Screening of rice gene pool for resistance to pyriculariosis by phenotyping and DNA genotyping

T Korotenko¹, Zh Mukhina¹, S Garkusha¹, E Savenko¹ and O Bragina¹

¹Federal State Budgetary Scientific Institution «All-Russian Rice Research Institute», Belozerny village, 3, Krasnodar, 350012, Russia
E-mail: korotenko.tatyan@mail.ru

Abstract. When creating new resistant varieties of rice of an intensive type, an important aspect of the breeding process is the study of the diversity of genetic resources. In the Krasnodar Territory, in Russia and in the world as a whole, the most harmful and common disease of rice is pyriculariosis (Pyricularia oryzae Br.et Cav.). The aim of this research was to study the genetic diversity accumulated in the collection of the rice institute (ARRRI, Krasnodar city) for the resistance to the causative agent of pyriculariosis to create a new source material with genes for a wide spectrum of resistance. In the course of studies, the results of phenotypic (1337 samples), immunological (775 samples) and molecular screening (115 samples) of the rice gene pool of Oryza sativa L. were obtained for the resistance to the pathogen of pyriculariosis. The rate of development of the disease (RDD, %) over the years of testing the general plasma varied within 5.6-85.0 %. The proportion of varieties medium resistant to the fungus M. Grisea was significantly higher in almost all countries except Italy and Brazil, where the number of resistant forms was at the level of medium resistant. In the general plasma of Bulgaria, Uruguay, Greece, India and African countries, stable forms were generally absent. The world variety of rice genotypes showing resistance to the local pathogen population has been shown. Screening of the rice gene pool for the presence of the Pi-9 and Pi-k genes made it possible not only to identify carrier donors, but also to establish their frequency of occurrence in the global general plasma. Against a provocative background, the identification of effective resistance genes was carried out using monogenic differentiator lines of Pyricularia oryzae races, 15 resistance genes were isolated.

1. Introduction

The range of domestic rice cultivation covers a significant territory between latitude 45° n. l. in the Krasnodar Region up to 49° s. l. in the Primorsky Region. In Russia, the climatic conditions of the Krasnodar Region are especially favorable for growing a heat-loving crop of rice. According to the rating of the regions of the Russian Federation for rice production, the Krasnodar Region accounts for more than 77% [1]. In recent years, rice cultivation in the Kuban has come close to a yield of 7.0 t/ha. The share of selection varieties of the All-Russian Research Institute of Rice (FSBSI ARRRI) in crops is consistently high - 90.9% (187.9 thousands ha). The largest area in the region is occupied by varieties: Rapan (39.4%), Hazar (8.2%) and Diamant (7.0%) [2].

It is known from the scientific press that, regardless of culture, the genetic uniformity of cultivated varieties creates conditions for the mass propagation of pests [3]. The degree of manifestation of resistance to the disease of the same variety for a number of years in vivo may be different. Therefore,
there is a constant search for new sources of disease resistance among ecotypes of various ecological and geographical origin.  

Pyriculariosis is the most formidable rice disease and is caused by a fungus Magnaporthe grisea (Hebert) Barr. The loss of gross grain harvest usually reaches 20%, and more with epiphytoties. The problem of selection for immunity to fungal diseases is complicated by the multiplicity of strains of Pyricularia and the narrow genetic basis of disease resistance of modern varieties. The studies of the resistance of varieties to the Pyricularia oryzae pathogen, the most harmful disease of the culture, conducted in almost all rice-producing countries [4]. Particular attention is paid to the control of genes encoding resistance to the pathogen of pyriculariosis [5]. Currently, over 100 genes and more than 350 loci of quantitative traits determining resistance to P. Oryzae [6].

To date, certain successes have been achieved in the field of rice cultivation in the creation and introduction into industrial production of rice varieties with pyramidal genes of resistance to pyriculariosis. First variety of rice if Blastonic (1992) with race-specific resistance to pyriculariosis, created on domestic hybrid material. In subsequent years, varieties with similar resistance were transferred to the State Ortho-Testing: Vityaz (1994), Talisman (1995), Snezhinka (1996) and Vodoley (1998) [7]. Mid-ripening rice variety Patriot (2016) is resistant to shedding and lodging, has the Pi-b resistance gene, high cereal quality and yield of 17.1 c / ha more than that of the standard Rapan variety [8].

To reduce the severity of pyriculariosis, varieties with group resistance to a complex of phytopathogens are created. Using innovative biotechnologies for immunological selection, Rostov scientists have created variety samples with five resistance genes: Pi-1+Pi-2+Pi-33+Pi-b+Pi-ta [9].

Many works are devoted to the study of the genetics of resistance to the causative agent of pyriculariosis by DNA marking. Scientists have found that the distribution of alleles of genes resistant to the Pyricularia or. depends on climatic factors and the variety of cultivated varieties [10-17]. Therefore, to improve varieties in terms of increasing resistance to Magnaporthe g. fungus races of the insufficiently resistant local varieties, it is necessary to expand their genetic basis. The role of collections and their information base is growing, containing information on SSR genotyping of the gene pool of agricultural crops.

Considering that alleles that could significantly improve modern varieties genetically are found in collections of genetic resources with a very low frequency (Hyten et al., 2006), it is necessary to combine phenotypic assessment of character carriers with molecular labeling methods in studies with genetic resources.

It is world famous that the Russian academician N.I. Vavilov identified seven centers of biodiversity and with his staff gathered from these places a world collection of plants [18]. To date, this collection has more than 324 thousand samples of cultivated plants and their wild relatives. This collection is the national treasure of the country, the basis of food security and serves as a rich source material for breeding. Over the past decade, scientists have considered the exchange of general plasma as a necessary component in increasing the yield of varieties and increasing resistance.

