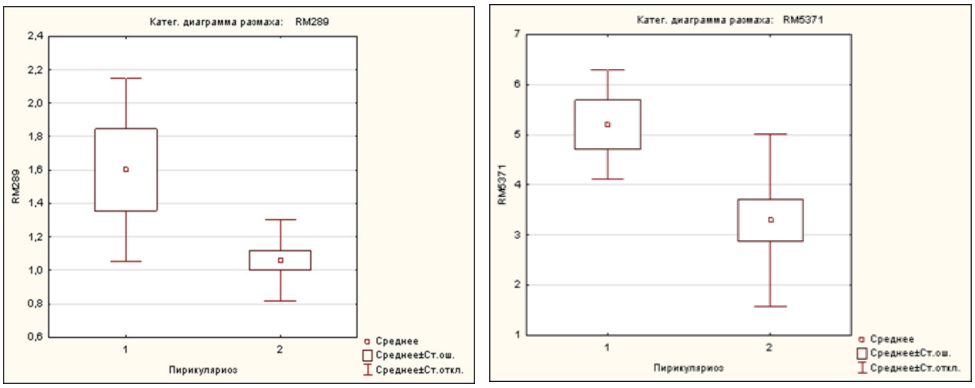

Fig. 1. Separation groups of Russian varieties with different resistance to blast using SSR molecular markers: a) RM289; b) RM5371

A decrease in the significance level to 0.09 increased the sensitivity of the method and made it possible to isolate another 5 loci with a possible arrangement of genes that determine stability (Table 2).

Table 2. SSR markers that reliably distinguish between groups of rice varieties contrasting in their resistance to blast

SSR marker	SS Effect*	df.*	MS Effect*	SS Effect*	df.	Ср. квад.	F*	p*
RM259	0.13690	1	0.13690	49.3176	20	2.46588	0.05552	0.816123
RM444	0.15455	1	0.15455	0.8000	20	0.04000	3.86364	0.063383
RM126	0.65508	1	0.65508	4.1176	20	0.20588	3.18182	0.089644
RM53	0.41925	1	0.41925	7.0353	20	0.35176	1.19185	0.287934
RM255	0.05348	1	0.05348	1.7647	20	0.08824	0.60606	0.445388
RM5361	1.73743	1	1.73743	11.0353	20	0.55176	3.14887	0.091204
RM5508	5.78396	1	5.78396	85.6706	20	4.28353	1.35028	0.258909
RM6024	0.15455	1	0.15455	0.8000	20	0.04000	3.86364	0.063383
RM2770	0.13690	1	0.13690	41.3176	20	2.06588	0.06627	0.799483
RM13	0.21390	1	0.21390	5.0588	20	0.25294	0.84567	0.368740
RM25	0.07701	1	0.07701	1.7412	20	0.08706	0.88452	0.358188
RM227	0.01337	1	0.01337	0.9412	20	0.04706	0.28409	0.599906
RM286	2.25936	1	2.25936	31.0588	20	1.55294	1.45489	0.241820
RM3428	0.02620	1	0.02620	4.5647	20	0.22824	0.11481	0.738267
RM5371	14.03422	1	14.03422	52.3294	20	2.61647	5.36380	0.031292
RM7187	0.00000	1	0.00000	0.0000	20	0.00000		
RM6314	0.19305	1	0.19305	5.6706	20	0.28353	0.68088	0.419019
RM6811	2.84973	1	2.84973	17.7412	20	0.88706	3.21256	0.088216
RM5638	0.00053	1	0.00053	19.3176	20	0.96588	0.00055	0.981461
RM5707	0.48128	1	0.48128	23.8824	20	1.19412	0.40305	0.532717
RM8243	0.00000	1	0.00000	0.0000	20	0.00000		
RM6410	0.10481	1	0.10481	4.2588	20	0.21294	0.49221	0.491025

RM463	0.08571	1	0.08571	8.2000	20	0.43158	0.19861	0.660885
RM289	1.13155	1	1.13155	2.1412	20	0.10706	10.56943	0.004001
* SS Effect - sum of squares, df *- degree of freedom, MS Effect *- mean square, F * -Fisher test, p * significance level								

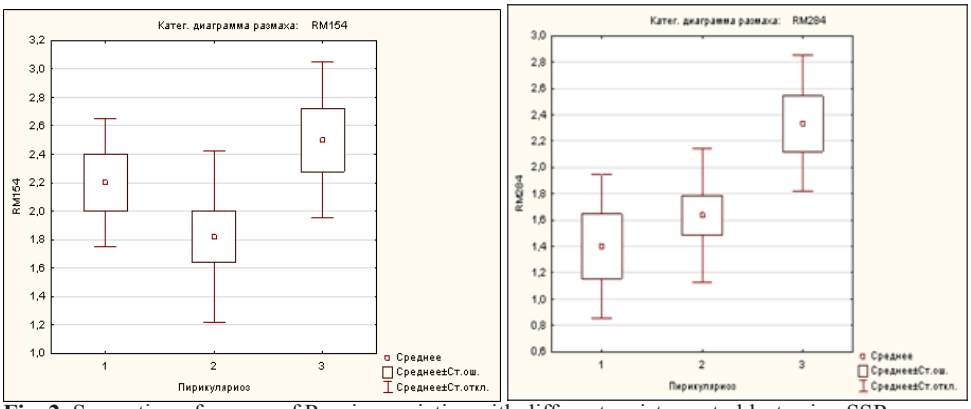

Fig. 2. Separation of groups of Russian varieties with different resistance to blast using SSR molecular markers: a) RM154; b) RM284

The division of varieties into 3 groups allowed us to isolate the locus on the eighth chromosome near marker RM284 (Figure 2, table 3). A decrease in the significance level (to 0.09) increased the sensitivity threshold of the method and revealed another locus with a possible effect on the formation of the trait.

