СЕЛЕКЦИЯ РИСА В РОССИИ С ИСПОЛЬЗОВАНИЕМ МАРКЕРОВ

RICE BREEDING IN RUSSIA USING GENETIC MARKERS

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Abstract: The article concentrates on studying tolerance to soil salinization, water flooding, and blast in Russian and Asian rice varieties, as well as hybrids of the second and third generations from their crossing in order to obtain sustainable paddy crops based on domestic varieties using DNA markers. Samples IR 52713-2B-8-2B-1-2, IR 74099-3R-3-3, and NSIC Rc 106 were used as donors of the SalTol tolerance gene. Varieties with the Sub1A locus were used as donors of the flood resistance gene: Br-11, CR-1009, Inbara-3, TDK-1, and Khan Dan. The lines C101-A-51 (Pi-2), C101-Lac (Pi-1, Pi-33), IR-58 (Pi-ta), and Moroberekan (Pi-b) were used to transfer blast resistance genes. Hybridization of the stress-sensitive domestic varieties Novator, Flagman, Virazh, and Boyarin with donor lines of the genes of interest was carried out. As a result of the studies carried out using molecular marking based on PCR in combination with traditional breeding, early-maturing rice lines with genes for resistance to salinity (SalTol) and flooding (Sub1A), suitable for cultivation in southern Russia, were obtained. Introgression and pyramiding of the blast resistance genes Pi-1, Pi-2, Pi-33, Pi-ta, and Pi-b into the genotypes of domestic rice varieties were carried out. DNA marker analysis revealed disease-resistant rice samples carrying 5 target genes in a homozygous state. The created rice varieties that carry the genes for blast resistance (Pentagen, Magnate, Pirouette, Argamac, Kapitan, and Lenaris) were submitted for state variety testing. The introduction of such varieties into production will allow us to avoid epiphytotic development of the disease, preserving the biological productivity of rice and obtaining environmentally friendly agricultural products.
Keywords: rice; salinity; submergence tolerance; blast; SSR markers; PCR analysis

1. Introduction

Rice *(Oryza sativa L.)* is the most important food crop for more than half of the world's population. Biotic and abiotic stressors are the main obstacles to increasing global crop production and expanding rice production. It was found that

only about 10% of the world's agricultural land is located in areas that do not suffer from stress factors [1].

Decreased rice yields in adverse climatic conditions threaten global food security. Genetic loci that ensure productivity in difficult conditions exist in the germplasm of cultivated plants, their wild relatives and species adapted to extreme conditions [2].

One fifth of the world's irrigated land is adversely affected by high soil salinity [3]. About 45 million hectares in the world are subject to soil salinization [4]. In the Russian Federation, rice is grown on an area of about 200 thousand hectares, of which about 80 thousand hectares are saline [5]. The decline in productivity on saline soils can be overcome by developing rice varieties tolerant to salinity and introducing them into agricultural production. Several non-allelic genes provide tolerance to salinity during ontogenesis [6]. The main locus of salt tolerance is Saltol, which was first identified in some rice varieties [7, 8]. This locus is mapped on chromosome 1, and its main function is to control the balance of Na + / K + ions in rice plants. [9].

One of the serious abiotic stress factors for rice, which inhibits plant growth and affects crop yield, is prolonged submersion of plants under water, which is most often subjected to large areas of land in rice-growing regions of Southeast Asia. Rice dies if total flooding lasts more than two weeks. A negative effect on the growth and development of rice plants at this time is exerted by a lack of oxygen (O2) and limited diffusion of carbon dioxide (CO2). Lack of light due to turbid flood water in the rice paddies during this period limits the ability of plants to photosynthesize and can even lead to their death [4-6].

Scientists in Asia have found the *Sub1A* gene, which regulates the response of plant cells to ethylene and gibberellin, leading to restriction in carbohydrate intake and dormancy of shoots under water, which contributes to tolerance to immersion [10, 11]. In Russia, this gene can be used to develop varieties resistant to a large layer of water during the germination phase, which will become an effective way to protect rice from weeds without herbicides. Most weeds die under water without oxygen, and rice can survive. To develop such varieties, it is necessary to combine in one genotype genes with increased energy of initial growth, the ability to anaerobic germination, resistance to prolonged flooding and lodging.

In all countries of the world, including Russia, blast is among the most dangerous fungal diseases of rice, which causes large yield losses in the years of epiphytoties. The most effective way to protect rice without fungicides is to grow blast-resistant varieties. More than 50 genes of resistance to this pathogen are known - *Pi-1, Pi-2, Pi-33*, *Pi-b*, *Pi-ta*, *Pi-z,* and etc[12]. Combining several effective resistance genes with their contribution on the genetic basis of the best varieties is an effective breeding strategy for resistance to variable fungal pathogens. Lines with a combination of 3-5 resistance genes show an increase and broadening of the spectrum of blast resistance in comparison with lines with separate genes. A number of successful breeding programs have already been carried out abroad to develop blast-resistant rice varieties by the gene pyramiding method using marker breeding [13].

Resistance to various biotic and abiotic factors is one of the traits that are difficult to assess, when the assessment of the breeding material is possible only in the presence of an appropriate stress factor. At present, when breeding agricultural plants for resistance, the splitting population obtained from the crossing of resistance sources with genotypes that have productivity is tested against a natural background or artificial infection is carried out under controlled conditions. This procedure, although it gives excellent results, is quite lengthy and costly. In addition, there are always susceptible plants that have escaped damage [14].