The homeland of rice culture is considered the South Asian center, which has a large genotypic variety. Asia is the largest continent by area, which gave the world the largest number of species (about 70%) of the entire cultural flora. The most significant working collections of seeds, oilseeds and cereals have been created and are maintained in Russian scientific organizations. The main gene pool of rice in Russia is concentrated in the Federal Research Center "All-Russian Institute of Plant Growing" named after N.I. Vavilov (St. Petersburg) - more than 10 thousand and the leading scientific institution in Russia on rice production "All-Russian Rice Research Institute" (Krasnodar) - more than 7.0 thousand. Both of these collections are the custodians of the unique genetic material of rice with a wide range of traits from different agroecological groups. To study the world variety of seed crops, in addition to traditional agronomic, immunological, physiological, biochemical and biological methods, modern classical morphometric and molecular-genetic, as well as computer express-methods of sample analysis are used that allow mass screening of the initial forms for the correct breeding strategy [18, 21].
Among the research works carried out with the gene pool of the All-Russian Scientific Research Institute of Rice, both applied and fundamental, using DNA marker analysis: the pyramidation of genes for resistance to pyriculariosis and the creation of more than 50 lines carrying genes for resistance to Pi-b, Pi-40, Pi-ta and Pi-z [8,10,19]. Perspective pyriculariosis-resistant samples with a complex of valuable traits that are promising for domestic breeding have been identified: high quality, productive Pyongyang 3 (Korea); highly-productive IR 78221, WAB 99-47 (Philippines) and JR 73694-41-2 (Thailand); coarse-grained productive Galileo (Italy), Avangard (Uzbekistan).

The strategy of using molecular markers for screening plant genetic resources for immunity with the target resistance genes to the Pyricularia or. Cav., as donors for marker selection, will accelerate the first stage of the breeding conveyor - obtaining new source material with identified genes and raising the ecological status of rice production.

The aim of the study is to summarize data on a comprehensive study of the resistance of collection samples to the Krasnodar population of the causative agent of pyriculariosis using various methods for selecting the promising sources for immunity selection and the donors of features.

2. Materials and methods

In 2014-2018, against the artificial infectious background of FSBSI ARRRI (All-Russian Scientific Research Institute of Rice), 775 collection rice varieties were tested for resistance to P. oryzae. Samples of two subspecies were taken for study - indica and japonica Kato Oryzas. L. from a unique scientific collection of the Institute with coverage of the main centers of rice biodiversity from nurseries of resistant varieties to biotic and abiotic environmental factors (IRCTN, IRSATON, IRBN). Introduced rice samples obtained in the framework of international treaties on scientific and technical cooperation between the countries of near and far abroad [20].

The base of experience, counts and observations, visual assessments, phenological observations, the study of the world collection was carried out according to the Methodological guidelines for the study of the world rice collection and the classifier Oryza L. (1982) «Russianstandart» [21]. A comprehensive field assessment of the rice gene pool was carried out in vivo in a field experiment in a collection nursery in an experimental irrigated area of the Rice Institute (Krasnodar) under the same conditions with equal standing density and on an optimal agricultural background. The size of the experimental plots in the collection nursery was 1.0 m², the distance between the rows in the plot was 15 cm, and between the plots 30 cm. Rapan and Flagman, widely used in the Kuban crops, were used as a standard.

The soil of the experimental plot is rice, meadow chernozem, slightly saline, hard loamy, arable horizon with a total humus content of 2.9%, easily hydrolyzed nitrogen - 4.3 mg / 100 g, mobile phosphorus - 4.1 mg / 100 g and mobile potassium - 21.9 mg / 100 g of soil, pH 7.7. Irrigation regime - shortened flooding. The agricultural cultivation culture in the experiment corresponded to the recommendations of the Rice Institute.

Phytopathological assessment of rice samples was performed against the provocative background of the infectious nursery of the laboratory of agriculture. Assessment of resistance to the pathogen of pyriculariosis P. oryzae - according to the methodology of the All-Russian Research Institute of Phytopathology (Moscow, 1988). The materials for the study were: collection variety samples - representatives of 29 rice-growing countries from 7 ecological-geographical groups with a vegetation period of 90 to 135 days, 2 standard varieties: susceptible Pobeda 65 and steady Avangard, as well as 7 varieties from an international set of differentiating varieties (China, Japan, India, Philippines), 4 domestic varieties and 26 monogenic rice lines (Japan and the Philippines, IRRI) with known resistance genes for identifying races of the pathogen of pyriculariosis: Pi-1, Pi-3, Pi-9, Pi-t, Pi-ta, Pi-ta2, Pi-a, Pi-b, Pi-i, Pi-19, Pi-20, Pi-40, Pi-k, Pi-kh, Pi-kp, Pi-ks, Pi-km, Pi-sh, Pi-z, Pi-z5, Pi-zt, Pi-5(t), Pi-7(t), Pi-11(t), Pi-12(t).

The collection of bio-material infected with pyricularia on the fields of rice-growing farms in the Krasnodar Region ensured the high quality of the synthetic pathogen population. Immunological evaluation of varieties for resistance to this disease was based on the creation of a rigid artificial infectious background. For infection, a natural fungal isolate of M. grisea was used, which was isolated
from samples of plants with well-defined symptoms of the disease. Dishes with samples were exposed in a thermostat at a temperature (27±1) °C. Observation of fungal growth began after 48 hours under a binocular magnifier at a 20-50-fold zoom. After the appearance of sporulation under a microscope with the (x-120) zoom, the identity of the fungus conidia to the M. grisea type was visually determined. Upon reaching the fungal colonies with a diameter of 0.5 to 0.75 of the size of the Petri dishes, they were scattered. Dry spore material was obtained from a pure 14-day culture of the fungus with a sporulation of at least 20 million conidia / Petri dish. Plants were inoculated with a spore suspension in the most vulnerable phases of rice development: tillering (5-7 leaves), paniculation - flowering. The consumption rate of dry spore material is 5 mg/m² with 100% spore germination, 2.5 mg per plot. Infection was carried out in the evening, during the period of dew and in the absence of wind.

