426. Physiology and Molecular Biology of Plants, 2020. № 26(2). – P.289-303.
Physiol Mol Biol Plants (February 2020) 26(2):289–303.
https://doi.org/10.1007/s12298-019-00737-6
RESEARCH ARTICLE

BIODIVERSITY OF PYRICULARIA ORYZAE CAV. IN RICE-GROWING REGIONS OF THE SOUTH OF RUSSIA USING PCR METHOD

Elena V. Dubina[1] · Andrey V. Alabushev[2] · Pavel I. Kostylev[2] · Elena S. Kharchenko[1] · Margarita G. Ruban[1] · Yuliya V. Aniskina[3] · Ilya A. Shilov[3] · Nazife S. Velishaeva[3] · Evgeniy P. Maximenko[4] · Yuliya A. Makukha[1]

1 All-Russian Rice Research Institute, 3 Belozerny Lane, Krasnodar, Russian Federation 350921
2 Agrarian Scientific Center ‘ ‘Donskoy’’, Nauchny Gorodok 3, Zernograd, Russian Federation 347740
3 All-Russia Research Institute of Agricultural Biotechnology, 42 Timiryazevskaya Street, Moscow, Russian Federation 127550
4 Federal State Unitary Elite-Seed-Production Enterprise ‘‘Krasnoe’’, All-Russian Rice Research Institute, Risoopytniy, Krasnoarmeyskiy District, Krasnodar Region, Russian Federation 353810

Received: 2 May 2019/Revised: 29 July 2019/Accepted: 22 November 2019/Published online: 1 January 2020 © Prof. H.S. Srivastava Foundation for Science and Society 2020

Abstract The purpose of this research by the way of investigating the molecular genetic structure of a highly variable fungal phytopathogen Pyricularia oryzae Cav., to determine effective genes for the development of a strategy for immunogenetic protection against rice blast in conditions of epiphytotic development of the disease in the south of Russia, which would combine high efficacy with both environmental friendliness and resource and energy saving, to ensure country’s food security. The knowledge of local pathotype diversity of Pyricularia oryzae Cav. and the (a)virulence genes in rice-growing regions of Russia may allow the prediction of new races and its interaction in local agro-ecology. The identification of virulence gene may become an indispensable theoretical basis for the development of genetic sources with long-lasting resistance to rice blast. Based on molecular and genetic approaches, the genetic structure and biodiversity of the phytopathogenic fungus Pyricularia oryzae Cav. in the south of Russia were considered. The monitoring was studied and it isolated 57 strains of the pathogen from the damaged herbal material collected from the fields in eight agro-ecological rice-growing regions of the Krasnodar Region (Russian Federation): Krasnoarmeysky, Kalinin-sky, Krymsky, Abinsky, Temryuksky, Seversky, Slavyan-sky districts, Krasnodar, Rostov Region (Russian Federation)—Proletarsky district and the Republic of Adygea (Russian Federation). A multiplex PCR technique was applied on the basis of fragment analysis to identify the virulent fungal isolates. 33 fungal genotypes with

unique genetic profiles were identified among the studied races of *Pyricularia oryzae* Cav. Their DNA profiles were created. The studied isolates of the pathogen of rice blast were classified using morphological and microbiology cultural features. Based on the phytopathological test using differentiation rice varieties, the quantitative and qualitative composition of (a)virulence genes in fungal races was established. Effective genes for pathogen resistance, which are recommended for breeding programs for the development of rice varieties resistant to rice blast, were identified in the south of Russia.

Keywords Rice • Rice blast • Isolates • PCR • Microsatellite markers • DNA profile • (A)virulent genes

Introduction

Rice blast infested by imperfect fungi Pyricularia oryzae Cav. (Magnaporthe grisea (Herbert) Barr), of order Hyphomycetales, is one of the most malicious diseases of rice and very common in all rice-growing regions. In its development, Pyricularia oryzae Cav. goes through a vegetative stage represented by mycelium and conidia and a perfect ascigerous stage (Magnaporthe grisea (Herbert) Barr) (Kovalenko et al. 1988).

Apart from rice, this pathogen is virulent for more than eighty species of cultivated and wild plants. Control of the distribution of rice blast using resistant varieties is rather complicated due to high variability of the pathogen and favourable conditions for the development of the disease in agrocenosis (alternative host than rice).

Three decades ago phytopathologists used only differentiation pathogenic varieties to analyse (a)virulence and genetic variability of pathogens. Currently, the emergence of molecular genetic methods ensure the obtaining of DNA ‘‘imprints’’ of the rice blast pathogen, Magnaporthe grisea (Herbert) Barr, which opened a new page in the study of its genetic diversity (Yankovsky 1996).

Several genetic maps of this pathogen have been created, including, based on different types of polymorphism, such as RAPD, RFLP, REMAP, isozyme, telomere, etc. (Castiglioni et al. 1998; Kumar and Zeigler 1995; Pad-manabhan et al. 1970; Resurreccion et al. 1994). A physical map of the seventh chromosome of pathogen was created using the BAC clones and attached to the RFLP marker card. The sequencing of all 7 chromosomes of Pyricularia oryzae Cav. was carried out. (Ahn et al. 1996; Bryan et al. 2000).

In the 1990s, the marker analysis of Pyricularia oryzae Cav. for most cases was based on the restriction fragment length polymorphism and discrete clonal lines were normally analysed (Lang et al. 2001; Launder et al. 1987). In 1993, Bernardo et al. analysed the population of Magna-porthe grisea (Herbert) Barr using the RAPD method and found a good correlation between the major groups of isolated RAPD and RFLP (Arase et al. 2000; Ayres et al. 1997). Later, pairs of primers were developed to amplify the mobile elements present in the genome of Pyricularia oryzae Cav. (Jensen et al. 2001; Kaye et al. 2003). At the next step, the analysis of isolates of Magnaporthe grisea (Herbert) Barr, isolated from affected rice plants and eleusine, showed that the studied pathogen populations did not have common DNA

‘‘fingerprints’’. To date, a variety of molecular markers of various types has been created to study the pathogen of rice blast (Conaway-Bormans et al. 2003; Fuji et al. 1999; Xia et al. 1993).

The polymorphism of simple, DNA tandem repeat sequences (SSRs, microsatellite repeats) has several advantages. Microsatellite sequences are found throughout the eukaryotic genome. They are based on the polymerase chain reaction, highly reproducible, codominant and mul-tiallelic (Levy et al. 1993). When compared to other marker systems, microsatellite markers showed better genetic characteristics of Aspergillus fumigatus, Saccharomyces cerevisiae, Fusarium oxysporum and Fusarium circinatum (Khush et al. 2001).

The purpose of the research was to study the genetic structure and biodiversity of a highly variable fungal phytopathogen, Pyricularia oryzae Cav., in the south of Russia based on the results of PCR using SSR-markers and to identify effective resistance genes. The phytopatholog-ical testing was used to determine (a)virulence genes of the pathogen for rice-growing regions in the south of Russia. For this purpose, a set of 30 rice differentiation varieties of pyricularia race was used. During identification of (a)virulence genes based on phytopathological testing using a set of differentiation varieties of pyricularia races, the resistance genes to Pyricularia oryzae Cav., effective for the south of Russia, were identified. Such studies are of particular interest, as they provide a forecast of the appearance of new races and pathogenic types of Pyricularia oryzae Cav. and identification of (a)virulence genes in the rice-growing regions of Russia. The latter is a necessary theoretical basis for the creation of genetic sources with long-lasting resistance to rice blast.

Materials and methods

The material for molecular genetic studies included 57 isolates of the Pyricularia oryzae Cav. pathogen (Table 1), isolated from the affected herbal material collected from rice fields in 2015–2018 in eight ecological rice-growing regions of the Krasnodar Krai (Krasnoarmeysky, Kalininsky, Krymsky, Abinsky, Temryuksky, Seversky, Slavyansky districts, Krasnodar), Rostov Region (Proletarsky district) and the Republic of Adygea, which all located in Russian Federation (Fig. 1).

The phytopathological testing was used to determine (a)virulence genes of the pathogen for rice-growing regions in the south of Russia. For this purpose, a set of 30 rice differentiation varieties of pyricularia race obtained from the International Rice Research Institute was used (Table 2) (IRRI, Philippines, Kovalenko et al. 1988).

Differentiation varieties (Table 2) were grown in the field of the nursery of the FGBNU All-Russian Research Institute of Rice and the rice irrigation system of the FGUESP Krasnoye (Krasnoarmeysky district). At the stage of 5–6 leaves (38–40 days after sowing), rice plants were inoculated with a suspension of Pyricularia oryzae Cav. conidia with a Tween 80 adhesive (0.0001%). The concentration of spores in the suspension was 105 conidia/mL, which corresponds to 10–14 spores in the field of view of the Biolar microscope with a 1259 zoom. The reaction to inoculation with a suspension of Pyricularia oryzae Cav. was evaluated on the seventh day using a method developed by the All-Russian Research Institute of Phy-

topathology (Kovalenko et al. 1988).