Table 3. SSR markers that reliably distinguish groups of rice varieties contrasting in their resistance to blast

Variety Groups	SSR marker (chromosome); chromosome locus	
	Significance Level 0.05	Significance Level 0.09
Two groups of varieties	RM 289(5); 36,2cM RM 5371(6); 25,8 cM	RM 126(6); 57cM RM 5361(5); 6,3cM RM 444(9); 3,3 cM RM 6811(6); 115,6 cM RM 6024(5); 17,7cM
Three groups of varieties	RM 284(8); 104,3cM	RM 154 (2); 4,8cM RM 289(5); 36 cM

The data obtained are consistent with the results of previous studies in this area [16]. According to the literature [15-16], many genes for resistance to blast are localized in the chromosomal regions that we have identified (Figure 3).

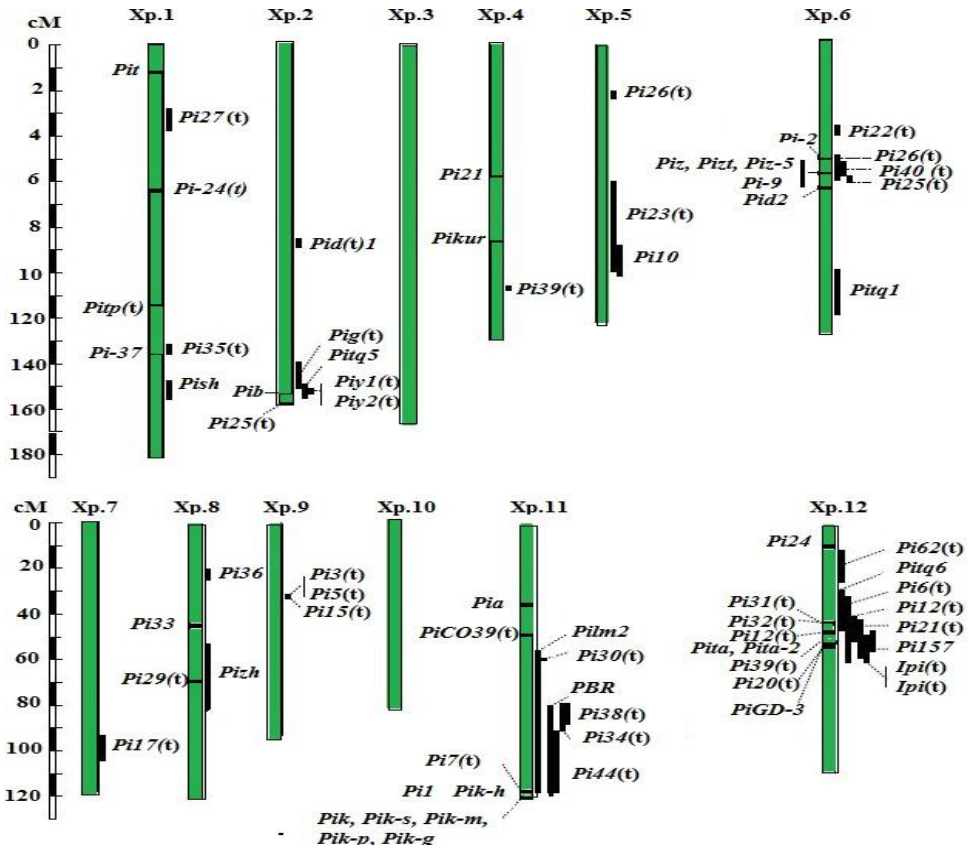

Fig. 3. Map of the distribution of genes for resistance to blast (*O.sativa*) http://www.jircas.affrc.go.jp [15]

So on the sixth chromosome there is a large cluster of resistance genes, which includes eleven genes (Pi-2, Piz, Pizt, Piz-5, Pi-9, Pid2, Pi22 (t), Pi26 (t), Pi40 (t) Pi25 (t), Pitq1). On the fifth chromosome, in the region where the marker RM 289 is located, the Pi26 (t) gene is located, and the markers RM 5361 and RM 6024 contain the Pi23 (t) and Pi10 loci. On the eighth chromosome in the marker region, polymorphism, which allowed us to reliably distinguish groups of samples with contrast resistance to blast, contains the genes Pi26, Pi33, Pi29 (t). Clusters of five to seven resistance genes were also detected on the second and ninth chromosomes.

Therefore, in order to reduce the laboriousness of evaluating breeding material when identifying sources of vertical resistance, the variability of Pi genes in the identified chromosomal regions should be studied first. On the ninth chromosome, the stability of the studied samples can be due to the presence of the genes Pi-3 (t), Pi5, Piii, Pi28 (t), Pi15 (t). The locus Pi-d (t) 1 is located closest to the second, but it is also possible to determine the trait due to the operation of the cluster, which includes the genes: Pi-g (t), Pitq5, Piy1 (t), Piy2 (t), Pib.

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