The stability of rice varieties was determined by the defeat of leaves, nodes, stems and panicles and the disease development index was evaluated (IDD, %). The affection of plants with the leaf form of pyriculariosis was determined 10 and 20 days after inoculation, and the nodes and panicles of rice plants were determined in the phase of milk-wax and full ripeness of grain. Two indicators were taken into account: the type of reaction (in points) and the intensity of plant damage (in %). The International Rice Institute Point Scale was used (IRRI, Philippines) according to the degree of damage to plants, where 0 - no damage (type of resistance: highly resistant); 1-3 points - single brown dots (stable forms); 5 - 6 points - typical spots of pyriculariosis, affected from 10 to 25% of the area (medium resistant); 7 - 9 points - from 50 to 100% of the leaf area is affected (susceptible and unstable forms). According to the DII (disease intensity index, %), varieties are classified into categories: resistant - 0-25%, medium-resistant - 25.1-50% and unstable -> 50%. Samples of local forms and introduced were analyzed by DNA marking. DNA was isolated from the selected rice leaves under laboratory conditions of the All-Russian Research Institute of Rice. For genotyping of 115 rice samples, molecular markers k8823 and pB8 associated with the Pi-9 and Pi-k genes, respectively, controlling resistance to the pathogen of pyriculariosis were used [6,14-17]. PCR analysis was performed using domestic sets made by SibEnzyme, Russia. To visualize the PCR of the product, electrophoretic separation of DNA fragments was performed. During electrophoresis, DNA fragments migrate in a 2.5% agarose gel based on 0.5 × Tris borate buffer under the influence of electric field forces. The results of agarose gel DNA electrophoresis were recorded in the presence of ethidium bromide, an intercalating compound that forms a stable compound with DNA fragments, which appears in ultraviolet light at 290-330 nm in the form of luminous bands when the gel is irradiated with a UV transilluminator. Fragments of the analyzed DNA appear in the form of luminous orange-red stripes.

3. Results

According to the Food and Agriculture Organization FAO, about 774 thousand of rice samples were stored in gene banks of the world for the period of 2009, for the period of 2013 the number of stored samples of cultivated and wild rice was about 900 thousand [18]. The collection of the All-Russian Rice Research Institute (ARRRI) in 2018 totaled more than 7.0 thousand samples of sowing rice (Oryza sativa L.) adapted to the agro-climatic conditions of southern Russia, 82 botanical varieties from 40 rice-growing countries of the world (8 ecological and geographical zones - EGG). In the structure of the gene pool there are both ancient and modern rice varieties, as well as selection lines for competitive testing, individual selection from hybrid populations, mutants, regenerates, digaploids and foreign varieties of near and far abroad.

The institute conducts scientific research on the conservation and study of ecotypes, the exchange of general plasma with breeding centers and gene-banks of different countries [20]. In the structure of the gene pool, more than 53% of the samples are representatives of the European environmental group, 34% are varieties and samples of the Philippine EGG (from the international institute IRRI), and about 10% are varieties of Asian origin. According to their taxonomic affiliation, the collection contains about 56% of specimens of the italica Alef variety of subspecies japonica Kato – these are short- and medium-grain varieties, more than 15% of samples of mutica Vav and gilanica gust. varieties of subspecies indica Kato (long-grain varieties).
The selection of disease-resistant parent forms for breeding programs depends on the environmental conditions of the Kuban and the genetic structure of the P. oryzae fungus population. In this regard, we approached differentially the methods of evaluating and selecting the source material: we used phenotyping in vivo field experience and on a provocative background, as well as the method of DNA genotyping to identify resistance genes.

775 rice samples from 29 countries (Table 1) were exposed to the immunological evaluation on the infectious background by inoculation by a synthetic population of a pathogen in the period 2014-2018.