Based on the results of the studies, the virulence of fungal isolates was analysed according to the type of infection (the size of the infected rice leaf plate) (Kovalenko et al. 1988); the pathogen types of the Krasnodar and Rostov populations of Pyricularia were determined and the quantitative and qualitative ratio of the virulence genes was interpreted. The main condition for the study of any pathosystem is the possibility of pure pathogen culture isolation, which is based on the methods for obtaining isolates and monoisolates. The fungal culture was isolated from affected rice tissues (nodes of the stem, leaves, parts of the panicle) collected from rice fields, according to the guidelines developed by the All-Russia Institute of Phytopathology (Kovalenko et al. 1988).

Table 1 Origin and morphological and cultural characteristics of strains of Pyricularia oryzae Cav. 2016–2018

Strain no.	District	Date of selection	Part of	Character of colony growth	Colony Colour (upper	SubstrateColour (lower myceli-
2	Temryuksky	27.06.2016	Leaf	Inhomogeneous low dense and a little	From light gray to gray	Black
8	Proletarsky (Rostov Region)	30.06.2016	Leaf	Felted	From light gray to olive	From light grey to black
7-16	Crimean	15.07.2016	Leaf	Inhomogeneous low shaggy and low	From light gray to	Dark brown
11	Crimean	30.07.2016	Leaf	Inhomogeneous, sectors with felted, shaggy and low	From gray to brown, with white zones	From discoloured to intensely black
12	Crimean	30.07.2016	Leaf	Low dense	Gray	Black
21	Krasnoarmeysky	13.08.2016	Joint	Inhomogeneous powdery with high	From light gray to dark	From dark brown to black
31	Krasnoarmeysky	14.07.2016	Leaf	Felted	Darkgrey	Black
4.1	Krasnoarmeysky	27.06.2016	Leaf	Low felted	Gray (mousy)	Brownishblack
37	Kalininsky	16.07.2016	Leaf	Inhomogeneous—sectors with	From light gray to dark	From brown to black
39	Kalininsky	16.07.2016	Leaf	Low powdery (‘‘radiate’’)	From dark gray to	Black
50	Kalininsky	18.07.2016	Leaf	Powdery and low shaggy	Light gray	Brown
5	Abinsky	15.07.2016	Leaf	Low felted	From light olive to gray	Black
38	Abinsky	30.07.2016	Head	Low powdery	From dark gray to	Black
6	Abinsky	15.07.2016	Leaf	Inhomogeneous felted and powdery	From gray to gray-brown	Black
3-17	Slavyansky	27.07.2017	Leaf	Low felted	From light gray to gray	From discoloured to dark

4-17	Slavyansky	27.07.2017	Leaf	Low felted	From light gray to gray	Fromdiscoloured to brown
5-17	Slavyansky	27.07.2017	Leaf	Low powdery, con-centric	Gray	Light brown
6-17	Slavyansky	27.07.2017	Leaf	Low felted	From light gray to dark	From light brown to brown
7-17	Slavyansky	27.07.2017	Leaf	Low dense	From light gray to olive	From discol-oured to in-
9-17	Slavyansky	27.07.2017	Leaf	Low felted	From light gray to gray	From light brown to dark
14-17	Kras-noarmeysky	10.08.2017	Leaf	Inhomogene-ous—sectors with	From light gray to dark	From discol-oured to black
17-17	Kras-noarmeysky	10.08.2017	Joint	Lowdense and a little fluffy	From light gray to gray	Frombrown to black
19-17	Kras-noarmeysky	10.08.2017	Joint	Lowfelted and a little fluffy	Light gray	From dark brown to black
20-17	Kras-noarmeysky	10.08.2017	Joint	Inhomogeneous low dense with white	From white to gray	Black
21-17	Kras-noarmeysky	10.08.2017	Joint	Lowdense and a little fluffy	Gray	Black
22-17	Kras-noarmeysky	10.08.2017	Joint	Inhomogene-ous—sectors with	From light gray to olive	From light brown to black
23-17	Kras-noarmeysky	10.08.2017	Leaf	Inhomogene-ous—crumbly	From light grey to dark	From discol-oured to in-
26-17	Kras-noarmeysky	10.08.2017	Joint	Inhomogene-ous—low dense and	From light gray to gray	From discol-oured to
27-17	Krasnodar, Belozerny	15.08.2017	Leaf	Inhomogene-ous—low felted and	From light gray to	From discol-oured to intense
33-17	Kras-noarmeysky	16.08.2017	Joint	Inhomogene-ous—powdery and	From light olive to dark	From discol-oured to intense
34-17	Kras-noarmeysky	16.08.2017	Joint	Inhomogene-ous—high crumby	From light gray to dark	Intense black
37-17	Kalininsky	25.08.2017	Leaf	Inhomogene-ous—shaggy and	From light gray to	From light black to black
38-17	Kalininsky	25.08.2017	Stalk of	Inhomogene-ous—crumby shaggy	From light gray to gray	From discol-oured to dark
39-17	Seversky	25.08.2017	Stalk of	Inhomogene-ous—sectors with	From gray to dark olive	From discol-oured to intense
40-17	Seversky	25.08.2017	Stalk of	High crumby shaggy ‘‘radiate’’	From white to brownish ol-	From discol-oured to intense
55-17	Crimean	30.08.2017	Stalk of	Inhomogene-ous—crumby shaggy	From light gray to grey	From discol-oured to dark
56-17	Krasnodar, Belozerny	4.09.2017	Stalk of	Low powdery ‘‘ra-diate’’ concentric	Gray	Black
58-17	The Republic of Adygea	19.09.2017	Stalk of	Inhomogene-ous—crumby shaggy	From white to brownish-gray	From light black to intense black
1.8	Kras-noarmeysky	22.06.2018	Leaf	Low felted	From light gray to dark	From light brown to dark
3.8	Kras-noarmeysky	22.06.2018	Leaf	Low felted and crumby fluffy	From white to gray (mousy)	From light brown to brown

4.8	Jrasnodar, Belozerny	2.07.2018	Leaf	Inhomogene-ous—crumby and	From white to gray and light	From discol-oured to dark
5.8	Kras-noarmeysky	5.07.2018	Leaf	Inhomogene-ous—sectors with	From white to gray	From discol-oured to dark
6.8	Kras-noarmeysky	5.07.2018	Leaf	Low felted	Dark gray concentric	From light brown to black
9.8	Kras-noarmeysky	5.07.2018	Leaf	Inhomogene-ous—sectors with	From light white to gray	From discol-oured to
10.8	Kras-noarmeysky	9.07.2018	Leaf	Inhomogene-ous—low powdery	From white to light gray and	From discol-oured to dark
11.8	Kras-noarmeysky	9.07.2018	Leaf	Inhomogene-ous—sectors with	From light gray to gray	From light brown to
12.8	Kras-noarmeysky	9.07.2018	Leaf	Low felted	From light gray to olive	From dark brown to black
14.8	Kras-noarmeysky	9.07.2018	Leaf	Inhomogene-ous—crumby shaggy	From light gray to dark	From light brown to dark
15.8	Kras-noarmeysky	9.07.2018	Leaf	Low dense and a lit-tle fluffy	From light gray to gray	From discol-oured to dark
18.8	Seversky	24.07.2018	Head	Inhomogene-ous—sectors with	From white to gray-olive	From light brown to intense
20.8	Abinsky	24.07.2018	Leaf	Low dense	From light gray to dark	From brown to dark brown
21.8	Abinsky	24.07.2018	Leaf	Low felted and low powdery	From white to dark gray	From dark brown to black
24.8	Slavyansky	26.07.2018	Head	Inhomogene-ous—shaggy and	From light gray to gray	From light brown to dark
25.8	Slavyansky	26.07.2018	Head	Inhomogene-ous—powdery with	From light gray to dark	From light brown to black
26.8	Slavyansky	1.08.2018	Head	Low felted ‘‘con-centric’’	From dark gray to olive	From discol-oured to intense
28.8	Krasnodar	17.08.2018	Stalk of head	Inhomogene-ous—crumby shaggy and low powdery	From gray to dark gray	From discol-oured to black
29.8	Kalininsky	30.08.2018	Joint	Crumbly fluffy with felted zones	From dark gray to olive	From light black to intense black

