

Development of rice variety with blast resistance genes *Pi-b* and *Pi-z* basing on DNA-technologies

Andrey Ogly^{1*}, Viktor Kovalev¹, Olesya Bragina¹, and Ivan Suprun²

¹Federal Scientific Rice Centre, Krasnodar, Belozerny, 3, 350921, Russia

²North Caucasian Scientific Center for Horticulture, Viticulture, Winemaking, Krasnodar, Russia

Abstract. The article presents stages of development and testing new rice variety Nautilus with blast resistance genes *Pi-b* and *Pi-z*. Basing on the use of DNA-marking methods, breeding material carrying the target genes was developed. The obtained material was studied in the links of breeding process in the field. Based on the results of field testing, 6 lines were identified, of which VNIIR 6016 turned out to be the best in terms of a complex of economically valuable traits. In 2016, this sample was transferred to the State variety testing under the name Nautilus. In 2019, a new rice variety Nautilus was included in the register of breeding achievements and approved for use in the Russian Federation in Krasnodar region. The study of variety Nautilus in 2019-2020 in an ecological test confirmed its high resistance to blast, in comparison with other released varieties. It took 10 - 12 years to develop the variety.

1 Introduction

Obtaining a high and economically viable yield of good quality is the main goal of rice farms. Achievement of this goal largely depends on the development and introduction into production of new high-yielding rice varieties with high grain and milled rice quality, resistant to various stress factors. Blast resistance of the varieties is especially important in the conditions of modern rice cultivation, since in the years of epiphytotic, up to 30% of the yield is lost, and the additional costs of treating crops with fungicides lead to an increase in the unit cost of production [1]. In this regard, the development of rice varieties with increased blast resistance, against the background of the urgent need to biologize agriculture, is an urgent direction in the breeding of this crop.

One of the strategies for obtaining rice varieties with increased and long-term blast resistance is to combine several genes in one genotype, which provides resistance to a wide range of pathogen races [2-5]. This problem can be solved by modern advances in DNA marking technology, which make it possible to assess and select the original breeding material by genotype based on molecular analysis.

The aim of the work was to develop a new rice variety with pyramided blast resistance genes *Pi-b* and *Pi-z* based on the Russian variety Khazar using DNA technologies.

* Corresponding author: ogly_a@mail.ru

2 Materials and methods

The blast resistance genes *Pi-b* and *Pi-z* were used in the work. The late-ripening Japanese variety BL-1 served as the donor of the *Pi-b* gene, and variety Zenit - of the *Pi-z* gene. Donor samples were obtained at the USU "Collection of genetic resources of rice and vegetable and melon crops" of FSBSI Federal Scientific Rice Centre. Development of lines carrying these genes was carried out on the basis of the domestic variety Khazar, which is widespread in Krasnodar region. Plants were hybridized by the TVELL method with pneumocastration [6]. The *Pi-b* gene was identified using a primer combination developed in the laboratory of biotechnology of Federal Scientific Rice Centre [7]. The *Pi-z* gene was identified using a SNP DNA marker developed by Hayashi K. et al. [8]. The presence of both genes in one genotype was determined using multiplex PCR [9]. DNA extraction was performed by CTAB method [10], PCR analysis - by standard methods [11]. Amplification products were analyzed by electrophoresis in a 2% agarose gel based on 0.5 x Tris borate buffer, visualized with ethidium bromide and photographed in ultraviolet light.

The study of lines with blast resistance genes was carried out on the rice irrigation system of the experimental production plot of Federal Scientific Rice Centre and in the ESPES "Krasnaya" on the predecessors of fallow and alfalfa for 2 years.

The breeding nursery was established with *WINTERSTEIGER "Rowseed"* cassette seeder with single-row plots 2.5 m long with a seeding rate of 100 grains per row and 22 cm spacing [12].

The control nursery was seeded with *WINTERSTEIGER "Plotseed XL"* center-seeder. Plot area 6.0 m². The seeding rate is 700 germinating grains per 1 m².

Sowing of competitive variety test was carried out with *WINTERSTEIGER "Plotseed XL"* central seeder, plots with an area of 18.0 m² and a seeding rate of 700 germinating grains per 1 m². Repetition 4-fold with a randomized way of placing the plots [13]. Yield calculation was carried out by the method of continuous threshing with a small-sized combine DKC-515, recalculated to standard moisture content.