| Country of origin of rice varieties | Total samples studied | Varying the Index of Disease Development, % | The average index of disease development (IDD), % by varieties |
|-----------------------------------|-----------------------|--------------------------------------------|---------------------------------------------------------------|
| Russia                            | 390                   | 21.0-85.0                                  | 38.4 ± 2.2                                                   |
| China                             | 45                    | 5.6-73.0                                   | 33.1 ± 6.3                                                   |
| Japan                             | 9                     | 22.2-55.0                                  | 35.5 ± 2.0                                                   |
| Philippines                       | 97                    | 7.8-71.0                                   | 41.6 ± 5.4                                                   |
| Afghanistan                       | 1                     | 55.0-60.0                                  | 57.5 ± 0.5                                                   |
| Italy                             | 32                    | 6.7-41.0                                   | 24.1 ± 1.6                                                   |
| Kazakhstan                        | 12                    | 6.5-58.0                                   | 30.2 ± 5.3                                                   |
| Azerbaijan                        | 3                     | 25.0-57.1                                  | 37.3 ± 6.1                                                   |
| Madagascar                        | 8                     | 27.0-77.0                                  | 58.4 ± 5.2                                                   |
| France                            | 34                    | 6.7-73.0                                   | 28.4 ± 2.5                                                   |
| Korea                             | 24                    | 5.6-61.0                                   | 23.7 ± 2.2                                                   |
| Vietnam                           | 5                     | 21.2-64.0                                  | 50.1 ± 4.2                                                   |
| Uzbekistan                        | 4                     | 37.0-43.5                                  | 40.2 ± 2.5                                                   |
| Bulgaria                          | 4                     | 38.0-46.5                                  | 42.0 ± 3.4                                                   |
| Uruguay                           | 2                     | 44.0-58.0                                  | 50.6 ± 4.0                                                   |
| USA                               | 11                    | 10.0-44.0                                  | 26.8 ± 3.3                                                   |
| Greece                            | 2                     | 40.5-50.0                                  | 43.2 ± 1.8                                                   |
| Romania                           | 5                     | 22.0-51.0                                  | 28.4 ± 2.0                                                   |
| Hungary                           | 3                     | 38.0-55.0                                  | 42.1 ± 2.1                                                   |
| Turkey                            | 17                    | 21.1-53.0                                  | 37.0 ± 2.2                                                   |
| India                             | 3                     | 44.1-50.0                                  | 45.2 ± 1.1                                                   |
| Thailand                          | 4                     | 22.0-45.0                                  | 28.7 ± 3.4                                                   |
| Australia                         | 1                     | 20.6-24.0                                  | 23.0 ± 1.0                                                   |
| Brazil                            | 2                     | 6.7-27.8                                   | 18.4 ± 3.5                                                   |
| Nigeria                           | 5                     | 38.5-61.0                                  | 50.1 ± 4.2                                                   |
| Egypt                             | 1                     | 44.0-50.0                                  | 47.6 ± 2.0                                                   |
| Puerto Rico                       | 1                     | 34.0-41.5                                  | 39.5 ± 1.5                                                   |
| Chile                             | 2                     | 23.0-50.0                                  | 25.0 ± 1.6                                                   |
| Ukraine                           | 48                    | 15.0-65.0                                  | 42.1 ± 2.5                                                   |

The disease development intensity (DII, %) over the years of researches of collection samples ranged from 5.6 - 85.0%. The largest variation in the index of the disease development was observed in rice varieties from China, Philippines, France and Ukraine. Varieties from Afghanistan, Madagascar, Uruguay, Nigeria, and Egypt are marked by a stable high degree of susceptibility. On average, during the research period, a relatively low pathogen susceptibility against a provocative background was observed in varieties from Italy, France, Korea, USA, Brazil, and Chile.

The stability or susceptibility of a grain crop is judged by two criteria: the intensity of plant damage and the manifestation of externally visible reactions. In this regard, there are two approaches to assessing sustainability:

1) taking into account the intensity of the manifestation of the disease;
2) identification of indicators of immunity or their absence.

The data obtained for four years of researches on the intensity of the disease development of varieties of different ecological and geographical origin allowed us to differentiate all the studied varieties into the three groups by countries (Figure 1). The proportion of medium tolerant varieties was significantly higher in almost all countries except Italy and Brazil, where the number of resistant forms was at the level of medium tolerant.

![Figure 1. Differentiation of the studied samples of world selection by categories of resistance to the Krasnodar population of the causative agent of pyriculariosis (2014-2018).](image)

The minimum number of resistant forms was found among the general plasma of Ukraine, and in the general plasma of Bulgaria, Uruguay, Greece, India and African countries there were no stable forms at all. The largest proportion of resistant forms to the local population of the causative agent of pyriculariosis is among varieties originating from Italy, Kazakhstan, Korea, Brazil and Chile. The studied set of varieties from Russia, China and Japan gave a similar type of reaction against a provocative background. Moreover, the data obtained do not allow to draw a conclusion about geographical patterns in the manifestation by rice ecotypes of resistance to the causative agent of pyriculariosis in the conditions of the Kuban due to the unrepresentativeness of the sampling from a number of countries.

Since the modern concept of selection consists in combining traditional methods with new directions in the field of basic researches, the molecular genetic assessment and synthesis of new initial forms will ensure the creation of genotypes with a high resistance potential to a complex of adverse environmental
factors. The goal of the next stage of the study is to determine the presence of genes for resistance to pyriculariosis in rice varieties of world selection using DNA markers for subsequent selection of gene donors for pyramidation them into genotypes of Kuban selection.

The use of molecular markers allows accelerated preselection selection in collections for resistance to the pathogen of pyriculariosis. In order to search for donors of the Pi-9 and Pi-k genes that determine resistance to a wide spectrum of pathogen races [10,16,22,23], the collection was screened using DNA markers.

To screen a set of 115 collection rice samples for interest genes, the following were used:

- k8823 marker (sequence of primers to identify the target gene Pi-9:
  straight, F-GTTGTGGGTTCCTCTATAACA;
  back, R - GCATGACAGATGGAAGTGTAGATGG);
- pB8 marker (sequence of primers to identify the target gene Pi-k:
  straight, F- CCGGACTAAGTACTGGCTCGATA,
  back, R - CCCAATCTCCAATGACCCATAAC).

Gene Pi-9 was localized on 6th chromosome; gene Pi-k was localized on 11th chromosome.

As a positive control during DNA genotyping, three differentiating varieties from an international set of varieties IRRI (Филиппины) were used: No 28-14, 60-14 IRBL9-W (gene Pi-9 carriers) and No № 31-14 IRBLk-Ka (gene Pi-k carriers). Phytopathological tests conducted with these differentiating varieties showed their immunity to local races of the pathogen. Table 2 presents only identified gene donors of the set of studied varieties (115 pieces).