The use of DNA markers allows speeding up the assessment and conducting selection without phenotypic assessment, at an early stage, regardless of external conditions. In recent years, great progress has been made in the development of molecular marking technologies and their application to control complex agronomic traits using marker breeding [15]. The technology of molecular marking of resistance loci makes it possible to quickly select plant forms with target genes without using provocative backgrounds [16]. The identification of molecular markers linked to genes of resistance to these factors facilitates breeding work. The use of DNA markers brings the breeding of agricultural plants to a qualitatively new level, making it possible to evaluate genotypes directly, and not through phenotypic manifestations, which, ultimately, is realized in the accelerated development of varieties with a complex of valuable traits [14]. Therefore, it is relevant to develop new rice varieties by marking [17].

The purpose of the study was the development of initial rice material using DNA markers for breeding highly productive varieties resistant to biotic and abiotic environmental stress factors: soil salinity, prolonged flooding, and blast.

2. Materials and methods

We used samples from the collection of the Institute of Agricultural Genetics (Vietnam) as donors of the transferred salt tolerance gene: IR 52713-2B-8-2B-1-2, IR 74099-3R-3-3, and NSIC Rc 106, which were crossed with the early--maturing Krasnodar variety Novator. These varieties carry the Saltol locus, which has been mapped near the centromeric region of the first chromosome. RM493 and RM7075 [18] were used as flanking SSR markers of this locus, with the greatest difference in the length of microsatellite repeats between the parental forms.

Varieties with the *Sub1A* locus were used as donors of the flooding resistance gene: BR-11, CR-1009, Inbara-3, TDK-1, Khan Dan. The early ripening variety Novator and rice lines with introgressed genes for blast resistance *Pi-2* and *Pi-33* were also taken as recipients. The *Sub1A* locus is mapped to an interval of 0.06 morganides in chromosome 9 [11]. We used microsatellite markers for the Sub 1A gene - CR25K and SSR1A. The *Sub1A* gene was identified by molecular marking based on PCR using specific primers.

When transferring blast resistance genes, lines C101-A-51 (*Pi-2*), C101-Lac (*Pi-1*, *Pi-33*), IR-58 (*Pi-ta*), Moroberekan (*Pi-b*) were used. To identify the Pi-1 gene, we used primer pairs of flanking microsatellite SSR markers RM224 and RM144, the Pi-2 gene – RM527 and SSR140, the Pi-33 gene – RM310 and RM72, the *Pi-b* and *Pi-ta* genes – intragenic markers developed in laboratory of biotech-

nology, Federal Scientific Rice Centre. They are localized on chromosomes 11, 6, 8, 2, 12, respectively (Table 1) [19, 20].

Table 1. Nucleotide sequences of codominant markers for identification of the allelic status of genes Pi-1, Pi-2, Pi-a, and Pi-b.

Resistance Gene	**Chromosomal Localization of Gene**	**Marker**		**Sequence**
Pi-2	6	Rm 527	F	GGC TCG ATC TAG AAA ATC CG
			R	TTG CAC AGG TTG CGA TAG AG
		SSR140	F	AAG GTG TGA AAC AAG CTA GCA
			R	TTC TAG GGG AGG GGT GTA GAA
Pi-33	8	Rm 72	F	CCG GCG ATA AAA CAA TGA G
			R	GCA TCG GTC CTA ACT AAG GG
		Rm310	F	CCA AAA CAT TTA AAA TAT CATG
			R	GCT TGT TGG TCA TTA CCA TTC
Sub1A	9	Sub1A203	F	GAT GT GT GGAGGAGAAGT GA
			R	GGTAGAT GCCGAGAAGT GTA
		Rm 7481	F	CGACCCAATATCTTTCTGCC
			R	ATTGGTCGTGCTCAACAAG
SalTol	1	Rm 493	F	GTACGTAAACGCGGAAGGTGACG
			R	CGACGTACGAGATGCCGATCC
		Rm 7075	F	TATGGACTGGAGCAAACCTC
			R	GGCACAGCACCAATGTCTC

The early ripening released rice varieties Boyarin, Flagman and Virage served as the paternal form. During plant hybridization, pneumocastration of maternal forms and pollination by the twel method were used [21]. Hybrid plants were grown on checks of Federal State Unitary Enterprise "Proletarskoe", Rostov region and the Federal State Unitary Elite Seed-growing Enterprise"Krasnoe" of the Federal Scientific Rice Centre, Krasnodar region. From the selected rice leaves, genomic DNA was isolated under laboratory conditions at the Federal Scientific Rice Centre, the Academy of Biology and Biotechnology of the Southern Federal University, and the All-Russian Research Institute of Agricultural Biotechnology, according to the manufacturer's instructions. PCR products were separated by electrophoresis in 2.5% agarose and 8% acrylamide gels. The experimental data were statistically processed using Microsoft Excel and Statistica 6 software.

The account of the degree of damage to plants (in percentages) was carried out on the 14th day after inoculation, in accordance with the express method for assessing rice varietal resistance to blast. The assessment was carried out taking into account two indicators: the type of reaction (in points) and the degree of dam-

age (in percentages), using the ten-point scale of the International Rice Research Institute [12]:

- resistant: 0-1 points - no damage, small brown spots, covering less than 25% of the total leaf surface;
- medium-resistant: 2-5 points - typical elliptical blast spots, 1-2 cm long, covering 26-50% of the total leaf surface;
- susceptible: 6 - 10 points - typical blast spots of elliptical shape, 1-2 cm long, covering 51% or more of the total leaf surface.

