Phenotyping and genotyping of rice breeding material by grain quality traits and blast resistance

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Abstract. The article discusses phenotyping and genotyping of 86 rice breeding samples for key grain quality traits, as well as identification of the allelic state of the blast resistance gene Pi-z. The study is aimed at developing rice varieties with high grain quality and resistance to blast, the most harmful disease in all rice-growing regions. Based on the results of phenotyping and genotyping, seven promising variety samples were selected - prototypes of varieties with good grain quality and resistance to the pathogen.

1 Introduction

Rice is the most important food crop in the world - it feeds more than 3 billion people and provides more than 30% of the food calories consumed by humanity. Currently, its crops are located in 118 countries on an area of 163 million 534 thousand hectares. In terms of yield and gross harvest, rice ranks second after corn among all grain crops.

The largest rice producers in the world are China and India - about 29% and 21% of the world volume, respectively. Indonesia, Bangladesh, Vietnam, Thailand, the Philippines, Brazil, the USA and other countries produce slightly less.

In Russia, the Krasnodar region is the main rice-producing region, which accounts for about 80% of the country's gross rice production. In 2024, rice sown areas in Kuban amounted to 116 thousand hectares [1-8].

The main criteria for developing new varieties are rice grain quality and stable, high yield. The mass of 1000 seeds is an indicator of quality, which is also directly related to yield, since large grain most often increases the gross yield per hectare.

Rice grain quality is a set of characteristics that determine its technological value, nutritional properties and suitability for consumption. These indicators are used to classify and standardize rice grain, as well as to determine its price on the market. Understanding and monitoring rice grain quality indicators are important for producers, processors and consumers, as they directly affect the economic efficiency of production, as well as the nutritional value and safety of the final product [8].

The key indicators of rice quality are the vitreousity and fracturing of the endosperm and the filminess of grain, as they directly affect the total milling yield and the head rice

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content during processing. Vitreousity characterizes the density and transparency of the endosperm. Grain with high vitreousity is less susceptible to destruction during processing. Fracturing, in turn, is associated with internal defects of the grain (the presence of microcracks), which also negatively affects the head rice content during processing. Filminess of the grain indicates the content of shells that must be removed during processing and is an important varietal characteristic.

Thus, in breeding work, the analysis of these indicators and the selection of the most economically valuable samples according to these quality characteristics is an important stage in the development of rice varieties with high grain quality and good milling yield.

One of the limiting factors that significantly affects rice yields and poses a major threat to stable rice production is rice blast, caused by the hemibiotrophic fungus Pyricularia oryzae Cavara. This disease affects most rice-growing countries, posing a serious threat to food security in rice-producing regions of the world [7]. P. Oryzae infects more than 50 species of grasses, and among agriculturally important crops, the pathogen infects rice, wheat, rye, barley and millet [6].

Extensive genetic studies of rice blast resistance have identified over 100 resistance genes and 500 QTLs associated with resistance to the disease. Among them is the Pi-z gene, one of several effective genes in the Krasnodar rice-growing zone. Controlling blast by breeding rice varieties carrying resistance genes is seen as the most effective strategy to combat the disease [7]. In this regard, identification of these genes in the breeding material with subsequent selection of resistant forms is the most important component of the breeding process. Molecular genetic methods for identifying target resistance genes are a highly effective aid.

2 Materials and methods

In this study, phenotyping of 86 rice breeding samples was carried out according to the studied rice grain quality traits, followed by identification of the allelic state of the effective blast resistance gene Pi-z using molecular genetic methods for targeted selection of prototype varieties with specified traits in the program for developing blast resistant rice varieties with high grain quality, carried out at FSBSI "Federal Scientific Rice Centre".

Phenotyping of rice breeding samples according to the studied rice grain quality traits was carried out on certified equipment in accordance with GOSTs [2-4. Volume – 86 samples.

Phenotyping was carried out on certified equipment in accordance with GOST and instructions for scientific instruments. The material under study was grown in a breeding nursery, at the experimental production site (EPS) of FSBSI "Federal Scientific Rice Centre".

The mass of 1000 absolutely dry grains was determined using the ELVIZ-2 moisture analyzer, the ASESh-8-2 air-heat measuring unit, the SLY-C automatic seed counter, and the Cas CUW-420H electronic laboratory scales [4].

Vitreousity and fracturing were determined in transmitted light using DSZ-3 and DSZ-2M diaphanoscopes [4].