**Table 2.** Identification of Pi-9 and Pi-k genes for resistance to pyriculariosis by molecular markers in the rice general plasma from various ecological and geographical zones of world rice cultivation.

| Catalogue number | Sample name   | Ecological and geographical group | The presence of the Pi-k gene according to DNA analysis | The presence of the Pi-9 gene according to DNA analysis |
|------------------|--------------|-----------------------------------|------------------------------------------------------|------------------------------------------------------|
| FSBSI ARRRI      |              |                                   |                                                      |                                                      |
| 01977            | Kulon 7381   | European                          | yes                                                  | no                                                   |
| 02281            | Avangard     | Central Asian                     | yes                                                  | no                                                   |
| 03835            | Mutant 168-87| European                          | yes                                                  | no                                                   |
| 03838            | Chirnogi 16  | European                          | yes                                                  | no                                                   |
| 03884            | Krasnodarsky 86 | European                   | yes                                                  | no                                                   |
| 04060            | Talisman     | European                          | no                                                   | yes                                                  |
| 04072            | Snejzhinka   | European                          | no                                                   | yes                                                  |
| 04124            | Taibonnet    | Latin American                    | no                                                   | yes                                                  |
| 04207            | Deshan B     | South Asian                       | yes                                                  | no                                                   |
| 04209            | Norin RL-22  | Philippinian                      | yes                                                  | no                                                   |
| 04210            | Onda         | Latin American                    | no                                                   | yes                                                  |
| 04389            | Pyongyang 21 | Eastern                           | yes                                                  | no                                                   |
| 04390            | Pyongyang 3  | Eastern                           | yes                                                  | yes                                                  |
| 04391            | Pyongyang 22 | Eastern                           | yes                                                  | no                                                   |
| 04434            | Б 33-38.7    | European                          | no                                                   | yes                                                  |
| 04520            | Volano       | European                          | yes                                                  | no                                                   |
| 04649            | Mu 07-980    | South Asian                       | no                                                   | yes                                                  |
| 04660            | Kaz NIIR-7   | Central Asian                     | yes                                                  | yes                                                  |
| 04673            | Marzhan      | Central Asian                     | yes                                                  | no                                                   |
| 04678            | Kuro-mochi   | Eastern                           | yes                                                  | yes                                                  |
| 04689            | Long ting15  | South Asian                       | no                                                   | yes                                                  |
| 04692            | Long-ting 12 | South Asian                       | no                                                   | yes                                                  |
| 04693            | Mu 07- 1111  | South Asian                       | yes                                                  | no                                                   |
| 04709            | Yir 8458     | European                          | yes                                                  | no                                                   |
| 04726            | Magic        | European                          | yes                                                  | no                                                   |
Donors of genes for resistance to the causative agent of pyriculariosis were identified by DNA genotyping of varieties among representatives of European, Eastern, South Asian, Central Asian, Philippinian, and Latin American ecological and geographical groups from the collection of the Institute of Rice.

It is believed that in the areas of world rice cultivation, the distribution of rice resistance genes to pyriculariosis is determined by the variety of cultivated varieties. Correspondingly, the maximum diversity of Puricularia oryzae virulence genes is revealed in countries with an optimal climate for pathogen development and a diversity of varietal composition [3, 7].

Figures 2 and 3 present the visualization of the results of PCR analysis for the identification of the Pi-9 and Pi-k genes in collection rice samples.

**Figure 2.** Identification of the Pi-9 gene by PCR Analysis Using the Dominant Marker pB8.

Note: M- molecular weight marker, 1. Kulon 7381, 2. Avangard, 3. Mutant 168-87, 4. Chirnogi 16, 5. Maisky 88, 6. Krasnodarsky 86, 7. Talisman, 8. Snezhinka, 9. Negative control IRBL20- IR 24, 10. Positive control IRBL9-W.
Figure 3. Identification of the Pi-k gene using the k8823 dominant marker.

Note: M - molecular weight marker, 1. AA 26072/09 Bluchelle, 2. IR 83260, 3. CR 547, 4. Duar, 5. Dular, 6. Carajas, 7. Giza 177, 8. IR 78221-19-6, 9. Positive control IRBLk-Ka, 10. Negative Control IRBL19-A

Further, in our studies, we evaluated the immunity and biological properties of the donor varieties of the Pi-k and Pi-9 genes. The standard mid-ripening Avangard variety with the Pi-k gene showed resistance to the pathogen against an infectious background with the intensity of disease development of 8.4% and against a natural background, type 1 of resistance (table 3). Rice plants with the necessary combination of resistance genes are rare, while resistance can negatively correlate with economically valuable features.

Evaluation of the complex of characteristics of donor varieties isolated by pathogen resistance using microsatellite markers from the gene pool of the rice collection shows (Table 4) that, according to the length of the growing season, they belong to different ripeness groups: from early to late ripening. Most of the varieties carrying the Pi-k and Pi-9 genes of foreign selection are mid-ripening varieties, i.e. the rate of development of their plants and ripening of grain coincides with domestic zoned varieties.
Table 3. Characterization of gene donors from the collection of the Institute of Rice (Krasnodar) to Pyricularia oryzae on a natural and infectious background and biological properties, 2017-2018.