The intensity of disease development (IDD, %) was calculated by the formula:

$$IDD = \sum (a \times b) / n \times 9, \quad (1),$$

where IDD – intensity of disease development, %; $\sum$ (a*b) – the sum of the products of the number of infected plants multiplied by the corresponding damage point, n is the number of recorded plants (pcs).

Depending on the damage point, all varieties are conventionally divided into 4 groups: resistant, intermediate, susceptible, and strongly susceptible.

3. Results and Discussion

The development of blast-resistant varieties and their rapid introduction into production is the most promising solution in the fight against this disease. However, the development of resistant varieties is one of the most difficult areas of breeding. The causative agents of the disease have a great potential for variability, which, combined with their colossal reproduction capabilities, provides the pathogen with the highest adaptive capabilities. Combining several effective genes of resistance on a genetic basis of the best varieties widely used in production is an effective breeding strategy for long-term resistance to variable fungal pathogens.

Based on the use of DNA marker breeding (marker assisted selection (MAS) – breeding with use of DNA markers towards genes of interest), we introduced 5 blast resistance genes into domestic rice varieties adapted to the agro-climatic conditions of rice cultivation in southern Russia.

A series of crosses made it possible to obtain rice lines based on varieties Boyarin, Flagman and Virage with introgressed and pyramided blast resistance genes *Pi-1, Pi-2, Pi-33, Pi-ta* and *Pi-b* in a homozygous state. During all cycles of backcrossing, the transfer of the dominant alleles of each such gene in the offspring was controlled by closely linked molecular markers. Plants with no resistance alleles in the genotype were discarded.

At the first stage of work in 2005 at ARC “Donskoy” 6 hybrids were obtained from crossing the varieties Boyarin and Virage with three donors of blast resistance carrying the *Pi-l, Pi-2* and *Pi-33* genes. After analysis at the Federal Scientific Rice Centre, homozygous forms were identified for the dominant alleles.

At the second stage of work (2008), after crossing among themselves the *Pi-1 + 33* x Boyarin and *Pi-2* x Boyarin hybrids, it was possible to obtain forms with three pyramided genes simultaneously: *Pi-1, Pi-2* and *Pi-33* in a homozygous state.

At the third stage of work (2010), they were hybridized with varieties – donors of *Pi-ta* and *Pi-b* genes for combining 5 genes. There were two types of crosses: [(*Pi-1 + 2 + 33*) x *Pi-ta*] x *Pi-b* and *Pi-1 + 2 + 33* x (*Pi-ta* x *Pi-b*).

Leaves were selected from the best F_2 hybrid plants for DNA analysis at All-Russian Research Institute of Agricultural Biotechnology and Federal Scientific Rice Centre using one marker for each gene. Based on the analysis results, it was possible to isolate two rice samples that were homozygous for all five dominant alleles. Reanalysis of the leaves of these samples confirmed last year's results, i.e. homozygosity for the dominant alleles of all five loci.

Figures 1, 2 show the panicles of two lines 1225/13 and 1396/13, which show the presence of dominant alleles at five loci in the homozygous state *Pi-1*, *Pi-2*, *Pi-33*, *Pi-b* and *Pi-ta*.

Line 1225/13 is early maturing, matures in 110 days, dwarfy (80 cm), with a small panicle (15 cm) (Figure 1).



Figure 1 - Panicle of early ripening line 1225/13

The second line 1396/13 is mid-ripening, the period to maturity is 120 days, taller (100 cm), with a large long panicle (22 cm) (Figure 2)



Figure: 2 - Panicle of mid-ripening line 1396/13

Against the infectious background in the Federal Scientific Rice Centre, the index of disease development (IDD) in this line was only 1.4%, while the variety Novator was damaged by 67.7%. The results of the analysis made it possible to

send these lines to the breeding nursery 2014-2015 for testing for yield and blast resistance. Sample Pentagen (1396/13), carrying 5 genes for blast resistance, is widely used in hybridization with high-yielding Russian varieties.

In the process of work at the Federal Scientific Rice Centre in 2007-2008, crosses were carried out and F_1 hybrids were obtained from the combination (Flagman x C101-Lac) x (Flagman x C-101-A-51), which have blast resistance genes *Pi-33* and *Pi-2* in their genotypes, respectively. The resulting F_1 generation was used in backcrosses with recipient parental forms. It should be noted that F_1 plants had a high degree of sterility (up to 95%). After the first series of backcrosses in 2008, the BC_1 and BC_2 generations were obtained in the artificial climate chambers. In BC_1-populations, fertility increased and averaged about 50%. Starting from the first backcrossing, marker control was carried out for the presence of transferred donor alleles in the hybrid offspring. In 2009 the plants of BC_3 and BC_4 generation were obtained. Among these plants, we selected the forms with the shortest growing season and the highest panicle fertility. From stage BC_4F_1 (the first self-pollination of rice plants, which makes it possible to transfer the donor allele to a homozygous state), individual selection was carried out. Segregation for Pi-2, Pi-33, and Sub1A genes fit into the Mendelian framework: in the second generation as a result of DNA analysis of the obtained plants, the ratio was 1:2:1 by genotype and 3:1 by phenotype.

Plants were selected that are closest in morphotype to the recipient parental form and have donor genes for resistance to the pathogen *Pyricularia oryzae Cav* in their genotype in a homozygous state [22].