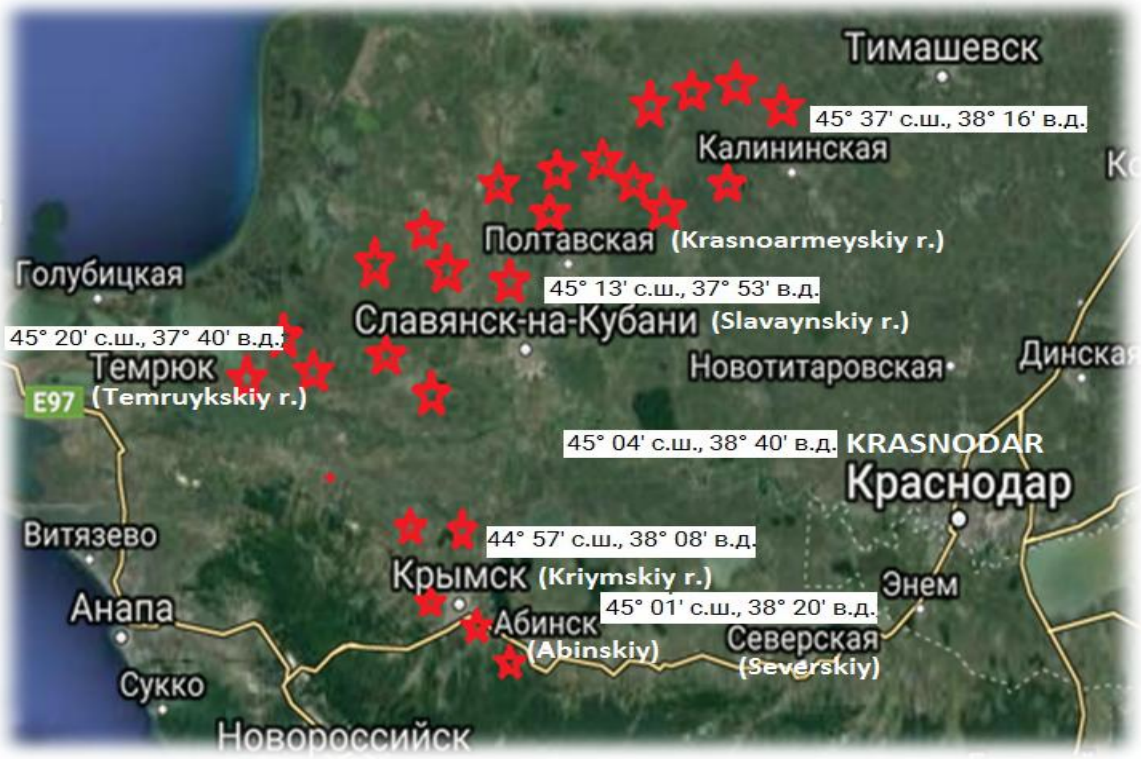


Fig. 1 Map of selection of herbarium material of rice plants with signs of affection by Pyricularia oryzae Cav.

Samples of the affected tissue were washed with sterile water and placed on sterile carrot-sugar agar in Petri dishes, which were then placed in a wet (100%) chamber at a temperature of 27 ± 1 °C, optimal for the formation of conidia of *Pyricularia oryzae* Cav. The resulting pure culture was cultivated in a thermostat prior to distribution of the fungal colony over the entire surface of the agar medium and the isolates were described using morphological and cultural characteristics. The *Pyricularia oryzae* Cav. isolates were further divided into morphological groups. The DNA *of Pyricularia oryzae* Cav. for molecular genetic studies was isolated from the mycelial mixture of monoconidial culture of Pyricularia oryzae Cav. using CTAB method (Murray and Thompson 1980).

Table 2 Determination of (a)virulence genotype of the rice blast population

No.	Registra-tion no.	Name of variety	Re-sistance gene	Variety reaction		Virulence gene	
				RIS of All-Russian Research Institute of Rice	Federal State Unitary Elite Seeds Enterprise ‘‘Krasnoye’’ of the All-Russian Rice Research Insti-tute	RIS of All-Russian Research Institute of Rice	Federal State Unitary Elite Seeds Enterprise ‘‘Krasnoye’’ of the All-Russian Rice Research Insti-tute

1	11366	Sha-tiao-tsao	Pi-k^{s}	R	R		
2	11367	Ishikari-shiroke	Pi-i	S	S	Av-i$^{?}$	–
3	11370	Usen	Pi-a	R	MS	–	–
4	11373	Yaschiro-mochi	Pi-ta	S	MS	Av-ta$^{?}$	–
5	11375	Toride-1	Pi-z^{t}	S	S	Av-z$^{t?}$	Av-z$^{t?}$
6	11377	Minehikari	Pi-m	R	MS	–	–
7	11382	CO39	No R gene	R	R	–	–
8	11385	IRBlK-KU [CO]	Pi-k	R	R		
9	11388	IRBlKm-TS [CO]	Pi-k^{m}	S	S	Av-k$^{m?}$	
10	11389	IRBlKp-K60 [CO]	Pi-k^{p}	MS	MS	–	–
11	11390	IRBl1-La [CO]	Pi-1	R	R	–	–
12	11391	IRBl7-M [CO]	Pi-7(t)	R	MS	–	–
13	11392	IRBl sh-Ku [CO]	Pi-sh	S	S	Av-sh$^{?}$	Av-sh$^{?}$
14	11395	IRBl ta-Ja [CO]	Pi-ta	R	MS	–	–
15	11396	IRBl taME [CO]	Pi-ta	MS	S	–	Av-ta$^{?}$
16	11399	IRBl ta2-IR64 [CO]	Pi-ta^{2}	S	S	Av-ta$^{2?}$	Av-ta$^{2?}$
17	11400		Pi-z^{5}	S	MS	Av-z$^{5?}$	
18	11405	IRBl9-W [LT]	Pi-9	S	S	Av-9$^{?}$	Av-9$^{?}$
19	11406	IRBl3-CP 4 [LT]	Pi-3	S	R	Av-3$^{?}$	
20	11409	IRBl K3 [LT]	Pi-kh	S	R	Av-kh$^{?}$	
21	11431	IRBl -Fu	Pi-z	R	R	–	–
22	11433	IRBl zt-T	Pi-z^{t}	S	MS	Av-z$^{t?}$	–
23	11436	IRBL b-B	Pi-b	R	MS	–	–
24	11437	IRBlt-K59	Pi-t	S	S	Av-t$^{?}$	Av-t$^{?}$
25	11445	IRBl12-M	Pi-12(t)	R	MS	–	–

26	11446	IRBl19-A	Pi-19	S	S	Av-19?	Av-19?
27	11451	IRBl11-Zh	Pi-11(t)	R	R	–	–
28		Bl-1	Pi-b	MS	MS	–	–
29		IR-36	Pi-ta	MS	MS	–	–
30		LAC-C101	Pi-33, Pi-1	R	R	–	–
31	St	Innovator	–	S			
32	St stable	-	R				

RIS, Rice Irrigation System; R (resistance), variety resistant to rice blast; S, non-resistant; MS, medium-resistant; Innovator, control of non-resistant rice variety; Sonet, control of resistant rice variety

In this method, cetyl trimethylammonium bromide was used as a detergent in a lysis buffer. To identify isolates of the pathogens causing rice blast, 30 pairs of primers with specific fluorescent dyes were synthesized (Table 3, Sintol, Russia) (Adreit and Santoso 2007). The SSR used in this study were evenly distributed across all seven chromosomes *of Pyricularia oryzae* Cav. (Li et al. 2007; Ahn et al. 1996; Kaye et al. 2003).

Their nucleotide sequences are taken from the NCBI genetic resources database, available at http://blast.ncbi. nlm.nih.gov/Blast.cgi (Table 3).

These markers were highly polymorphic (73% of the locus), which allows its use for genomic mapping, population studies and DNA ‘‘profiling’’ of the rice blast pathogens in all rice-growing countries worldwide (Adreit and Santoso 2007). The position on the genetic map for all 41 microsatellite loci uniformly distributed over all seven *Pyricularia grisea Sacc.* chromosomes was determined as shown in Fig. 2 (Zheng et al. 2008).

PCR was performed in a volume of 25 **u**l containing 1 **x** PCR buffer, 1.25 units of Taq DNA polymerase 100 umol dNTP (NPO DNA-Technology LLC, Russia), 10 pmol of each primer and 5 **u**l of DNA solution. Amplification was carried out in a detection thermocycler (model of thermo-cycler was CFX-BioRad, USA) according to the program: 95 °C—10 min (94 °C—30 s, 55 °C—30 s, 72 °C—30 s) 30 cycles.

Fragment analysis was carried out using the automatic genetic analyser ‘‘NANOFOR-05’’ at the Institute of Analytical Instrumentation RAS in Russia, supported by the Centre for Collective Use ‘‘Biotechnology’’ of the All-Russian Research Institute of Agricultural Biotechnology.