| Sample Name       | Country of origin | Disease development index (DDI), % | Degree of stability | Type of sustainability on a natural background, score | The duration of the growing season, days | Mass of 1000 grains, g |
|-------------------|-------------------|-----------------------------------|---------------------|----------------------------------------------------|----------------------------------------|------------------------|
| Avangard - standart | Uzbekistan       | 8.4                               | R                   | 1                                                  | 120                                    | 26.4                   |
| Pobeda 65 – standart | Russia            | 63.0                              | N/S                 | 5                                                  | 105                                    | 25.0                   |
| Kulon 7381         | Russia            | 7.8                               | R                   | 1                                                  | 118                                    | 30.1                   |
| Mutant 168-87      | Russia            | 10.1                              | R                   | 1                                                  | 110                                    | 32.9                   |
| Chirnogi 16        | Romania           | 6.7                               | R                   | 1                                                  | 105                                    | 24.5                   |
| Krasnodarsky 86    | Russia            | 23.3                              | R                   | 3                                                  | 115                                    | 30.2                   |
| Talisman           | Russia            | 16.7                              | R                   | 1                                                  | 118                                    | 25.2                   |
| Snezhinka          | Russia            | 12.4                              | R                   | 1                                                  | 120                                    | 23.4                   |
| Thaibonne          | USA               | 26.7                              | M/T                 | 3                                                  | 120                                    | 22.6                   |
| Deshan B           | China             | 7.4                               | R                   | 1                                                  | 115                                    | 24.7                   |
| Norin RL-22        | Philippines       | 22.3                              | R                   | 3                                                  | 120                                    | 34.6                   |
| Onda               | USA               | 16.6                              | R                   | 1                                                  | 115                                    | 24.7                   |
| Pyongyang 21       | Korea             | 15.4                              | R                   | 3                                                  | 125                                    | 25.2                   |
| Pyongyang 3        | Korea             | 8.2                               | R                   | 1                                                  | 130                                    | 26.0                   |
| Pyongyang 22       | Korea             | 5.6                               | R                   | 1                                                  | 130                                    | 25.7                   |
| Б 33-38.7          | Russia            | 20.0                              | R                   | 3                                                  | 116                                    | 20.2                   |
| Volano             | Italy             | 6.7                               | R                   | 1                                                  | 125                                    | 38.8                   |
| Mu 07-980          | Primorye          | 19.4                              | R                   | 3                                                  | 95                                     | 24.3                   |
| Kaz NIIR 7         | Kazakhstan        | 7.2                               | R                   | 1                                                  | 115                                    | 28.6                   |
| Marzhan            | Kazakhstan        | 26.4                              | M/T                 | 3                                                  | 110                                    | 28.0                   |
| Kuro-mochi         | Japan             | 22.2                              | R                   | 3                                                  | 98                                     | 21.3                   |
| Long ting 15       | China             | 6.2                               | R                   | 3                                                  | 95                                     | 25.4                   |
| Long Ting 12       | Japan             | 13.2                              | R                   | 1                                                  | 98                                     | 26.5                   |
| Mu 07- 1111        | China             | 17.8                              | R                   | 1                                                  | 95                                     | 28.0                   |
| Yir 8458           | Ukraine           | 8.9                               | R                   | 1                                                  | 98                                     | 26.7                   |
| Magic              | Romania           | 16.7                              | R                   | 3                                                  | 105                                    | 29.0                   |
| Chinese            | Primorye          | 23.0                              | R                   | 3                                                  | 102                                    | 29.8                   |
| Arsenal            | Italy             | 22.3                              | R                   | 1                                                  | 120                                    | 27.3                   |
| Carnise            | Italy             | 26.5                              | M/T                 | 3                                                  | 120                                    | 43.0                   |
| 820146 TR-556-7-1  | France            | 22.5                              | R                   | 1                                                  | 112                                    | 27.8                   |
| SR 18336-5-5       | Korea             | 22.2                              | R                   | 3                                                  | 105                                    | 25.0                   |
| Carajas            | Brazil            | 28.9                              | M/T                 | 1                                                  | 135                                    | 25.2                   |
| AA 3227/07 GZ      | Philippines       | 1.1                               | R                   | 1                                                  | 135                                    | 22.6                   |
| 4120-205           | Philippines       | 23.3                              | R                   | 1                                                  | 130                                    | 21.4                   |
| IR 78221-19-6-82-B | Philippines       | 3.4                               | R                   | 1                                                  | 115                                    | 22.3                   |
| Duar               | Philippines       | 6.7                               | R                   | 1                                                  | 145                                    | 23.7                   |
| Blucbelle          | Brazil            | 23.3                              | R                   | 1                                                  | 125                                    | 23.0                   |
| IR 83260           | Philippines       | 2.4                               |                     |                                                   |                                        |                       |

Note: Type of resistance to the causative agent of pyriculariosis during visual assessment on a natural background (field experiment): 1-3 points-resistant (R), 5 points - medium-tolerant (M/T), 7 points - unstable (N/S), 9 points - highly susceptible (H/S).

Almost all genotypes showed resistance to plant lodging in the environmental conditions of the Kuban. By grain size variation in varieties is from 21.3 to 43.0 grams. It was noted that both small-grain and large-grain forms of rice of the subspecies japonica and indica show resistance to the fungus M. grisea.
In the selection of rice varieties resistant to fungal diseases, the following strategy was chosen: monitoring the varietal resistance to the pyriculariasis population against an artificial infectious background, selecting the recipient parent form, selecting effective resistance genes for the formation of immunity, and predicting the stability of the variety with this combination of genes [3, 19-22]. Creating varieties based on one effective gene can lead to races that overcome this resistance. In 2018, we have continued researches to identify effective resistance genes using monogenic differentiator lines. The gene pool of sources of immunity with known genes (the international set of differentiator-varieties) was included in the FSBSI ARRI collection (Krasnodar) from IRRI (Philippines) and is widely used

Table 4. Phenotyping and phytopathological testing of a set of differentiating varieties of Magnoporthe grisea races to the local, synthetic population of the pathogen, 2018.