Figure 3 shows the results of PCR analysis for identification of the *Pi-33* blast resistance gene in the BC_4F_3 hybrid material.

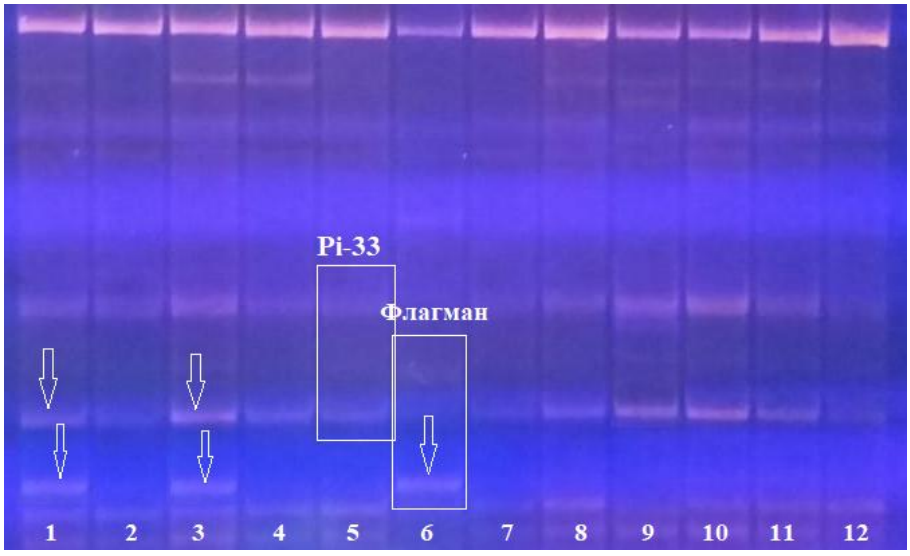


Figure: 3. Electrophoregram of genomic DNA amplification products at loci RM310 and RM72: 1-4, 7-12 – analyzed hybrid BC_4F_3 plants, 5 – donor line of the *Pi-33* gene C101-Lac, Flagman – maternal form.

The figure shows that plants number 2, 4, 7-12 are homozygotes for the dominant allele; plants number 1 and 3 are heterozygous.

In 2015-2016 the resulting rice lines with introgressed blast resistance genes *Pi-2* and *Pi-33* were crossed with the variety Khan Dan (Vietnamese breeding) to introduce into their genotypes the *Sub1A* gene, linked to the trait of resistance to prolonged flooding, as a factor in weed control for obtaining breeding material with combined genes for disease resistance and tolerance to prolonged immersion of plants under water. In 2017-2020, F_4 and BC_2F_3 generations were obtained using climatic chambers at Federal Scientific Rice Centre (All-Russian Rice Research Institute).

To increase economic efficiency and reduce labor costs, multi-primer systems have been developed to identify two genes (*Pi* and *Sub1A*) in a hybrid material simultaneously.

At the first stage, when selecting DNA markers for reliable interpretation of PCR products and identification of nonspecifically amplified fragments, the following parameters were taken into account: the annealing temperature of specific pairs introduced into the reaction mixture; the difference in the size of PCR products synthesized during amplification with specific primer pairs (at least 100 base pairs) and the self-complementarity of primer sequences.

The results of testing the combination of primer pairs flanking the marker regions of the *Pi-2* + *Sub1A* genes are shown in Figure 4.

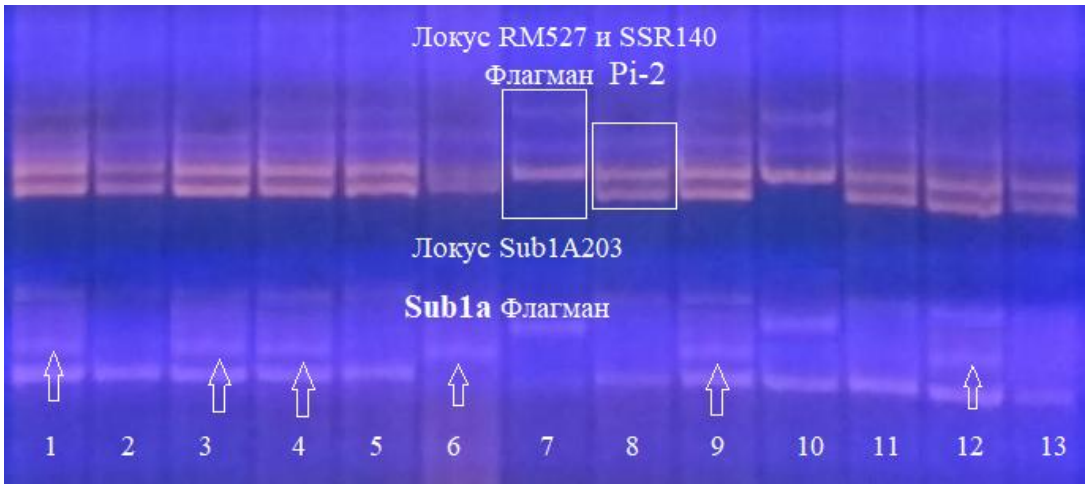


Figure: 4. Electropherogram of multiplex PCR of genomic DNA amplification products at loci RM527 and SSR140 for the *Pi-2* gene and at the Sub1A203 locus for the *Sub1A* gene: 1-5, 9- 13 – analyzed hybrid plants of BC_2F_3 – generation, 6 - Khan Dan, donor of the Sub1A gene; 7 – Flagman – maternal form; 8 – C101-Lac, donor line of *Pi-2* gene.