The assessment of the resistance of the numbers of the control nursery and the competitive test to blast was carried out according to the method of the "International Rice Research Institute IRRI" [14].

The statistical evaluation of the results of scientific research was carried out according to B.A. Dospekhov [15].

3 Results

At the initial stage of work on development of rice variety with the *Pi-b* and *Pi-z* blast resistance genes, backcross lines based on variety Khazar were obtained, carrying these genes separately (Figure 1).

To determine the presence of target genes in hybrid plants, DNA analysis was carried out and, on its basis, the necessary plants were selected, which were used for backcrosses with variety Khazar. Combining genes in one genotype was carried out using crosses of hybrid plants with the *Pi-b* gene from the BC3F2 population and BC3F2 plants with the *Pi-z* gene. Subsequently, lines with both blast resistance genes were isolated by multiplex PCR. For breeders, not one trait is of great importance, but a whole complex of qualitative and quantitative traits of genotypes, manifested in conditions close to production, therefore, the samples obtained were studied in the field in breeding, control nurseries and competitive testing. Based on the results of a comprehensive assessment, 6 varieties were identified that exceeded the standard variety Rapan in terms of basic parameters, the best among which was VNIIR 6016 (Table 1).

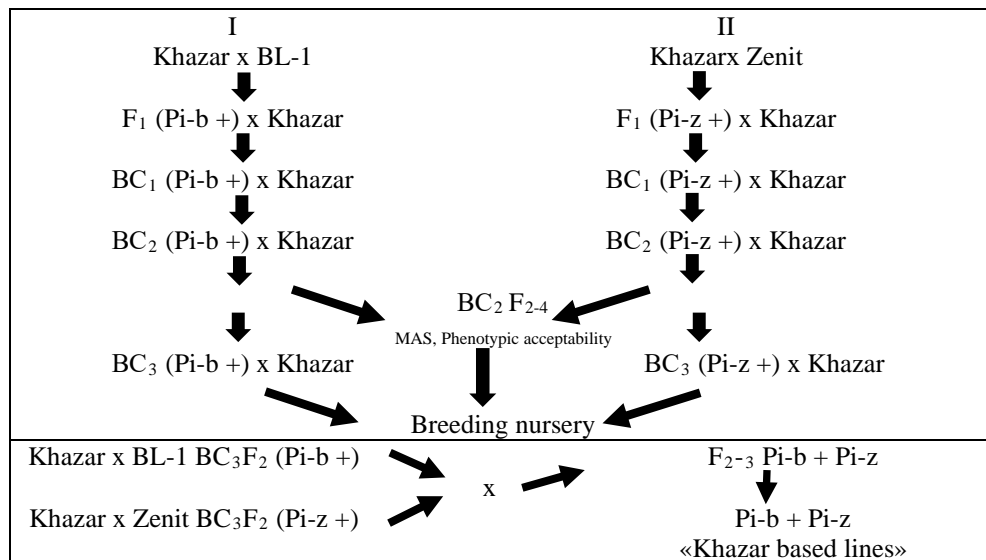


Fig. 1. Scheme of obtaining lines with blast resistance genes Pi-b and Pi-z.

Table 1. Brief characteristics of varieties with blast resistance genes Pi-b and Pi-z, according to the results of competitive testing in 2013 - 2014.

Variety	Yield		Vitreous- sity, %	Milling yield, %	Blast resistance	
	2013	2014			genes	degree
	т/га	т/га				
Rapan st.	6,20 *	9,50	94	70,3		S
VNIIR 5662	8,70	9,80	96	72,1	Pi-b	MR
VNIIR 40/4-1	10,20	9,60	86	67,4	Pi-b	MR
VNIIR 21/3-8	9,40	10,40	97	69,7	Pi-b + Pi-z	R
VNIIR 6013	10,70	9,50	95	72,0	Pi-b + Pi-z	MR
VNIIR 6016	11,30	9,60	92	74,6	Pi-b + Pi-z	R
VNIIR 6017	10,20	9,50	94	70,1	Pi-b + Pi-z	R

Further testing of the sample in 2015 and 2016 confirmed its advantages, mainly in terms of yield and blast resistance, in comparison with the standard variety Rapan, the most common in the Russian Federation (table 2). On average, over three years of competitive testing, VNIIR 6016 showed a yield of 9,9 t/ha, which is 1,3 t/ha more than that of Rapan. When artificially infected, VNIIR 6016 was affected by blast disease by 20,9 % less. This served as the basis for transferring the sample VNIIR 6016 for state testing under the name Nautilus in 2016.