Pathogen isolates were clustered ranked into groups according to their genetic affinity and correlation between *Pyricularia oryzae* Cav. genotypes. The analysis was calculated according to phytopathological test and its molecular (microsatellite) ‘‘finger print’’ (Li et al. 2007).

Results

The research was conducted in the laboratory of information, digital and biological technology of All-Russian Rice Research Institute. As a result of investigation of 25 rice-growing farms in eight ecological regions of the Krasnodar Krai, and the ‘‘Proletarskoe’’ plot and production farm of the Rostov Region, herbarium material with signs of disease was collected from (Fig. 1).

When selecting parental pairs for breeding programs to create rice blast-resistant varieties of rice, it is important to have information about the type of resistance of the donor variety that would provide long-term protection against the disease. Therefore (a)virulence genes to Pyricularia oryzae Cav. should be determined for a certain rice-growing area in the south of Russia.

A set of differentiation varieties of rice blast obtained from the International Rice Research Institute (IRRI, Philippines) based on the phytopathological testing method was used in the studies. Differentiation varieties were seeded in the infectious nursery of the All-Russian Research Institute of Rice (Krasnodar), and in the rice irrigation system of Federal State Unitary Elite Seeds Enterprise ‘‘Krasnoye’’ of

the All-Russian Rice Research Institute of Krasnoarmeysky district. The phyto-pathologi-cal test results are presented in Table 2. The inoculation was carried out with a mixture of isolates of the Krasnodar population of Pyricularia oryzae Cav. pathogen by the method described in the ‘‘Materials and methods’’ section.

The affected leaves, stem nodes, panicles of rice plants, 57 strains (populations) of Pyricularia oryzae Cav. with various sporulation capabilities were isolated from the affected leaves, stem nodes, panicles of rice plants. Their characteristics are presented in Table 1.

According to morphological and cultural attributes, the majority of Pyricularia oryzae Cav. pathogen strains studied in 2015–2018 has non-homogeneous colony growth and can be divided into the following morphotypes (clusters):

I: Strains with low, powdery, concentric growth pattern of the grey colony

II: Strains with low felty (slightly fluffy) growth pattern of the light grey or grey and grey-olive colony

III: Strains with low dense (slightly fluffy) growth pattern of the light grey to grey colony

IV: Strains with flocculent, felty and low powdery growth pattern of the light grey to dark grey and olive colony

V: Strains with high, loose, flocculent, ‘‘radiant’’ growth pattern of the white to brownish-olive colony

VI: Strains with heterogeneous sectors with loose, flocculent and low felty growth pattern of the light grey to dark grey and olive colony

VII: Strains with heterogeneous sectors with powdery and felty growth pattern of the light olive to dark grey colony

VIII: Strains with heterogeneous low-density growth pattern with white areas of the white to grey colony

IX: Strains with low, powdery, ‘‘radiant’’, concentric growth pattern of the grey colony

X: Strains with low dense and slightly fluffy growth pattern of the light grey and grey colony

XI: Strains with low felty and slightly fluffy growth pattern of the light grey to grey and olive colony

Table 3 Microsatellite markers used to study the biodiversity of the pathogen of rice blast, Pyricularia oryzae Cav.

Primer	Primer sequence	Microsatellite pattern	Annealing T, °C	Chromosome (genome contig)	Reference for genomic sequence
Pyrms 7-8	F(NED):gcaaataacataggaaaacg R: agaaagagacaaaacactgg	CT/GA29	53	7 (557)	Kaye et al. (2003)
Pyrms 15-16	F(NED): ttcttccatttctctcgtcttc R: cgattgtggggtatgtgatag	(CT)n	53	3 (152)	Kaye et al. (2003)
Pyrms 37-38	F(NED): accctaccccactcatttc R: aaggatcagccaatgccaagt	CA/GT6 ? CT/GA12	53	2 (1054)	Kaye et al. (2003)

Pyrms 39-40	F(NED): cgcatacaggaaa- gccaaga R: ctgac-	(CA)n	53	1(628)	Kaye et al. (2003)
Pyrms 43-44	F(FAM): tcagtaggcttggaatt- gaaaa R: cttgattggtggtggtgttg	TA12	55	4 (864)	Kaye et al. (2003)
Pyrms 45-46	F(FAM): ccac- tttatagcccacccagt R:	(TA)n	55	7 (203)	Kaye et al. (2003)
Pyrms 47-48	F(HEX): tcacatttgcttgctggagt R: agacagggttgacggctaaa	(TA)n	55	4 (197)	Kaye et al. (2003)
Pyrms 59-60	F(NED): ttctcagtaggctt- ggaattga R: ctt-	(TA)n	55	4 (204)	Kaye et al. (2003)
Pyrms 61-62	F(NED): gaggcaacttgg- catctacc	(GA)n	55	3 (315)	Kaye et al. (2003)
Pyrms 63-64	F(NED): ttgggatcttcggtaa- gacg	CT/GA15	55	1 (2196)	Kaye et al. (2003)
Pyrms 67-68	F(NED): agcaagcagga- gatgcagac R: gtttggctgg-	(CA)n	55	7 (200)	Kaye et al. (2003)
Pyrms 77-78	F(HEX): gaagtattgcacacaaacac	CA/GT24?	55	3 (1135)	Kaye et al. (2003)
Pyrms 81-82	F(FAM): ccttgttttccccctgtgta R: tagccaaatgcccattatcc	(ACT)n	55	4 (452)	Kaye et al. (2003)
Pyrms 83-84	F(FAM): gtctgcctcgactccttcac R: agcccaaaaacagaaagcaa	TCA/AGT13	55	2 (1895)	Kaye et al. (2003)
Pyrms 87-88	F(FAM): agactt- gttactcgggtcttga R:	(TGC)n	53	1 (614)	Kaye et al. (2003)
Pyrms 93-94	F(NED): cctcgactccttcac- caaaa	(ATC)n	55	2 (407)	Kaye et al. (2003)
Pyrms 99-100	F(NED): caccactttatggcgcagt R: acctaggtaggtatacatgttgtt	ACC/TGG20	53	5 (718)	Kaye et al. (2003)
Pyrms 101-10	F(FAM): ctgcgttcaacatgcctcta R: cttgatctgcggtatgagca	TG/AC25	53	6 (50)	Kaye et al. (2003)
Pyrms 107-10	F(FAM): gcagcaagcagcaa- tatcag R: gtgga-	(GA)n	53	2 (317)	Kaye et al. (2003)
Pyrms 115-11	F(NED): ttcgttcacctttggctct R: ttgttaagtgagcggacgtg	(GA)n	55	3 (203)	Kaye et al. (2003)
Pyrms 125-12	F(NED): ctctccggccaagattga R: ggttgttgggagaaagaacg	(CAA)n	55	7 (213)	Kaye et al. (2003)
Pyrms 233-23	F(FAM): tgagatggaccg- catgatta	CAG/CTC_{10}	55	5 (2059)	Dean et al. (2005)
Pyrms 319-32	F(NED): taagaccactggcg- gaatct	CAA/GTT_{6}	55	2 (1898)	Dean et al. (2005)
Pyrms 409-41	F(FAM): tcccagtacttgcccatctc R: atctcatatccgtcggtcgt	TA/AT23	55	4 (730)	Dean et al. (2005)
Pyrms 427-42	F(HEX): ctgtcaccacaaccaa- gacg R: ttgccctgatttgtcagtca	AT/TA16	55	5 (718)	Dean et al. (2005)
Pyrms 453-45	F(NED): gaccctcga- gaaatgatgga R: gtcaaa-	GAA/CTT6	55	7 (520)	Dean et al. (2005)
Pyrms 505-50	F(HEX): cctgtcgtagcacctttggt R: ttgttcctcgtcccatgttt	GAAA/CTTT5	55	6 (33)	Dean et al. (2005)
Pyrms 533-53	F(HEX): cttatcggaggtgcagaac R: cacggcatactgcatacgtg	TCTAGT/AGATCA 3	55	5 (1587)	Dean et al. (2005)

Pyrms 607-60	F(FAM): cccaagctccataa-taggctac R: tccgagactctttgga-	GCA/CGT13	55	1 (90)	Dean et al. (2005)
Pyrms 657-65	F(HEX): atcag-tcgaacccacaaagc R: atgtgtg-	CA/GT12	55	6 (29)	Dean et al. (2005)

F forward primer (5′-nucleotide sequence), *R* reverse primer (3′-nucleotide sequence), *n* number of microsatellite motif repeats