The electrophoretogram shows that when PCR with such a combination of molecular markers is carried out, the target products specific for DNA markers of the desired genes are reliably amplified. Samples number 3 and 12 have dominant alleles of genes *Pi-2* and *Sub1A* in a homozygous state in their genotype; samples No 1, 4, 5, and 9 are homozygous for the *Sub1A* gene and have the *Pi-2* gene in the

genotype in a heterozygous state; sample 10 is recessive homozygote by two target genes and is rejected. Clear identification on the electrophogram makes it possible to reliably identify the presence of dominant alleles of target genes.

The introduction of such varieties into production will allow avoiding the epiphytic development of the disease, preserving the biological rice productivity, and obtaining environmentally friendly agricultural products.

Magnat is the first cultivar in Russia created at the Agrarian Research Center Donskoy together with the Federal Scientific Rice Centre by the method of marker selection from a hybrid population (C101A-51 × Boyarin) × (C101 LAC × Boyarin) with transfer of blast resistance genes. Sample C101 LAC is a donor of the genes Pi-1 and Pi-33, and C101A-51 is a Pi-2. The growing season is 125 ± 1 days and the plant height is 96 ± 2 cm. The panicle is erect and compact, 17.5 ± 0.5 cm long, and bears 185 ± 5 spikelets. The grain is oval, 8.3 ± 0.2 mm long, 3.1 ± 0.1 mm wide, and 2.2 ± 0.1 mm thick and weighs 24.0 ± 2.0 mg. The yield of the Magnat variety was 8.25 t/ha, which is 1.1 t/ha higher than that of the Boyarin standard.

The rice variety Pirouette was bred at Agrarian Research Center Donskoy together with the Federal Scientific Rice Centre by the method of stepwise hybridization and marker breeding from a hybrid population (C101-A-51 (*Pi-2*) x Boyarin) x (C101-Lac (*Pi-1 + 33*) x Virazh). It contains three blast resistance genes: *Pi-1*, *Pi-2*, and *Pi-33*. The variety is mid-ripening, the growing season from the flooding to full ripeness is 124 ± 1 days. The average yield of the variety Pirouette was 9.57 t / ha, which is 1.13 t / ha higher than that of the standard variety Yuzhanin. Plant height is 88 ± 2 cm, panicle is erect, compact, 17.5 ± 0.5 cm long, carries 165 ± 5 spikelets. Spikelets are oval, 8.9 mm long, 3.7 mm wide. Weight of 1000 grains is 31.6 ± 2 g. The variety is resistant to lodging and shedding, cold-tolerant, germinates well from under a layer of water. Included in the Register of Breeding Achievements of the Russian Federation for the North Caucasus region since 2020.

The rice variety Kapitan was bred at Agrarian Research Center Donskoy in cooperation with the Federal Scientific Rice Centre by the method of triple backcrossing and marker breeding from the Flagman x IR-36 hybrid population. The variety is mid-ripening, the growing season from the flooding to full ripeness is 120 ± 1 days. On average, over the years of competitive testing, the yield of the variety Kapitan was 8.13 t / ha, which is 0.64 t / ha higher than that of the variety Yuzhanin. A higher yield of this variety is formed due to a more grained panicle and an increased weight of the caryopsis. The average height of plants is 90 ± 2 cm, the panicle is semi-inclined, compact, 18.5 ± 0.5 cm long, the average number of spikelets is 140 ± 10 pieces (Figure 5). The grains are oval, 9.5 ± 0.2 mm long and 3.6 ± 0.1 mm wide. The average weight of 1000 grains is 35.0 ± 2.1 g. The variety carries the *Pi-ta* gene, is resistant to blast, lodging and shedding. The variety has been under state testing since 2019.

The rice variety Argamak was bred at Agrarian Research Center Donskoy by individual multiple selection of plants with the largest panicles from a hybrid population Il. 14 (*Pi-1, Pi-2, Pi-33*) × Kuboyar. The variety belongs to the mid-ripening

group, the growing season from flooding to full ripeness is 119 days. On average, over the years of competitive testing (2017-2019), the yield of the variety was 8.79 t / ha, which is 1.59 t / ha higher than that of variety Yuzhanin. The maximum yield was formed in 2019 - 10.1 t / ha, 2,55 more than the standard. The high yield of this variety is formed due to the greater grain content of the panicle than that of the standard and the increased density of the stem. Plant height is 93 ± 2 cm on average; the panicle is erect, compact, and 16 ± 0.5 cm long; the average number of spikelets is 142 ± 6 pieces. The grains are oval, 8.4 ± 0.2 mm long, 3.3 ± 0.1 mm wide. Weight of 1000 grains – 31.1 ± 1.9 g. The variety is resistant to blast, lodging and shedding. It has been tested at State varietal testing since 2020.



Figure 5. Rice panicles of the variety Kapitan

The rice variety Lenaris (Federal Scientific Rice Centre) had shown high adaptability, non-lodging, and the possibility for straight combine harvesting. Its yield was 10.6 t/ha. Plants had high spikelet fertility and short stems (77 ± 5 cm) and were resistant to the Krasnodar population of P. oryzae as well. Their panicle is slightly drooping and compact; its length is 18 ± 1.0 cm. The mass of 1000 grains is about 30.4 ± 1.8 g.

