# **CROP PRODUCTION**

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# THE BREEDING OF RICE VARIETIES TOLERANT TO SALINITY

Research article

## Abstract

Rice resistance to soil and water salinity plays an important role in agriculture, as it affects the productivity of plants in areas with high salt content. This article provides information on the identification of samples with the Saltol tolerance gene from hybrid rice populations obtained from hybridization of the Russian variety Novator with Asian donors of this gene. In laboratory analysis of salt resistance, rice seeds were germinated in glasses with a 1.5% solution of table salt and distilled water. The ratio of the mass of young 10-day plants in the experiment to the control was used as an indicator of stability. In the course of the work, samples tolerant to salinization were selected. PCR analysis showed that most of them contained the gene Saltol in their genotype. The best lines were tested for productivity in the field on plots of 25 m2 in 2-fold repetition. The elements of the crop structure are also studied. Two samples 7328/17 and 7322/17 from a combination of IR-52713-2B-8-2B-1-2 x Novator in an average of two years showed a yield of 6.82-7.53 t/ha, while the variety-standard Uzhanin – 6.25 t/ha). The increase was 0.57-1.28 t/ha.

Keywords: rice, hybrid, salt tolerance, PCR analysis, yield.

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## ВЫВЕДЕНИЕ СОРТОВ РИСА, ТОЛЕРАНТНЫХ К ЗАСОЛЕНИЮ

Научная статья

#### Аннотация

Устойчивость риса к засолению почвы и воды играет важную роль в сельском хозяйстве, поскольку влияет на продуктивность растений на участках с повышенным содержанием солей. В данной статье приведена информация по выявлению образцов с геном толерантности к засолению Saltol из гибридных популяций риса, полученных от гибридизации российского сорта Новатор с азиатскими донорами этого гена. При лабораторном анализе солеустойчивости семена риса проращивали в стаканах с 1,5%-м раствором поваренной соли и дистиллированной водой. Отношение массы молодых 10-дневных растений в опыте к контролю использовали как показатель устойчивости. В ходе работы были отобраны образцы, толерантные к засолению. ПЦР-анализ показал, что большинство из них содержали в своем генотипе ген Saltol. Наилучшие линии прошли испытание на продуктивность в полевых условиях на делянках площадью 25 м2 в 2-кратной повторности. Изучены также элементы структуры урожая. Два образца 7328/17 и 7322/17 из комбинации IR-52713-2Б-8-26-1-2 х Новатор в среднем за два года показали урожайность 6,82-7,53 т/га, тогда как сорт-стандарт Южанин – 6,25 т/га). Прибавка составила 0,57-1,28 т/га.

Ключевые слова: рис, гибрид, солеустойчивость, анализ ПЦР, урожайность.

## 1. Introduction

Selection for resistance to abiotic and biotic stresses is an important way to combat the decline in yield. Rice, Asia's most beloved grass, feeds most of the world's population. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's population and about two-thirds of the world's population live [12]. The green revolution helped solve

global food demand, but this is not enough to satisfy the growing population of the 21st century. To increase production needs, it is necessary to increase the acreage under rice. About 6.5% of the total land area is exposed to salt in the soil. The area exposed to salt load is increasing due to many factors, including climate change, rising sea levels, excessive irrigation without adequate drainage on inland lands, bedrock rich in harmful salts, etc. not used due to salinity and alkalinity problems.

Salinization is one of the major obstacles to the increase in rice production in areas where it is grown worldwide. Rice is classified as a culture especially sensitive to salt [15]. It has been established that the effect of salinization on rice growth is associated with the stage of plant development, the concentration and type of salt, the duration of its impact, soil pH, water regime, temperature, humidity and solar radiation [4].

Laboratory tests of rice varieties showed that the salt concentration in the solution during seed treatment is especially important for assessing the relationship between ion accumulation and salt tolerance. Salinization stimulated the accumulation of Na+, Na+ / K+, Cl- ions in shoots and roots and reduced the content of K+, NO3- in both organs at a NaCl concentration of 1.2%, but not always at 0.6%. A significant negative correlation between the content of Na + and the dry weight of the shoot and the root was shown at a NaCl concentration of 1.2%. A significant negative effect of Cl- ions on biomass growth and survival rate of rice plants and a positive relationship between the dry mass of the root and the K+ and NO3- content with high NaCl concentrations was also found [5].

Janaguiraman et al. (2003) reported that tolerant rice genotypes show a higher percentage of germination, root and sprout length, growth index, amylase and dehydrogenase activity with less anthocyanin accumulation in the roots [11]. Sankar et al. (2006) estimated the salt tolerance of rice varieties by germination and growth of seedlings under salt stress conditions with 1.6% NaCl. The CSR 23 and CSR 10 genotypes showed themselves well in terms of the germination rate, CO 43 and Nona Bokra, in terms of the share of grain in biomass, and CSSRI 60, in terms of the growth energy index [8].

In recent years, the creation of salt-resistant varieties has been proposed as a means of expanding agriculture in salineaffected regions [9]. Selection of rice varieties with genetically determined salt tolerance is implemented as the most promising, less resourcious, economically viable and socially acceptable approach. Salt tolerance is a multigene trait that allows plants to grow and form an economically significant crop with physiologically low and relatively constant levels of salt, in particular, NaCl [10]. Rice yields in saline areas are very low, <1.5 t / ha, but can be easily increased by at least 2 t / ha [14]. In India, salt-resistant rice varieties have been created: CSR, Panvel, Pokkali, Vytilla, Savitri, and others [7]. Similar work is underway in other countries.