The first morphotype includes strain No. 5-17 isolated from the affected herbarium material collected from the rice fields of the Slavyansky district. The second type includes strains Nos. 3-17, 4-17, 6-17, 8-17, 9-17, No. 27-17 isolated from the herbarium material collected in rice fields in the farms of the Slavyansky district and Krasnodar (village of Belozerny). The third morphotype includes strains Nos. 7-17 (Slavyansk district), 21-17, 26-17 (Krasnoarmeysky district). The fourth type includes strains Nos. 14-17, 22-17 (Krasnoarmeysky district), Nos. 37-17, 38-17 (Kalininsky district) and No. 39-17 (Seversky district). The fifth morphotype includes strain No. 40-17 isolated from the herbarium material with signs of rice blast, collected on the fields in rice-growing farms of the Sev-ersky district. The sixth type includes strains Nos. 23-17, 34-17 (Krasnoarmeysky district) and 55-17 (Krymsky district), and strain No. 58-17 (the Republic of Adygea). Strain No. 33-17 (Krasnoarmeysky district) belongs to the 7th type and has a heterogeneous powdery and felty colony and therefore may occupy an intermediate position between the fourth and seventh type. Strain No. 20-17 is allocated to the eighth type according to the morphological and cultural attributes. This strain has a heterogeneous low and dense structure of the colony with white areas. The ninth morphotype includes strain number 56-17 (Krasnodar, village of Belozerny); and the tenth and eleventh morphotypes include strains Nos. 17-17 and 19-17, respectively, from the Krasnoarmeysky district.

Thus, in the ecological regions studied, the greatest diversity of the phytopathogenic fungus Pyricularia oryzae Cav. was observed in the Krasnoarmeysk district, where 7 morphotypes of Pyricularia oryzae Cav. were isolated.

Figure 3 shows pictures of all eleven morphotypes of Pyricularia oryzae Cav. isolated in 2015-2017.

With comparing the three-year data in 2017, 4 new morphotypes of this pathogen (strains Nos. 17-17, 19-17, 20-17, 40-17) with a unique genetic profile were identified (Table 4).

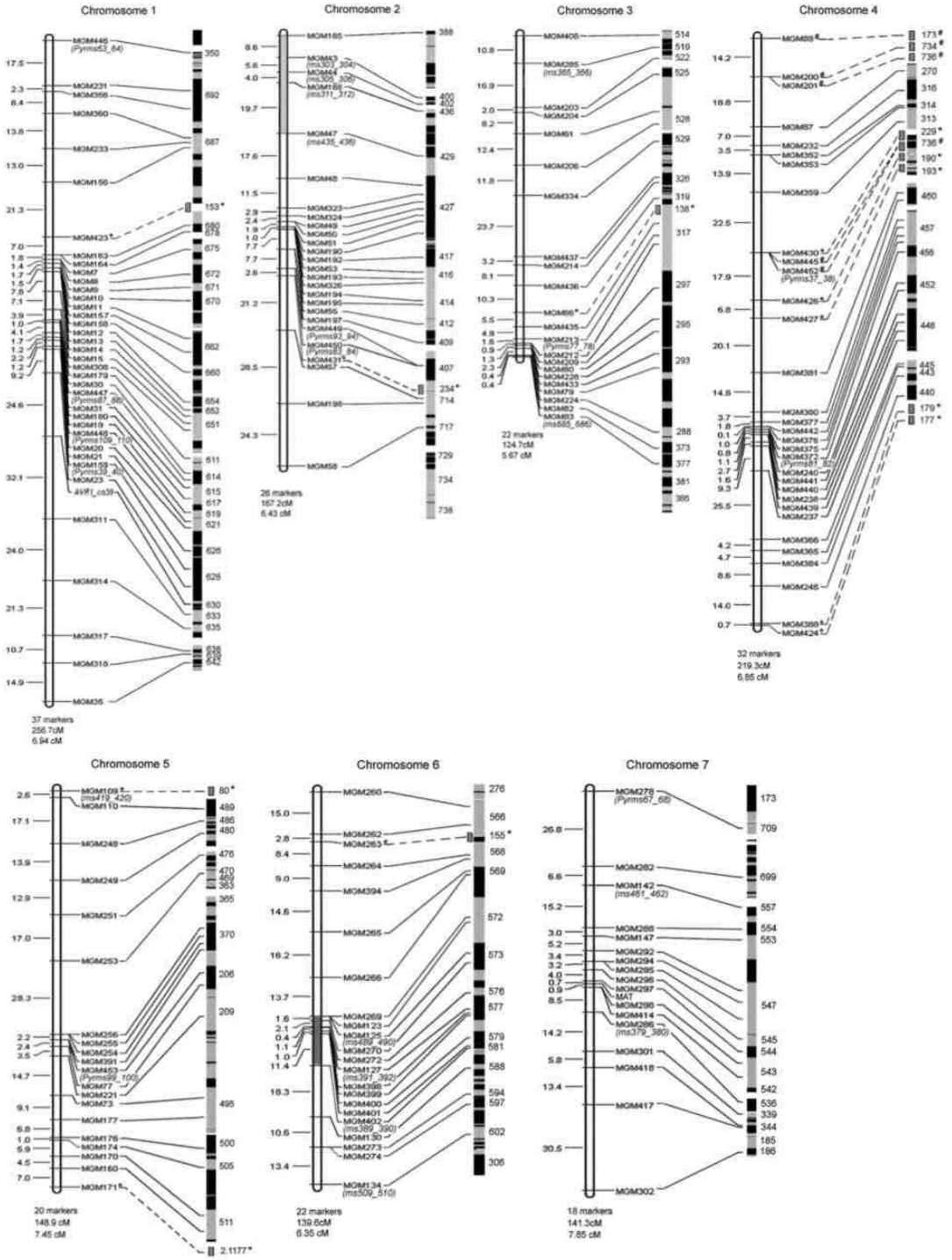


Fig. 2 Genetic map of Pyricularia oryzae Cav. created on the basis of physical map (option 5) and its comparison with physical map

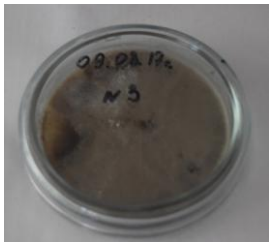
Previously published sequences of 18 pairs of primers (Adreit and Santoso 2007) were used for the genetic classification of Pyricularia oryzae Cav. Strains which were isolated in the territory of the Krasnodar Krai, the Rostov Region and the Republic of Adygea were subjected to by microsatellite analysis. Based on these primers sequences while the current study two multiprimer systems were developed for the analysis of 9 microsatellite loci simultaneously in one PCR sample.

As a result of the testing of these two SSR systems for analysing the genetic diversity of Pyricularia oryzae Cav. isolates from the affected herbarium material

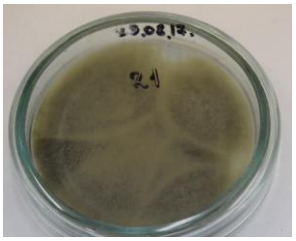
collected from rice fields in southern Russia, in the current study reproducible results were obtained for all 18 pairs of primers. However, out of these, only 6 pairs of primers (Pyrms 07-08, Pyrms 37-38, Pyrms 77-78, Pyrms 101-102, Pyrms 233-234, Pyrms 409-410, Pyrms 453-454, Pyrms 533-34, Pyrms 657-658) were found to be discriminatory for the Pyricularia oryzae Cav. strains studied.