In 2013-2014, Agrarian Research Center Donskoy conducted crosses and obtained F_1-F_2 hybrids of variety Novator with Asian donor rice varieties carrying the *SalTol* and *Sub 1A* genes. The hybrids of the second generation varied significantly in terms of quantitative traits: growing season (from early ripening to non-flowering), plant height (75-122 cm), panicle length (14-25 cm), number of filled grains (80-206 pcs.), number of spikelets (99-300 pcs), panicle density (4.4-16.6 pcs / cm), 1000 grains weight (26.3-34.9 g), grain weight per panicle (2.1-5.5 g) and etc.

Hybridization of the salt-sensitive domestic variety Novator with lines IR52713-2B-8-2B-1-2, IR74099-3R-3-3, IR61920-3B-22-2-1 (NSIC Rc 106) - *SalTol* locus donors was carried out. The first generation of hybrids was used to generate an F_2 hybrid population. From the populations of plants of the second generation, 90 early ripening samples with well-ripened grains (30 in each combination of crossing) were selected, which were analyzed by PCR for the presence of introduced *SalTol* alleles. As an example, Figure 2 shows the data of electrophoretic analysis of PCR products with the Rm493 marker. The donor allele of the parental line NSIC Rc 106, designated as 2.2, was found in a homozygous state in sample 282. The rest of the plants, whose amplification spectra are presented in this electrophoregram, carried the alleles of the donor and the variety Novator, that is, they were heterozygous for the *SalTol* locus (Fig. 2). Similar results were obtained during DNA analysis of the studied rice samples with the RM7075 marker (Figures 6 and 7).

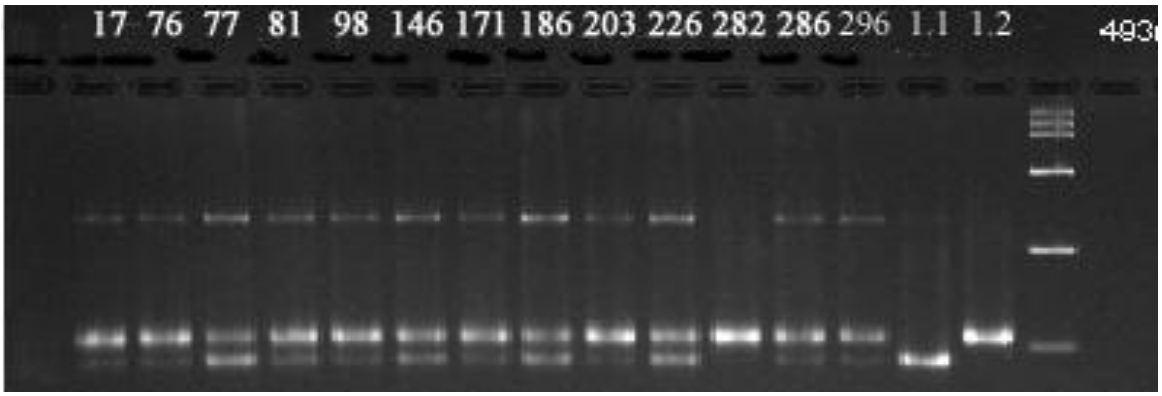


Figure 6. Electropherogram of genomic DNA amplification products with RM 493: 1.1 – Novator; 2.2 – NSIC Rc 106; 37-50 – hybrid plants NSIC Rc 106 × Novator; DNA marker (100-1500 bp).

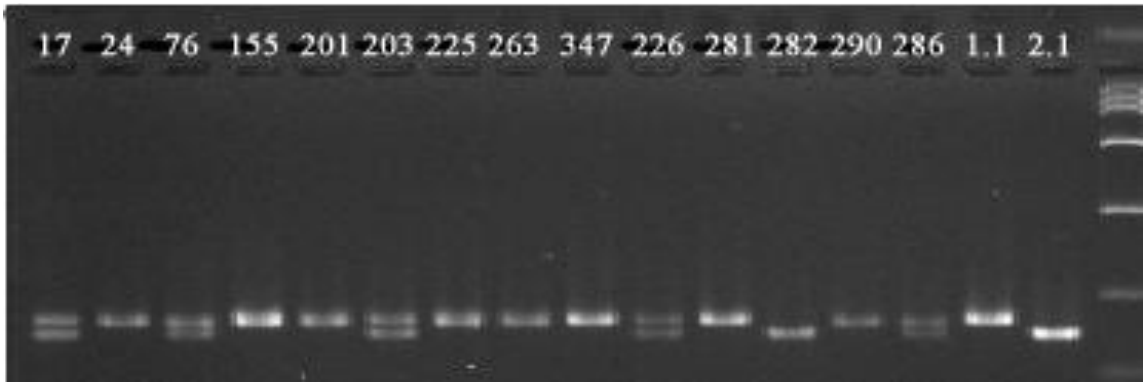


Figure: 7. Electropherogram of genomic DNA amplification products with RM 7075: 1.1 – Novator; 2.2 - NSIC Rc 106; 37-50 – hybrid plants NSIC Rc 106 × Novator; DNA marker (100-1500 bp).

In general, according to the results of DNA analysis of F_2 hybrids, 17 plants homozygous for the dominant allele of the *SalTol* locus were identified, 29 sam-

ples carried *SalTol* in a heterozygous state, the rest of the studied plants showed only recessive alleles inherited from the variety Novator.