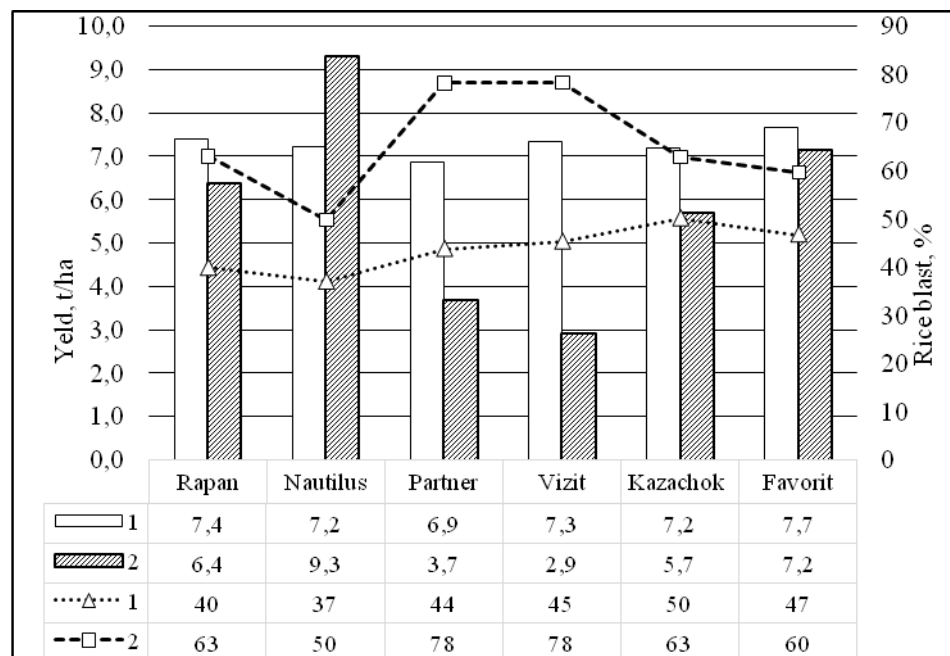
The new rice variety Nautilus successfully passed the state test in 2017 - 2018 and, as a result, was included in the register of breeding achievements approved for use in Krasnodar region since 2019.

In 2019 - 2020, the variety Nautilus was studied in an ecological test in the ESPES "Krasnaya" by the predecessor of alfalfa for 2 years and in Federal Scientific Rice Centre by the predecessor of fallow. The experiments included varieties Rapan, Vizit, Kazachok, Favorit and Partner, developed in the same way as Nautilus, but having only the Pi-b gene in the genome [16].

Table 2. Brief characteristics of the new variety Nautilus (VNIIR 6016) based on the results of competitive variety testing

Trait	Nautilus				Rapan, st.*				± κ *
	2014	2015	2016	mean value	2014	2015	2016	mean value	
Yield, t/ha	9,64	10,31	9,75	9,90	9,30	7,66	8,84	8,6	1,3
Duration, days	113	115	114	114	114	115	113	114	0
Plant height, cm	90,9	96,7	98,3	95,3	93,1	97,7	97,5	96,1	-0,8
Mass of 1000 grains, g	27,9	27,4	29,1	28,1	29,2	28,8	30,2	29,4	-1,3
Vitreosity, %	94,0	97,0	84,0	92,0	98,0	97,0	93,0	96,0	-4,0
Grain index, (l/b)	2,1	2,1	1,9	2,0	2,1	2,1	1,9	2,0	0
Total milling yield, %	71,6	73,7	69,4	71,6	72,3	71,4	70,2	71,3	+0,3
Head rice content, %	93,5	87,7	94,8	92,0	93,7	79,0	89,7	87,5	+4,5
Rice blast, %	18,9	25,6	26,7	23,7	42,5	35,5	55,7	44,6	-20,9

For two years in the FSBI ESPES "Krasnaya", a high infectious background of blast infection was formed on the predecessor of alfalfa, and at FSBSI Federal Scientific Rice Centre the development of the disease was lower (Figure 2). As a result, in the field in the FSBSI Federal Scientific Rice Centre the yield of varieties was slightly different and varied mainly within the range of 6,88 – 7,39 t/ha. The highest yield here was formed by the variety Favorit – 7,66 t/ha (LSD₀₅ = 0.48).