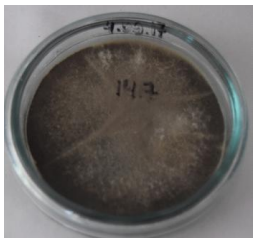
A



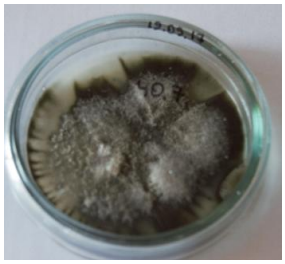
B



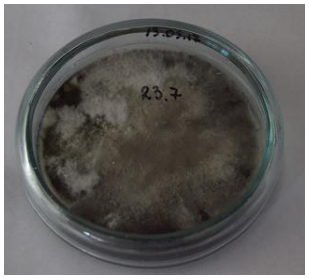
C



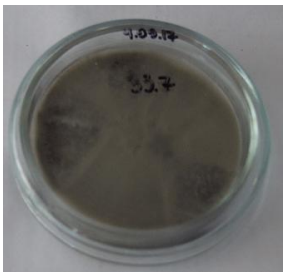
D



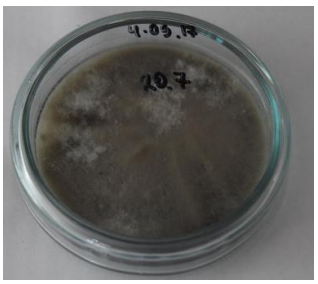
E



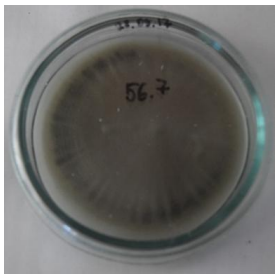
F



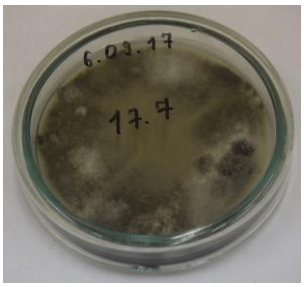
G



H



I



J

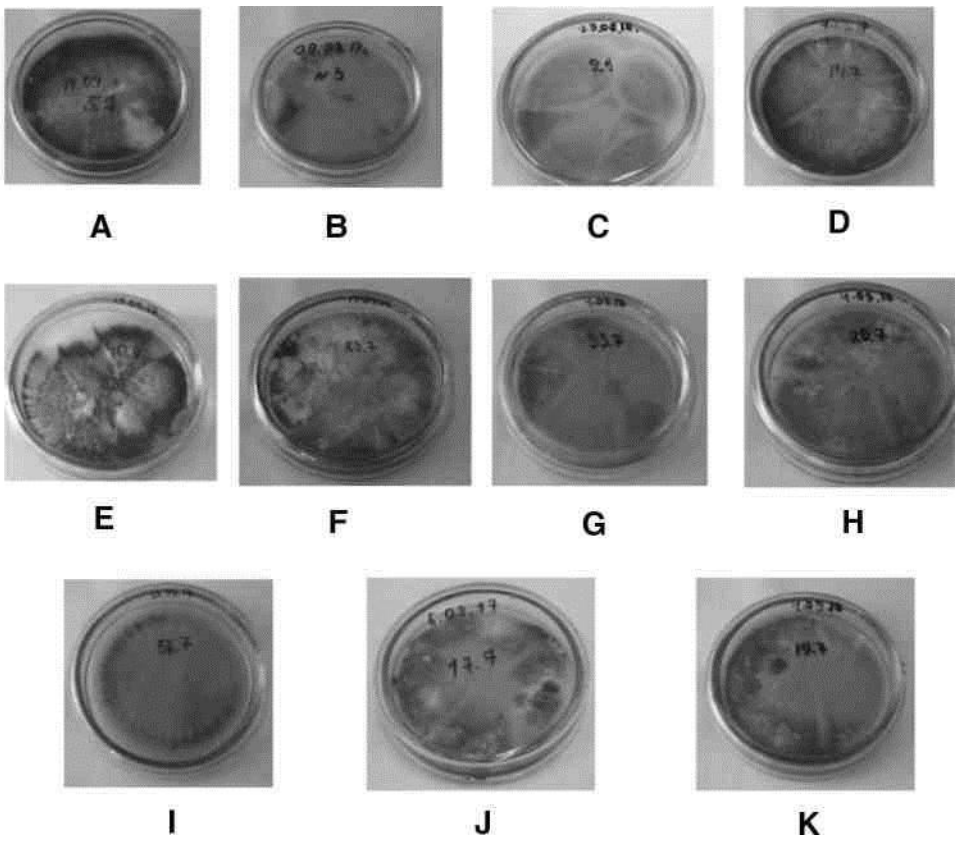


Fig. 3 Morphological and cultural attributes of the strains of rice blast pathogen Pyricularia oryzae Cav.

Table 4 Multi-primer PCR system for the genetic analysis of isolates of the rice blast pathogen from different ecological rice-growing regions of the Krasnodar Krai, the Rostov Region and the Republic of Adygea

No.	Primer	Indication	Fluorescent dye	Genotype 1 DNA fragment, base pair	Genotype 2 DNA fragment, base pair	Genotype 3 DNA fragment, base pair	Genotype 4 DNA fragment, base pair
1	Pyrms 07-08	A	R6G	130	138	130	124
2	Pyrms 47-48	B	ROX	176	174	176	172
3	Pyrms 83-83	C	FAM	179	182	179	187
4	Pyrms 43-44	D	FAM	222	205	222	212
5	Pyrms 99-100	E	R6G	220	214	223	200
6	Pyrms 427-428	F	ROX	224	222	224	208

Blue – fluorescent dye FAM; Green – fluorescent dye R6G; Red – fluorescent dye ROX

Table 5 Classification of rice blast pathogen Pyricularia oryzae Cav. strains based on the obtained genetic profiles and their DNA profiles

Genotype	A 07-08	B 47-48	C 83-84	D 43-44	E 99-100	F 427-428	Strain no.
1	124	171	186	210	199	206	K4-16
2	128	175	178	223	223	223	37-17
3	130	175	178	219	223	223	5-17
4	130	175	178	221	220	223	K1-16
5	132	175	178	221	223	223	55-17
6	134	175	178	223	223	223	18-16
7	138	173	180	204	211	219	48-16
8	138	173	180	204	214	221	3-17, 4-17, 14-17, 34-17
9	138	175	180	204	214	221	27-17
10	138	173	180	204	226	221	40-17
11	138	173	178	206	188	221	39-17
12	138	173	180	206	214	221	19-17
13	138	173	180	206	214	223	56-17
14	138	173	180	208	214	221	26-17
15	140	173	180	204	214	221	17-17, 21-17
16	140	173	180	208	214	221	33-17
17	140	173	180	225	220	221	20
18	146	179	180	214	211	223	7-16
19	130	176	179	222	220	224	12,21,37,38, 50,11,30,32
20	138	174	182	205	214	222	5, 31, 39, 7, 6
21	130	176	179	222	223	224	2, 5, 8
22	124	172	187	212	200	208	4

Based on these six SSR primers, a multi-primer PCR system was developed (Table 4), which was used to classify strains of Pyricularia oryzae Cav. isolated in the Krasnodar Region, the Rostov Region and the Republic of Adygea (Dubina et al. 2015).

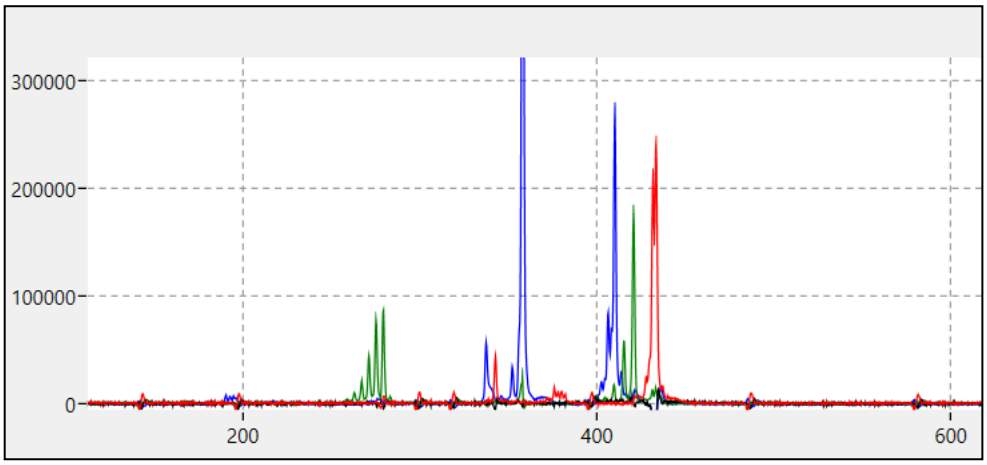
As a result of the analysis, individual genetic profiles were obtained for each strain (Table 5, Fig. 4). Samples of DNA isolates were used as positive controls, with known genetic profiles belonging to four different genotypes: K1 – strain No. 12, K2 – strain No. 5, K3 – strain No. 8 and K4 – strain No. 4. Repeated analysis of these samples gave reproducible results. Among 57 strains studied, 22 genotype variants were identified (Table 5).