Segregation for *SalTol* genes did not fit into the Mendelian framework, since the sample was unrepresentative due to selection. Plants with recessive alleles of the gene prevailed, and the number of salt-tolerant dominant homozygotes was less than the expected number. This is due to the linkage of *SalTol* genes with genes unfavorable for plants in our conditions: photosensitivity, late maturity, spikelet shedding, and spinosity.

Testing plants for resistance to salinity in the early stages of development is a quick, common method based on simple criteria. It was shown that at the initial vegetation stage, the length of the root and shoots, and seed germination are potential indicators of resistance to the effects of increased salt concentrations [18,19]. Evaluation of the potential salt tolerance of the studied rice hybrids and their parental forms revealed significant variations in salinity tolerance depending on the genotype. The greatest decrease in seed germination - 52% - was found in the salt-sensitive variety Novator. The line NSIC Rc 106 and second generation plants, which were homo- and heterozygous for the *SalTol* locus, showed the highest resistance by seed germination (germination decrease of less than 5%). The donor lines IR52713-2B-8-2B-1-2, IR74099-3R-3-3 and hybrid combinations obtained on their basis with the *SalTol* gene in a homozygous state also showed high resistance for this trait.

The least suppression of growth indices, as well as in the case of seed germination, was recorded in lines NSIC Rc 106, IR52713-2B-8-2B-1-2, IR74099-3R-3-3 and *SalTol* homozygous plants from the F_2 generation, and the greatest decrease in the length of shoots and roots under salt stress was shown in the variety Novator and in hybrid plants that did not inherit the *SalTol* locus according to molecular analysis data. Thus, DNA analysis made it possible to simplify the breeding scheme and obtain salt-tolerant F_2 hybrids carrying the *SalTol* locus in a homozygous state. These results indicate that the developed codominant markers of the *SalTol* locus RM 493 and RM 7075 are an effective tool for marker-assisted selection of salt-tolerant forms based on domestic rice genotypes.

Rice samples with *SalTol* genes in 2018-2020 were studied in a control nursery and in competitive variety testing, productive forms were identified.

At the same time, in 2013, hybrids were obtained by crossing variety Novator with donors of the *Sub 1A* gene. The Asian varieties turned out to be late-ripening, photosensitive and did not flower under our conditions. Hybridization was carried out only with the help of artificial climate chambers. The first generation in 2013 was characterized by a high degree of sterility (90-95%) and brown color of flowering scales during maturation, which indicates significant genetic differences between the parental forms. In the second generation in 2014, a huge spectrum of splitting was observed in terms of the growing season, plant height, panicle length and shape, number of spikelets, and spinosity (Table 2).

Table 2 - Variations in quantitative traits in F_2 hybrids from crossing submergence resistant samples with the variety Novator, 2014

Trait	Crossing combination			
	BR 11 x Novator	CR-1009 x Novator	Inbara 3 x Novator	TDK-1 x Novator
Plant height, cm	71-129	57-131	60-149	45-138
Panicle length, cm	11,5-27	10-26	9,5-32	9-27
Number of grains, pcs	10-220	2-201	4-343	4-180
Number of spikelets, pcs	57-322	38-273	18-411	17-261
Spikelet length, mm	6,1-10,1	6,8-9,8	6,1-11,9	7,2-11,3
Spikelet width, mm	2,3-3,8	2,5-4,0	2,1-3,8	2,3-3,9
Mass of 1000 grains, g	11-38	10-35	12-37	13-39
Mass of grain from the panicle, g	0,72-5,54	0,03-5,90	0,06-5,42	0,07-6,09

Note: The average value is indicated in brackets.

This wide range of variability is not observed in other crops. This is due to the genetic and ecological-geographical remoteness of the crossed forms. In each combination, about 400 plants were selected for morphometric and genetic analysis. Among the F_2 hybrids, we managed to select the best plants according to many traits, combining early maturity, optimal plant height, good grain size in panicles, non-shattering and fertility of spikelets (Table 3).

Table 3 – Selected F_2 hybrid plants from crossing submergence resistant samples with Novator, 2014

№, hybrid	Duration, days	Plant height, cm	Panicle length, cm	Number of grains in panicle, pcs.	Mass of 1000 grains, g
Novator, st	112	97,5	16,5	110	31,8
176(BxN)*	120	108,0	21,5	146	27,0
334 (BxN)	118	96,7	17,3	145	21,6
34 (CxN)	121	81,2	16,5	109	25,3
390 (CxN)	122	82,0	17,5	122	27,7
273 (IxN)	120	95,4	15,1	151	24,4
507 (IxN)	119	97,2	19,0	138	29,3
81 (TxN)	123	96,5	17,2	159	32,0
393 (TxN)	121	97,3	14,9	152	25,2

Note *: (BxN) – BR 11 x Novator, (CxN) – CR-1009 x Novator, (IxN) – Inbara 3 x Novator, (TxN) – TDK-1 x Novator

PCR analysis of leaves was carried out in 20 plants of each of the four hybrids, as a result of which forms with the *Sub 1A* flood resistance gene were isolated. The electrophoretic analysis of PCR products with the RM 7481 marker is shown in Figure 8. The donor allele of the parental line CR-1009 was homozygous

in samples 2, 3, 5, 9, 13 and 17, plants 2, 4, 6-8, 10, 11, 16, 18 and 19 were heterozygous at the *Sub1A* locus, that is, they carried both the alleles of the donor and the recessive alleles inherited from variety Novator. Thus, according to the results of PCR analysis with the RM 7481 marker, 14 homozygotes of F_2 plants at the *Sub1A* locus were identified, 40 samples carried *Sub1A* in a heterozygous state, 22 plants inherited only the recessive allele from the variety Novator.