The grain shape and its linear dimensions were determined on a scanner (image analysis system LA 2400, WinFOLIA using the Seedling computer program (Canada) [2].

A molecular genetic study to identify the allelic state of the target blast resistance gene Pi-z was conducted on 86 rice varieties phenotyped for grain quality traits. To identify the Pi-z gene, a dominant intragenic marker Z 565962 was used, the primer sequence of which was taken from the publicly available NCBI database [1]:

Z 565962 F	AAGAAATAATATTTTTGAAACATGGCAAAT

Z 565962 R CCATGGTGGTAACTGGTATGTG

To extract DNA from the samples, seven-day-old seedlings were used, obtained by incubation on moistened filter paper at a temperature of 25 - 27 °C. DNA extraction was performed using commercial kits on the Nexor 32M automated nucleic acid extraction station.

The PCR was set up with preliminary optimization of the experimental parameters for the real-time amplifier Quant Studio 5 according to the following method [5]:

Initial DNA denaturation at 94°C - 5 minutes,

the next 35 cycles:

15 seconds denaturation at 94°C,

30 seconds primer annealing at 60°C,

35 seconds elongation at 72°C,

Electrophoresis of the amplification products was performed in a 2% agarose gel, and the results of electrophoretic separation of the PCR products were visualized on a transilluminator using ethidium bromide (BrEtd).

3 Results and discussion

The results of phenotyping rice samples according to the studied grain quality traits, the mass of 1000 absolutely dry grains (a.d.g.), and the grain length to width ratio are presented in Table 1.

Table 1. Indicators of quality traits of rice breeding samples, yield of 2023.

№	Sample number	Mass of 1000 a.d.g., g	Filminess,%	Vitreousity,%	Fracturing, %	Grain length to width ratio (l/b)
1	KSI-1	23.,4	17.8	96	8	2.4
2	KSI-2	23.4	19.0	94	8	3.2
3	KSI-3	23.4	16.0	97	15	2.5
4	KSI-4	27.2	17.8	96	7	2.6
5	KSI-5	23.5	16.4	83	15	2.4
6	KSI-6	34.0	18.2	81	6	2.3
7	KSI-7	24.2	17.8	88	17	2,0
8	KSI-9	22.5	17.2	95	6	3.2
9	KSI-10	22.6	16.2	96	4	2.4
10	KSI-11	29.4	17.2	80	17	2.6
11	KSI-12	26.2	17.6	94	10	2.4
12	KSI-13	27.6	17.0	84	12	2.4
13	KSI-14	22.9	16.8	79	14	2.3
14	KSI-17	23.9	18.6	19	5	1.8

15	KSI-18	27.6	17.2	87	12	2.4
16	KSI-19	23.7	18.4	93	7	2.2
17	KSI-20	23.4	18.0	94	24	1.9
18	KSI-21	22.0	19.4	93	7	1.9
19	KSI-22	25.8	18.0	93	15	2.1
20	KSI-23	26.9	16.6	68	23	2.0
21	KSI-24	22.8	19.0	95	9	2.4
22	KSI-25	23.5	17.2	85	10	2.3
23	KSI-26	26.7	19.6	94	11	2.5
24	KSI-27	24.1	21.0	94	12	2.2
25	KSI-28	20.3	19.8	94	33	2.5
26	KSI-29	23.8	20.0	98	5	3.1
27	KSI-30	28.9	18.2	92	17	2.6
28	KSI-31	20.9	17.8	79	90	2.3
29	KSI-32	26.9	18.2	90	10	2.6
30	KSI-33	21.5	19.4	89	5	2.3
31	KSI-34	21.7	19.6	97	7	2.6
32	KSI-36	28.8	16.4	66	33	2.1
33	KSI-37	26.4	17.8	85	26	2.2
34	KSI-38	22.1	18.4	95	6	2.4
35	KSI-39	23.4	19.0	97	8	2.2
36	KSI-40	27.6	17.8	90	12	2.3
37	KSI-41	28.0	18.6	84	6	2.4
38	KSI-42	21.4	17.8	87	4	2.3
39	KSI-43	29.0	17.8	97	15	2.6
40	KSI-44	27.7	17.8	85	8	2.7
41	KSI-45	29.1	16.6	86	19	2.6
42	KSI-46	20.8	16.2	89	3	2.4
43	KSI-48	24.9	19.8	89	30	2.4
44	KSI-49	27.7	20.2	99	11	3.0