It can be seen from the table that the results of fragment analysis using SSR markers revealed significant genetic differences between the isolates studied in the current work. The analysis of the obtained PCR results and their description by morphological and cultural attributes showed some differences in the distribution of pathogen strains into types (morpho- and genotypes). The major research findings were as follows:

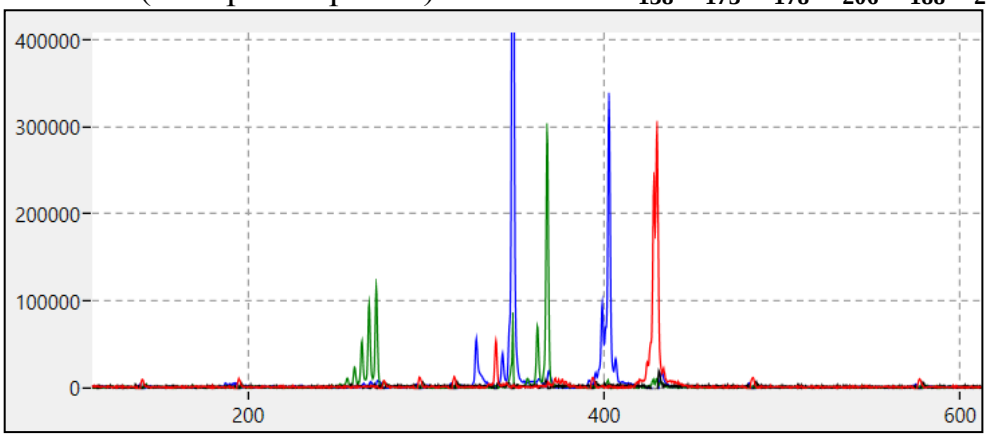
1. According to the genetic analysis results strains Nos. 3-17, 4-17, 14-17 and 34-17 have a similar genetic profile, but differ in morphological and cultural characteristics.
2. Strains 26-17, 21-17, 7-17 have the same morphotype, but different genetic profiles.
3. Strains Nos. 17-17 and 21-17 are similar in genotype, but different in morphotype. Strain number 9 belongs to one cluster with strain number 7-17 on the genetic profile, but they are different in morphological attributes.
4. Strains Nos. 37-17, 14-17, 39-17 are different in genotype, and included into the same cluster by morphotype. The other strains studied in the work correspond to their distribution into groups according to morphological and cultural attributes and genetic profiles.
5. Strains Nos. 2, 8, and 5 are assigned to genotype 21, which, when described by morphological and cultural characteristics, have the same growth pattern (powdery) and colour (light to dark grey) of colonies.
6. Strains Nos. 5, 31, 39, 7, and 6 are assigned to genotype 20. All of them, except for strain No. 39, have a felty growth pattern with the light grey or light olive to grey or olive colony in the description by morphological and cultural characteristics.
7. Strain No. 39 has a powdery and radiant colony growth pattern on the basis of cultural and morphological attributes.
8. According to the results of fragment analysis, strains Nos. 12, 21, 37, 38, 50, 11, 30, 31 are referred to genotype 19 with certain differences in morphological and cultural attributes: some strains have powdery growth with felty areas, and some strains are powdery, but with a low flocculent colony growth pattern (strains Nos. 37, 50).
9. Strain No. 4, characterized by a felt growth and a dark grey colony, had a unique genetic profile of the 22nd genotype.

The genetic profiles of some strains were shown as an example (Fig. 4).

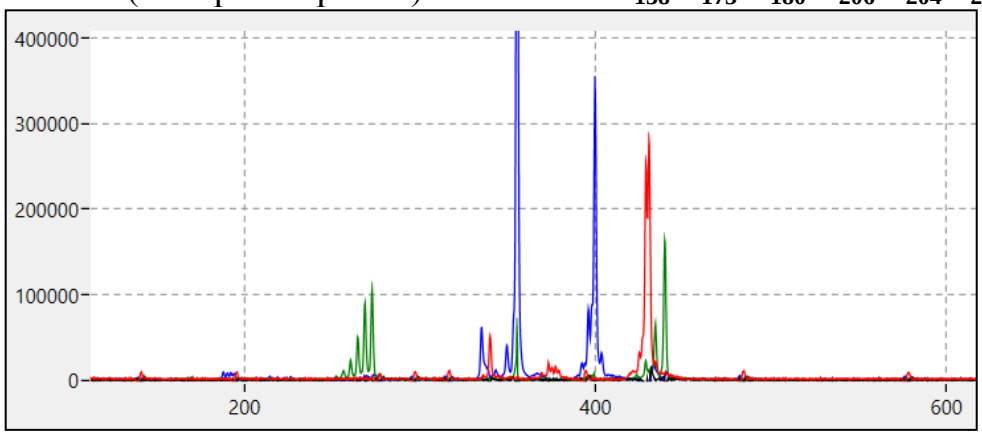
Штамм №33-17 (Красноармейский район) – генотип: $\mathbf{A_{140}\ B_{173}\ C_{180}\ D_{208}\ E_{214}\ F_{221}}$

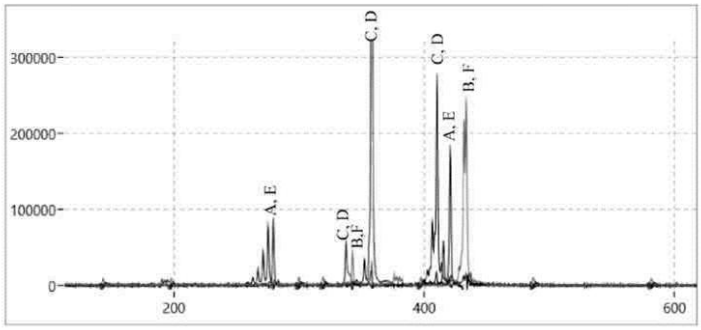


Штамм № 39-17 (Северский район)– генотип: **A$_{138}$ B$_{173}$ C$_{178}$ D$_{206}$ E$_{188}$ F$_{221}$**

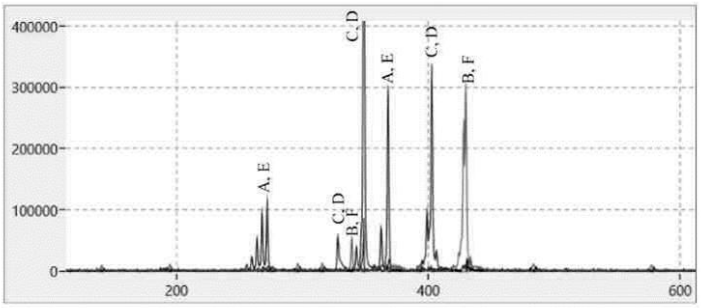


Штамм №40-17 (Северский район) – генотип: **A$_{138}$ B$_{173}$ C$_{180}$ D$_{206}$ E$_{204}$ F$_{221}$**

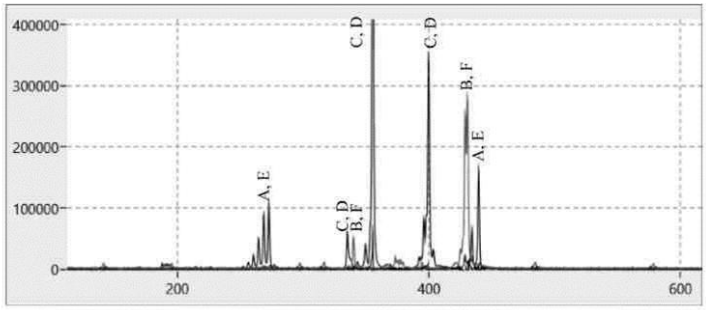




Strain number 33-17 (Krasnoarmeysky district) – genotype: A140 B173 C180 D208 E214 F221



Strain No. 39-17 (Seversky district) – genotype: A138 B173 C178 D206 E188 F221



Strain No.40-17 (Seversky district) – genotype: A138 B173 C180 D206 E204 F221

Fig. 4 Genetic profiles of the studied isolates of Pyricularia oryzae Cav., identified with DNA analysis

Thus, according to morphological and cultural attributes, Pyricularia oryzae Cav. strains studied are divided into 11 types (pathotypes), and 22 genotypes of the pathogen are isolated with DNA analysis. The differences between two methods can be explained by colony heterogeneity (by sectors) of some strains when the morphological and cultural attributes were described.

Despite the fact that DNA analysis is a more accurate method of identifying strains of Pyricularia oryzae Cav. since the study sample can be characterized according to the genotype, and this method has several advantages over the phenotype estimation, these two methods can complement each other when studying the biodiversity of the disease pathogen.

From the experimental data given in Table 5, it can be seen that the virulent genes for the rice-growing zone of Krasnodar are: Av-i$^{?}$, Av-ta$^{?}$, Av-z$^{t?}$, Av-k$^{m?}$, Av-sh$^{?}$, Av-ta $^{2?}$, Av-z $^{5?}$, Av-9$^{?}$, Av-3$^{?}$, Av-kh$^{?}$, Av-z $^{t?}$, Av-t$^{?}$, Av-19$^{?}$. Effective Pyricularia oryzae Cav.-resistant genes: Pi-k^{s}, Pi-a, Pi-m, Pi-k, Pi-1, Pi-7(t), Pi-z, Pi-b, Pi-12(t), Pi-11(t), Pi-33, Pi-1 which can be used as donors in breeding programs for the development of resistant rice varieties to rice blast for this cultivation

area.