Figure: 8. Electropherogram of the amplification products of rice genomic DNA with primer RM 4781. 1-19 – F_2 (Novator x CR-1009). Molecular weight marker 1 KB

Of the analyzed hybrid plants BR-11 x Novator, the Sub 1A gene (in homo- and heterozygous state) was present in nine, i.e. in a ratio of 9:11, although with monohybrid segregation it should have been 15: 5. In the hybrid combination CR-1009 × Novator, F_2 segregated in a ratio of 18: 2, i.e. almost all of the selected plants had the *Sub 1A* gene. In the hybrids Inbara-3 × Novator and TDK-1 × Novator, segregation took place in a ratio of 14: 6, or approximately 3: 1, i.e. close to Mendelian.

The deviations in segregation of the two combinations can be explained by the influence of selection and gene linkage. A total of 55 plants with the target gene in homo- and heterozygous state were isolated from 80 plants of four hybrids. The selected samples with the *Sub 1A* gene in 2015 were reproduced in the Federal State Unitary Enterprise “Proletarskoye” of the Rostov Region, where the best F_3 plants were selected from them for DNA analysis.

In F_3, significant morphological and biological segregation continued. Significant variation was noted for the growing season, plant height, size of panicles and caryopses, fertility, grain shedding, etc. The best forms were selected from them, leaves were taken for DNA analysis. At the next stage of work, in 2016-2020, constant lines carrying the *Sub 1A* gene in a homozygous state were selected, tested for yield and resistance to prolonged water flooding. As a result, rice varieties for herbicide-free technologies will be developed, vigorously overcoming a deep layer of water in the germination phase with minimal seed loss.

4. Conclusions

1. As a result of the studies carried out using molecular marking based on PCR in combination with traditional breeding, early maturing rice lines with genes for resistance to salinization *SalTol* and to flooding *Sub 1A*, suitable for cultivation in the south of Russia, were isolated.

2. Rice lines have been developed, the genotype of which contains five effective blast resistance genes (*Pi-1, Pi-2, Pi-33, Pi-ta,* and *Pi-b*). The introduction of such varieties into production will allow avoiding the epiphytotic development of the disease, preserving the biological productivity of rice and obtaining environmentally friendly agricultural products.

3. Samples of the F_4 and BC_2F_3 generations were obtained with combined blast resistance (*Pi*) and prolonged flooding tolerance (*Sub1A*) genes as a factor in the control of weeds in homo- and heterozygous state, which is confirmed by the data of their DNA analysis. The testing of the obtained rice breeding resources for resistance to prolonged flooding under laboratory conditions made it possible to select tolerant rice forms that will be studied in the breeding process for a complex of agronomically valuable traits. Their use will reduce the use of chemical plant protection products against diseases and weeds, thereby increasing the ecological status of the rice growing industry.

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СЕЛЕКЦИЯ СТРЕССОУСТОЙЧИВЫХ СОРТОВ РИСА С ПОМОЩЬЮ ДНК-МАРКЕРОВ

Статья посвящена исследованию толерантности к засолению почвы, затоплению водой, пирикуляриозу российских и азиатских сортов риса, а также гибридов второго-третьего поколения от их скрещивания с целью получения устойчивого риса на основе отечественных сортов с помощью ДНК маркеров. В качестве доноров гена толерантности к засолению *SalTol* использовали образцы IR 52713-2B-8-2B-1-2, IR 74099-3R-3-3 и NSIC Rc 106. В качестве доноров гена устойчивости к затоплению использовали сорта с локусом Sub1A: Br-11, CR-1009, Inbara-3, TDK-1, Khan Dan. При переносе генов устойчивости к пирикуляриозу использовали линии C101-A-51 (*Pi-2*), C101-Lac (*Pi-1, Pi-33*), IR-58 (*Pi-ta*), Moroberekan (*Pi-b*). Была проведена гибридизация чувствительных к стресс-факторам отечественных сортов Новатор, Флагман, Вираж, Боярин с линиями – донорами генов интереса. В результате проведенных исследований с помощью молекулярного маркирования на основе ПЦР в сочетании с традиционной селекцией выделены скороспелые линии риса с генами устойчивости к засолению *SalTol* и затоплению *Sub 1A*,

пригодные для выращивания на юге России. Проведена интрогрессия и пирамидирование генов устойчивости к пирикуляриозу *Pi-1, Pi-2, Pi-33, Pi-ta, Pi-b* в генотипы отечественных сортов риса. ДНК-маркерный анализ позволил выявить устойчивые к болезни образцы риса, несущие 5 целевых генов в гомозиготном состоянии. Созданы сорта риса, несущие гены устойчивости к пирикуляриозу: Пентаген, Магнат, Пируэт, Аргамак, Капитан, Ленарис, переданные на Государственное сортоиспытание. Внедрение таких сортов в производство позволит избежать эпифитотийного развития болезни, сохранить урожай риса и получать экологически чистую сельхозпродукцию.

Ключевые слова: рис, гены устойчивости к засолению, затоплению, пирикуляриозу, SSR-маркеры, ПЦР-анализ.