45	KSI-50	25.6	17.2	82	13	2.1
46	KSI-51	23.6	17.0	91	8	2.6
47	KSI-52	27.6	17.6	92	35	2.7
48	KSI-53	23.9	16.8	88	16	2.2
49	KSI-54	33.8	18.2	89	22	2.5
50	KSI-55	34.5	18.6	75	8	2.4
51	KSI-56	23.7	17.8	87	12	2.1
52	KPU-2-5	29.4	17.6	87	25	2.5
53	KPU-2-7	28.4	18.0	89	39	2.8
54	KPU-2-10	26.5	16.6	87	8	2.3
55	KPU-2-15	22.3	17.2	94	5	2.6
56	KPU-2-24	26.6	20.2	93	19	2.4
57	KPU-2-34	26.0	21.4	64	21	3.8
58	KPU-2-44	26.5	17.4	95	32	3.0
59	KPU-2-50	22.4	17.8	85	12	2.2
60	KSI-40 PIP	28.4	17.2	58	39	2.4
61	Utes PIP	25.9	19.0	93	11	2.8
62	Gamma PIP	22.3	18.4	92	12	2.2
63	KSI-17 PIP	28.3	18.2	81	22	2.4
64	KSI-54 PIP	22.9	17.4	86	22	2.3
65	KSI-6 PIP	24.2	18.4	64	33	1.9
66	Orion PIP	23.6	18.4	92	15	2.2
67	Rubikon PIP	23.4	16.8	99	20	2.3
68	Kornet PIP-1	23.6	19.6	94	11	2.2
69	Leader PIP	25.1	18.6	92	17	2.1
70	Dieta PR	24.3	19.0	3	13	1.7
71	Mars PIP-1	23.0	19.0	94	7	3.6
72	Atlet PIP	23.5	19.4	91	31	2.2
73	Zlata PIP-1	23.9	21.8	83	31	3.3
74	Snezhinka	23.0	19.2	96	9	3.8

	PR					
75	Kalvdiy PR	25.8	17.2	94	5	2.5
76	Lyubin PR	27.8	18.6	84	24	2.6
77	Lekar PIP	23.4	19.0	1	3	1.9
78	Anita-20 PR	34.5	19.4	87	21	2.3
79	Polus-5 PR	24.6	16.2	96	26	2.5
80	Helga PR	22.3	20.0	94	19	2.4
81	Atlant PR	23.5	19.4	91	31	2.2
82	KPU-2-19	23.3	17.0	94	9	1.9
83	Kumir (KSI-80)	20.7	17.6	74	33	2.2
84	Vita PIP	21.2	19.2	1	8	3.0
85	Titan	29.3	15.6	86	32	2.4
86	Azovskiy	21.1	19.6	83	18	2.2

Table 1 shows that samples No. 40, 36 and 29 are the most economically valuable breeding material in terms of the quality parameters studied, since they have a higher mass of 1000 grains and a high vitreousity index, while being characterized by low fracturing and filminess. These samples will show the highest milling yield and head rice content when processed. Sample No. 40 has a high mass of 1000 grains and low fracturing – 27.7 g and 8%, respectively, which makes it one of the best in terms of quality. Sample No. 36 has high vitreousity (90%) with relatively low fracturing and a fairly high mass of 1000 grains, which indicates its high quality.

Real-time PCR followed by electrophoretic separation of reaction products in agarose gel was used to evaluate rice samples phenotyped for grain quality traits for the presence of the blast resistance gene Pi-z. The results are presented in Figures 1–7.