Taking into account the ecological and geographical aspects of the rice-growing region of the Krasnoarmeysky district, virulence genes were determined as follows: Av-z$^{t?}$, Av-sh$^{?}$, Av-ta$^{?}$, Av-ta $^{2?}$, Av-9$^{?}$, Av-t$^{?}$, Av-19$^{?}$. As a

result of gene identification for resistance to rice blast based on phytopathological testing using differentiation rice varieties which overcome the Krasnodar population Pyricularia oryzae Cav. for this cultivation area are genes: Pi-k^{s}, Pi-a, Pi-k, Pi-1, Pi-3, Pi-kh, Pi-z, Pi-11(t), Pi-33. Donors with these effective resistance genes to pyricularia can be recommended for use in breeding programs for the development of disease resistant rice varieties.

Researchers from other countries have also been studying the biodiversity of Pyricularia oryzae Cav. in rice-growing regions of their countries. Kariaga et al. (2017) analyzed the genotypes of eight different isolates previously described morphologically using DNA analysis and sequencing of the its region from the rice genome. Results revealed variation with respect to colony colour, diameter, morphology and conidia shape and size demonstrating that there is a certain level of genetic diversity among isolates of P. oryzae from various regions of Kenya (Kariaga et al. 2017).

In a similar study conducted in Brazil using 57 isolates and 8 rice-growing regions a total of 21 strains were identified, with a prevalence for the IA group strains, in all collection sites, especially the IA-1 strain. From the studied isolates, it was concluded that there is a variability of P. oryzae, prevalence of the IA strain, a higher tolerance for the cultivar BRS Firmeza and that the Pi-2 gene confers resistance to rice blast in all isolates analysed (Martins Nunes et al. 2014).

The main difference from the above articles and the uniqueness of this study is that the effective genes for resistance to blast identified in this work can be recommended for use in breeding programs for the development of disease-resistant rice varieties in rice-growing zones.

Conclusions

As a result of this study, based on the fragment analysis method, the studied races of Pyricularia oryzae Cav. were divided into twenty-two genotypes. However, according to morphological and cultural features, the studied races of Pyricularia oryzae Cav. were divided into 11 types of pyricularia. This is due to the fact, that when described according to morphotypes, some races were characterised by heterogeneity of the colony (sectors). Despite the fact that DNA analysis is a more accurate method for the identification of Pyricularia oryzae Cav. races, as it allows sample characterisation by genotype and has a number of advantages over the ‘‘phenotype’’ evaluation, these two methods can complement each other for the studies of biodiversity of the pathogen.

During identification of (a)virulence genes based on phytopathological testing using a set of differentiation varieties of pyricularia races, the resistance genes to Pyricularia oryzae Cav, effective for the south of Russia, were identified. The existence of a large number of effective genes can be explained by the fact that for more than 25 years the All-Russian Rise Research Institute has conducted direction

of breeding of rice varieties resistant to pyricularia.

The effective resistance genes of Pyricularia, found during this study, can be recommended for use in breeding programs for the development of disease resistant rice varieties in the rice-growing areas of the Krasnodar Region: Pi-k^{s}, Pi-a, Pi-m, Pi-k, Pi-1, Pi-7(t), Pi-z, Pi-b, Pi-12(t), Pi-11(t), Pi-33 for the rice-growing areas of the Krasnoarmeysky district: Pi-k^{s}, Pi-a, Pi-k, Pi-1, Pi-3, Pi-kh, Pi-z, Pi-11(t), Pi-33.

Acknowledgments The studies were carried out with the grant of FGBU RFFS ‘‘p_a’’ No. 16-44-230178 ‘‘Study of the genetic structure of the pathogen population of Pyricularia oryzae Cav. and scientific rationale for immunogenetic protection of rice culture’’ in the laboratories of rice protection and biotechnology and molecular biology of the All-Russian Rice Research Institute, as well as in cooperation with the All-Russian Scientific Research Institute of Agricultural Biotechnology (Moscow) and the Agrarian Science Centre Donskoy (Zernograd).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adreit H, Santoso D (2007) Microsatellite markers for population studies of the rice blast fungus, Magnaporthe grisea. J Compil Mol Ecol Notes 131:667–670

Ahn SN, Kim YK, Han SS (1996) Molecular mapping of a gene for resistance to a Korean isolate of rice blast. Rice Genet Newsl 13:74–76

Arase S, Fujita K, Uehara T (2000) Light-enhanced resistance to Magnaporthe grisea infection in the rice Sekiguchi lesion mutants. J Phytopathol 148:197–203

Ayres NM, McClung AM, Larkin HPD (1997) Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germ plasm. Theor Appl Genet 94:773–781

Bryan GT, Wu KS, Farrall L (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. Plant Cell 12:2033–2045

Castiglioni P, Pozzi C, Heun M (1998) An AFLP-based procedure for the efficient mapping of mutations and DNA probes in barley. Genetics 149:2039–2056

Conaway-Bormans CA, Marchetti MA, Johnson CW (2003) Molecular markers linked to the blast resistance gene Pi-z, in rice for use in marker-assisted selection. Theor Appl Genet 107:1014–1020

Dean DB, Adesogan AT, Krueger N, Littell RC (2005) Effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrass silage. J Dairy Sci 88(3):994–1003

Dubina EV, Kostylev PI, Esaulova LV (2015) Development of multiplex PCR systems for identification of resistance genes to Magnaporthe grisea (Herbert) Barr and creation of new resistant forms (donors) of Oryza sativa L. to the pathogen. Grain Econ Russia 1(37):30–34

Fuji K, Hayano-Saito Y, Sugiura N (1999) Gene analysis of panicle blast re-

sistance in rice cultivars with rice stripe resistance. Breed Res 1:203–210

Jensen LB, Courtous B, Shen L (2001) Locating genes controlling allelopathic effects against barnyard grass in upland rice. Agron J 93:21–26

Kariaga MG, Wakhungu J, Were H, Muoma D, Onamu R, Wekesa C (2017) Rice blast Pyricularia oryzae Cav. isolates in Kenya characterized sequenced and registered. Int J Life Sci Res 5(3):23–30

Kaye C, Milazzo J, Rozenfeld S (2003) The development of simple sequence repeat (SSR) markers for Magnaporthe grisea and their integration into an established molecular linkage map. Fungal Genet Biol 40(3):207–214

Khush GS, Brar DS, Hardy B (2001) Rice genetics VI. Los Banos. http://books.irri.org/157808167X_content.pdf

Kovalenko ED, Gorbunova YuV, Kovaleva AA (1988) Methodical guidelines for assessing the resistance of rice varieties to the causative agent of pericularyosis. Nauka, Moscow

Kumar J, Zeigler R (1995) Mating behaviour of Magnaporthe grisea from Central Himalayas of India. Phytopathology 85:1201

Lang NT, Khush GS, Huang N (2001) Fine mapping for blast resistance gene in rice (Oryza sativa L.) using bulked segregant analysis. Omonrice 1:1–8

Launder ES, Green P, Abrahamson J (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage map of experimental and natural population. Genomics 1:174–181

Levy M, Correa-Victoria FJ, Zeigler RS (1993) Genetic diversity of rice blast fungus in a disease nursery in Colombia. Phytopathology 83:1427–1433

Li CY, Li JB, Liu L (2007) Development of minisatellite markers in phytopathogenic fungus, Magnaporthe grisea. Mol Ecol Notes 131:144–152

Martins Nunes CD, Salete Mota M, de Carvalho FIF, de Oliveira AC (2014). Variability of Pyricularia oryzae Cav. in irrigated rice genotypes. Pesqui Agropecu Trop, 44(3). http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1983-40632014000300014& lng=pt&tlng=pt

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 10:4321–4325

Padmanabhan SY, Chakrabarti NK, Mathur SC (1970) Identification of pathogenic races of Pyricularia oryzae in India. Phytopathology 60:1574–1577

Resurreccion AP, Villareal CP, Parco A (1994) Classification of cultivated rices into indica and japonica types by the izozyme, RFLP, and two milled-rice methods. Theor Appl Genet 89:14–18

Xia JQ, Corell JC, Lee FN (1993) DNA fingerprinting to examine microgeographic variation in Magnaporthe grisea (Pyricularia grisea) population in two rice fields in Arkansas. Phytopathology 83:1029–1035

Yankovsky NK (1996) Molecular genetic methods in the hands of a detective, or the experience of researching the remains of the family of the last Russian emperor. Soros Educ J 2:21–27

Zheng W, Finkel JS, Landers SM, Long RM, Culbertson MR (2008) Nonsense-mediated decay of ash1 nonsense transcripts in Saccharomyces cerevisiae. Genetics 180(3):1391–405

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.