| Sample Name   | Resistance gene | IDD, % | Degree of stability | The duration of the growing season, days | Plants height, cm |
|---------------|-----------------|--------|---------------------|------------------------------------------|------------------|
| Fukunishiki   | Pi-z            | 12.5   | resistant           | 125                                      | 106.0            |
| Maratelli     | Pi-2            | 30.0   | medium tolerant     | 135                                      | 73.0             |
| Zenit         | Pi-a, Pi-z, Pi-zt| 24.2   | resistant           | 150                                      | 96.0             |
| Ahi-asachi    | Pi-a, Pi-19t    | 20.1   | resistant           | 130                                      | 98.0             |
| Yashto-mochi  | Pi-62t, Pi-ta   | 10.5   | resistant           | 140                                      | 103.0            |
| IR 64         | Pi-3t, Pi-30t, Pi-33, Pi-28t, Pi-25t | 36.0 | medium tolerant     | 135                                      | 97.0             |
| Toride        | Pi-zt           | 21.5   | resistant           | 125                                      | 86.0             |
| CO39          | No R gene       | 37.5   | medium tolerant     | 142                                      | 92.0             |
| IRBLa-Ze      | Pi-a            | 17.8   | resistant           | 122                                      | 101.0            |
| IRBK-KA       | Pi-k            | 18.0   | resistant           | 125                                      | 128.0            |
| IRBLks-S      | Pi-ks           | 27.8   | medium tolerant     | 120                                      | 148.0            |
| IRBLkp-K60    | Pi-kp           | 12.3   | resistant           | 128                                      | 90.0             |
| IRBLkh-K3     | Pi-sh           | 16.2   | resistant           | 120                                      | 115.0            |
| IRBLkm-Ts     | Pi-km           | 14.5   | resistant           | 135                                      | 72.0             |
| IRBL 1 - La   | Pi-1            | 11.0   | resistant           | 140                                      | 68.0             |
| IRBLz-Fu      | Pi-z            | 40.0   | medium tolerant     | 145                                      | 98.0             |
| IRB1zt- Ir 56 | Pi-zt           | 27.5   | medium tolerant     | 135                                      | 105.0            |
| IRBLz5-CA 8   | Pi-z5           | 15.0   | resistant           | 120                                      | 92.0             |
| IRB1ta-ME     | Pi-ta           | 14.4   | resistant           | 135                                      | 90.0             |
| IRB1ta-K1     | Pi-ta           | 10.2   | resistant           | 118                                      | 93.0             |
| IRBLt-K59     | Pi-t            | 26.1   | medium tolerant     | 116                                      | 110.0            |
| IRBL7-M       | Pi-7(t)         | 24.8   | resistant           | 120                                      | 87.0             |
| IRBL5-m       | Pi-5(t)         | 17.5   | resistant           | 140                                      | 102.0            |
| IRBL11-Zh     | Pi-11t          | 22.0   | resistant           | 118                                      | 101.0            |
| IRBLta2-Pi    | Pi-ta2          | 38.0   | medium tolerant     | 118                                      | 123.0            |
| IRB1b-B       | Pi-b            | 25.0   | medium tolerant     | 125                                      | 140.0            |
| IRBL9-W       | Pi-9            | 11.1   | resistant           | 120                                      | 90.0             |
| IRBL3-CP4     | Pi-3            | 22.0   | resistant           | 122                                      | 94.0             |
| IRBL12- A     | Pi-19           | 24.0   | resistant           | 121                                      | 90.0             |
| IRBL20- IR 24 | Pi-20           | 30.1   | medium tolerant     | 140                                      | 87.0             |
| IRB1z5-Ca     | Pi-z5           | 11.1   | resistant           | 120                                      | 92.0             |
| IR65482-4-136-2-2 | Pi-40    | 14.4   | resistant           | 130                                      | 97.0             |
| IRBLsh-S      | Pi-sh           | 27.2   | medium tolerant     | 135                                      | 57.0             |
| B2-103        | Pi-21           | 22.0   | resistant           | 100                                      | 90.0             |
| JI-9 ARRI     | Pi-33           | 38.0   | medium tolerant     | 110                                      | 93.0             |
| JI-5 ARRI     | Pi-1+ Pi-33     | 23.0   | resistant           | 120                                      | 104.0            |
| Magnat        | Pi-1 and Pi-2   | 32.7   | medium tolerant     | 115                                      | 103.0            |

Note. The category: resistant - IDD <25%, medium tolerant - IDD -25.1-50%
for screening studies and selection works. The identification of effective pathogen genes was carried out by assessing the type of reaction of a set of indicator varieties of foreign selection with known genes.

In 2018, differentiator-varieties of piricularia races and monogenic lines against a provocative background (field experience) showed the degree of infection within the IDD: 10.5-40.0%. According to the length of the growing season, these are medium-late and very late-ripening forms of rice; according to the height of plants, these are medium-tall and tall forms.

4. Discussion
An immunological assessment of the genetic diversity of world breeding under the conditions of the Krasnodar Region identified 159 resistant forms out of 775 varieties, of 390 samples of the collection of Russian origin, 84 forms resistant to pyriculariosis were identified by means of phenotyping. Of 45 varieties originating from China, 12 forms resistant to the Krasnodar population of the race of the pathogen Magnaporthe grisea were identified. Sources of resistance were identified from the domestic general plasma: rice varieties Zlata, Mutant 168-87, Snezhinka, Viktoriya, Krasnodarsky 86, Kulon, Maisky 88, Olump, Privolny, Talisman and others. From the Chinese general plasma, the varieties were identified from phytopathological assessment: DeshanB, Mu 07-980, Mu 07-1111, Longting 16, Longting 18, Longting 12, Longting 13, Longting 20, XIAOMA CU (ACL 56158), IRIS 251-53324, Takanari, 39B / Gisa178. The identified initial forms represent a valuable pre-breeding resource for the creation of varieties with pyramidal genes of resistance to Pyricularia oryzae for the countries of Russia and China under the program of international cooperation.