Note: 1 – yield and rice blast in FSBSI «Federal Scientific Rice Centre»;
 2 – yield and rice blast in FSBI ESPES «Krasnaya».

Fig. 2. Yield and intensity of blast development in ecological variety testing

By the predecessor of alfalfa, the best was the new variety Nautilus, which formed 9,33 t/ha of rice under conditions of a high infectious background of blast disease (LSD₀₅ = 0,56). The rest of the varieties were less productive, especially Partner and Vizit (3,69 and 2,92 t/ha, respectively), which were most affected by blast disease (by 78%). One *Pi-b* gene

did not provide the variety Partner with increased and long-term resistance to the disease, probably due to the fact that it was developed on the basis of the susceptible variety Yantar [16].

4 Conclusion

Thus, based on the results of many years of work, combining DNA technology and methods of classical breeding, a new rice variety Nautilus (VNIIR 6016) with pyramided blast resistance genes has been developed, which has increased disease tolerance, high yield potential and good grain and milled rice quality.

The use of DNA technologies in breeding programs allows for targeted selection of genotypes according to the traits of interest, which can reduce the volume of the breeding material studied in breeding process nurseries, but today these technologies do not significantly reduce the time for developing rice varieties. The variety Nautilus has been developed since 2007, and its testing ended in 2018, that is, 12 years have passed. It takes about 10-15 years to develop rice varieties by the method of classical breeding (hybridization + selection).

References

1. G. Zelensky, *Fight against rice blast by creating resistant varieties* (KubSAU, Krasnodar, 2013)
2. G. Los, *Agricultural biology*, **12**, 107-109 (1987)
3. A. Khanna, V. Sharma, K. Ranjith, A. Shikari et al. *Theoretical and Applied Genetics*, **128**, 1243-1259, (2015) <https://doi.org/10.1007/s00122-015-2502-4>
4. S. Ashkani, M.Y. Rafii, H.A. Rahim and M.A. Latif. *Mol. Biol. Rep.* **40**, 2503-2515 (2013) <https://dx.doi.org/10.1007/s11033-012-2331-3>
5. M. Yadav, S. Aravindan, U. Ngangkham, S. Raghu, S. Prabhukarthikeyan, U. Keerthana et al., *PLOS ONE* 14 (3), (2019) <https://doi.org/10.1371/journal.pone.0213566>
6. *Methodological guidelines for assessing the resistance of rice varieties to the causative agent of blast* (All-Union Academy of Agricultural Sciences, Moscow 1988)
7. I. Suprun, E. Ilnitskaya, Zh. Mukhina, *Agricultural Biology*, **5**, 63-66 (2007) <http://agriscience.spsl.nsc.ru/journal/0131-6397/2007/5/63-66>
8. K. Hayashi, *Theoretical and Applied Genetics*, **108** (7), 1212-20 (2004) <https://doi.org/10.1093/nar/8.19.4321>
9. I. Suprun, V. Kovalev, V. Shilovsky, *Vestnik of the Russian agricultural science*, **3**, 50-52 (2012)
10. M. Murray, W. Thompson, *Nucleic Acids Research*, **8** (19), 4321-5 (1980)
11. D. Shibata, *Polymerase chain reaction and molecular genetic analysis of bioplates* (Molecular clinical diagnostics, Mir, Moscow, 1999)
12. A. Smetanin, V. Dzyuba, A. Aprod, *Methods of experimental work on selection, seed production and quality control of rice seeds* (Krasnodar, 1972)
13. V. Kovalev, N. Ostapenko, Abstracts of reports of the Conference of young scientists and specialists, Krasnodar, 10-12 (1987)
14. International Rice Research Institute (IRRI), *Standard evaluation system for rice*, (4th.ed. IRRI, Manila, 1996)
15. B. Dospikhov, *Methodology of field experience* (Kolos, Moscow, 1979)
16. V. Shilovsky, I. Suprun, A. Ogly, *Grain farming of Russia*, **5**, 29-32 (2016)