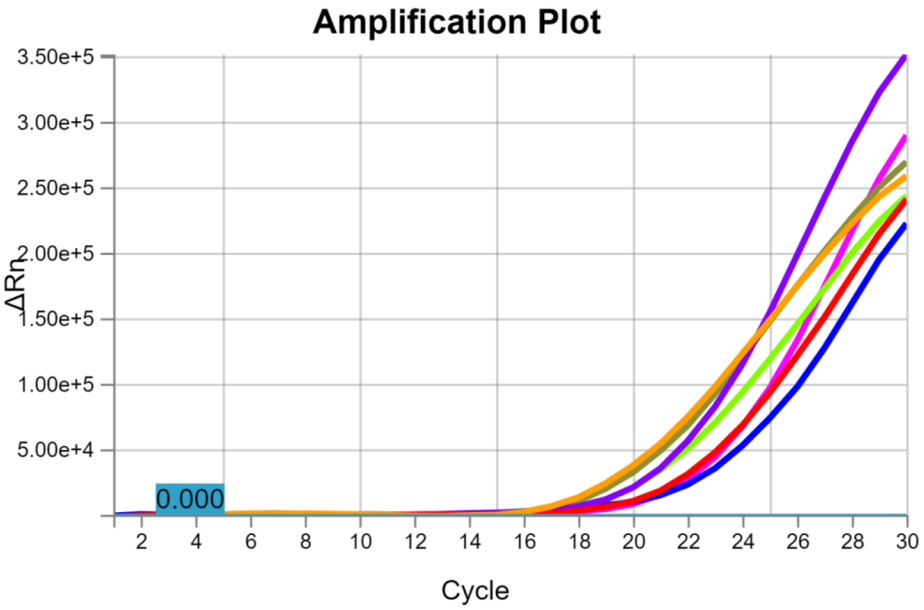

Fig. 1. Amplification curve of the dominant marker of the Pi-z gene. Note: ΔRn – amount of PCR product; Cycle – number of cycles.

The amplification curve of the studied samples shows that the PCR product gain occurred in 7 studied samples. The color correspondence of the parabolas and the numbers of the studied samples is shown in Figure 2. According to the results of real-time PCR, the dominant allele of the Pi-z gene was detected in 7 studied samples: No. 2, 8, 26, 58, 73, 74, 77. To confirm the PCR results, electrophoresis in agarose gel was performed. The results of electrophoretic separation are shown in Figures 3-8.

Fig. 2. Color matching of samples on the amplification curve.

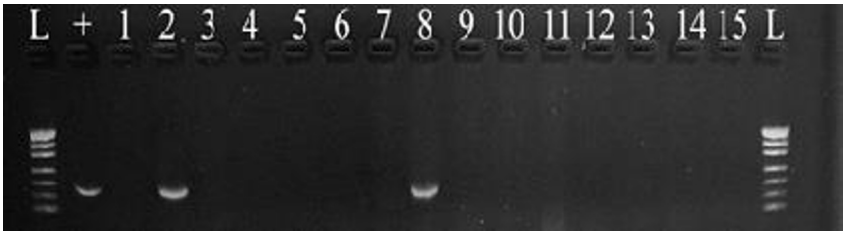

Fig. 3. Electrophoretic separation of the PCR product of the Pi-z gene. Samples 1-15. Note: L – DNA molecular weight marker; + – positive control; 1-15 – test samples.

Among the studied samples 1-15, the dominant allele of the Pi-z gene was found in samples No. 2, 8. The illumination of the band of these samples coincides with the positive control and corresponds to the desired length of the PCR product.

Fig. 4. Electrophoretic separation of the PCR product of the Pi-z gene. Samples 16-30. Note: L – DNA molecular weight marker; + – positive control; 16-30 – test samples.

In samples 16-30, the desired gene was detected in sample No. 26, which had a band of the desired length, corresponding to the positive control.

Fig. 5. Electrophoretic separation of the PCR product of the Pi-z gene. Samples 31-45. Note: L – DNA molecular weight marker; + – positive control; 31-45 – test samples.

In the studied samples 31-45, the Pi-z gene is absent according to the results of electrophoretic separation, which coincides with the results of real-time PCR.

Fig. 6. Electrophoretic separation of the PCR product of the Pi-z gene. Samples 46-60. Note: L – DNA molecular weight marker; + – positive control; 46-60 – test samples.

According to the results of electrophoresis of the studied samples 46-60, the Pi-z gene was detected in 1 sample - No. 58.

Fig. 7. Electrophoretic separation of the PCR product of the Pi-z gene. Samples 61-75. Note: L – DNA molecular weight marker; + – positive control; 61-75 – test samples.

Fig. 8. Electrophoretic separation of the PCR product of the Pi-z gene. Samples 76-86. Note: L – DNA molecular weight marker; + – positive control; 76-86 – test samples.

In Figures 7-8, the gene of interest was detected in 3 samples: No. 73-74, No. 77. The electrophoresis results coincide with the real-time PCR results.

Thus, the molecular genetic marker used in the study is informative for conducting real-time PCR on the studied rice breeding material.

Based on the detection results, the Pi-z gene was identified in 7 studied samples: No. 2, 8, 26, 58, 73, 74, 77. These samples are valuable breeding material and will be used in further stages of the breeding program.

3 Conclusions

Based on phenotyping and genotyping data, we have selected promising plants that combine high technological quality traits and also carry the dominant allele of the effective blast resistance gene Pi-z. These plants are prototypes of future rice varieties that are resistant to blast and have high quality milled rice.

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