A number of scientists note that the genes Pi-ks, Pi-I, Pi-k are widely spread among genes of resistance to the causative agent of pyriculariosis detected in introduced varieties. Genes Pi-z, Pi-zt, and Pi-ta are less common in the rice plasma [11,12,16,22].

Chinese scientists found in their studies that the monogenic line IRBL9-W with the Pi-9 gene showed resistance to 45 monospore isolates of the causative agent of pyriculariosis of different ecological and geographical origin, and the line with the Pi-2 gene was susceptible to 16 isolates, although the gene is attributed to genes of a wide spectrum of resistance [10].

From a wide variety of gene plasmas of the ARNII rice collection, 17 donors of the Pi-9 genes and 28 samples with the Pi-k gene were isolated. Thus, the frequency of occurrence of the Pi-k gene is higher in the studied sample of samples than the Pi-9 gene. In rice varieties Pyongyang 3 and SR18336-5-5 (Korea), Marzhan (Kazakhstan), Kuro-mochi (Japan), Chinese (Primorye), Arsenal (Italy), 2 genes for resistance to pyriculariosis were revealed, and the rice variety from Kazakhstan KazNIIR-7 has 2 genes for resistance to pyriculariosis and a cold resistance gene. The listed varieties are of particular breeding value in the rice collection.

Of the identified carriers of resistance genes, the varieties Thai bonne, Marzhan, Carnise, and Carajas turned out to be moderately susceptible with signs of damage against the provocative background of 2018. Probably, the presence of the Pi-k and Pi-9 genes in these varieties does not provide them with field stability in the conditions of the southern region of Russia. The immunological assessment of the degree of damage to pyriculariosis in varieties with identified markers is high, they are resistant to the disease - at the level of the Avangard standard (table 3). Index of disease development (IDD, %) in selected varieties is in the range of 1.1 - 28.9%, varieties with a reaction type at the level of medium-tolerant or slightly higher: Krasnodarsky 86, Thai bonne, Norin RL-22, Marzhan, Chinese, Carnise, Arsenal, IR 78221-19-6-82-BB, IR 83260.

The nature of the variability of the type of reaction of some differentiator-varieties with different resistance genes to infection in different years of research indicates the insufficient efficiency of these genes to the pathogen of the Krasnodar population. According to monitoring data, in the period of 2015-2017, in the environmental conditions of the Krasnodar Region, symptoms of infection with the pathogen of Pyricularia oryzae were absent in all cases in differentiator-varieties with genes: Pi-1, Pi-z, Pi-ta, Pi-z5, Pi-9, Pi-5 (t), Pi-t, Pi-19 [21].

A phytopathological assessment of gene carrier rice lines and varieties showed that in 2018, forms with genes showed resistance to the Krasnodar pathogen population: Pi-zt, Pi-a, Pi-k, Pi-1, Pi-z5, Pi-ta,
Pi-7 (t), Pi-5 (t), Pi-11t, Pi-9, Pi-3, Pi-19, Pi-21, Pi-40. The index of disease development in stable lines ranged from 10.2 to 24.8%. Varieties of rice with a combination of genes: Pi-a + Pi-19t; Pi-62t + Pi-ta; Pi-1 + Pi-33 had a degree of damage in the range of 10.5 -24.2%.

The susceptible type of reaction to the pathogen of the fungus M. Grisea in the local population was shown by indicator lines with genes: Pi-2, Pi-z, Pi-20, Pi-33 and Pi-ta2. The Magnat rice variety of Rostov selection with the Pi-1 and Pi-2 genes showed moderate resistance to pyriculariosis against a provocatively infectious background. In a number of reports, it is noted that genes that influence resistance to pyriculariosis are located together on chromosome 6, 11, and 12. The Pi-z gene is on the 6th chromosome along with the Pi-9, Pi-zt, and Pi-z5 genes. The Pi gene is localized on chromosome 11 along with the Pi-7 (t), Pi-k, Pi-ks, Pi-km, Pi-1 genes. The Pi-ta gene is localized on chromosome 12 along with the Pi-ta2 gene. The Pi-5 (t) gene is located on the 9th chromosome, and the Pi-21 gene on the 4th chromosome [17, 22].

This study has clearly demonstrated the effectiveness of the combination of phenotyping and DNA genotyping for the selection and improvement of source material in the selection of rice for immunity. A system for assessing the effectiveness of genes for a local pathogen population using an international set of standards carrying resistance genes allows us to develop a further strategy for large-scale screening of the gene pool for immunity and to identify a number of genes for selective improvement of rice genetic resources: Pi-1, Pi-z, Pi-a Pi-9, Pi-ta.

5. Conclusions
For the indirect selection of stable rice genotypes, two genes were genotyped, phenotyping against an infectious background of a set of varieties of wide geographical diversity and international monogenic races of piricularia differentiator lines. Identified in the course of the studies, resistance collection samples donors of the Pi-9 and Pi-k genes from geographically distant regions of rice cultivation turned out to be the most valuable source material for breeding varieties with race-specific and field resistance to pyriculariosis. For pyramination of the effective combinations of genes into the domestic rice gene plasma, donor lines Pi-1, Pi-a, Pi-z, Pi-9, and Pi-ta were determined, which, according to morphobiological and immunological characteristics, meet the selection requirements. To obtain a wide range of valuable breeding forms, rice genotypes with a high level of resistance were used in crosses: Chirnogi 16, Volano, Norin RL-22, Victoriya, Deshan B, Talisman, Long ting 15.

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