In Russia, this problem is also relevant, which makes it necessary to carry out selection work on the creation of saltresistant rice varieties. To do this, it is necessary to involve the best Asian samples in crosses with local varieties and to use modern methods of controlling gene transfer and salt tolerance diagnostics.

In the research institute of rice Gisheva N.G. (1999) conducted studies of the morphological traits of rice plants, quantitatively changing under the influence of excess salts in the soil, closely related to salt tolerance, and the assessment of variety samples for resistance to this stress. The data obtained are of practical importance for breeding, allowing for the evaluation of breeding samples for salt tolerance using a complex of morphological and physiological and biochemical characteristics [1]. Ladatko N.A. (2006) showed the effect of salinization and the level of nitrogen nutrition on the integral indicators of the photo-synthesis of varieties [2].

Scientists-breeders of the All-Russia Scientific Research Institute of Rice created varieties combining a high degree of tolerance to chloride soil salinization with economically valuable traits for cultivation using various technologies. In the period from 1999 to 2006 salt-resistant varieties Kurchanka, Fountain, Serpentine, Iceberg, Sonata were transferred to STV (state testing of varieties). The Sonata variety was created by the anther culture method from the hybrid population F3 Pokkali / Slavyanets [3].

The aim of our research was the creation of salt-resistant varieties of rice for the conditions of southern Russia.

#### 2. Methods

The early maturing variety of the All-Russia Scientific Research Institute of Novator rice and 3 Asian salt-resistant samples IR 52713-2B-8-2B-1-2-2B-8-2B-1-2, IR 74099-3R-3-3- served as the starting material. 3R-3-3 and NSIC Rc 106 carrying the Saltol gene, localized in the 1st chromosome of rice. These samples were crossed with the variety Novator in 2013 under the conditions of the climate chamber of the All-Russia Scientific Research Institute of Rice.

Hybrid plants were grown in the Rostov region on the checks of the Proletarskaya Experimental Station of the Agricultural Research Center "Donskoy". From selected leaves of rice, genomic DNA was isolated in the laboratory of marker selection of ARC "Donskoy" using the CTAB method [13]. Using PCR analysis based on the RM 493 marker [6], the presence of the target Saltol gene in the cleaved hybrid progeny was carried out. Amplification was carried out in an Applied Biosystems 2720 thermal cycler: denaturation  $-94^{\circ}C - 5$  minutes, 35 cycles ( $94^{\circ}C - 30$  sec.,  $60^{\circ}C - 30$  sec.,  $72^{\circ}C - 30$  sec.), final elongation  $-72^{\circ}C - 8$  minutes. PCR products were separated by electrophoresis on a 2.0% agarose gel, stained with ethidium bromide and photographed using a Bio-Rad GelDoc XR + instrument.

Samples of rice were tested for salt tolerance using a laboratory (cups) method. The seedlings were weighed at 10 days of age after their growth in distilled water and 1.5% NaCl solution. For differentiation of samples for salt tolerance at the development stage of the seedling, the scale developed by IRRI [17] was used: unstable - from 0 to 20%; poorly resistant - from 21 to 40%, medium-resistant - from 41 to 60%, stable - from 61 to 80%, highly resistant - more than 80%. In laboratory experiments, the parent variety Novator served as the standard, in the field, in the assessment of yield, the Uzhanin variety.

The study of the best lines of the complex signs was carried out in the control nursery on plots of 25 m<sup>2</sup> in 2-fold repetition. Accounting yield carried out in the field after harvesting plots combine. Statistical processing of the data was performed using Excel and Statistica 6.

## 3. Results

The first generation hybrids in 2013 were characterized by late ripeness and high sterility of spikelets, caused by the genetic remoteness of the crossed forms. The variety Novator belongs to the japonica sub-species, and the other specimens are indica. Hybrids of the second generation in 2014 varied significantly by the growing season (110-150 days), the length of the stem (70-120 cm), the length of the panicle (13-24 cm), the number of grain per panicle (81-200) and the number of spikelets per panicle (100-300), the mass of 1000 grains (26-35 g), mass of panicles (2-5 g), etc.

From these hybrid populations, panicles were selected from the best plants that had the optimal values of economically valuable traits: early ripeness, short stature, good horniness, low hollowness. DNA was isolated from their leaves to analyze the presence of the Saltol gene. Seeds from the analyzed plants of the second generation carrying the target gene in the homozygous and heterozygous state were sown in 2015 in the field for the 3rd generation. For the next PCR analysis, the best bushy plants with a well-watered panicle and ripened grain were taken from them. Work on the selection and analysis of the best samples lasted for several generations.

DNA analysis revealed differences between samples in the presence of Saltol gene alleles [16]. In 2016, out of 205 F4 plants analyzed, only 41 plants had the Saltol gene in the homozygous dominant state, 29 plants in the heterozygous state and 135 plants in the recessive state. An example of electrophoregram presented in Figure 1.



Figure 1 – Electrophoregram of F4 rice lines for the presence of the Saltol gene, 2016. Note: M - Thermo Scientific Gene Ruler 50 + bp molecular weight marker notes: H - Novator variety, YR - sample IR 74099-3R-3-3-3R-3 -3, B - H2O deionized (negative control of experience)

The donor IR 74099-3R-3-3-3R-3-3 and lines 7267 and 7275 had a dominant allele, 7270, 7274, 7278, 7281 and 7282 had a heterozygous allelic state, other lines had a recessive allele.

Checking them for salt tolerance in cups with a 1.5% solution of NaCl compared with the control (distilled water) revealed a significant diversity in the relative weight of seedlings (Figure 2).

100 43% 90 80 35% Number of samples, pieces 70 60 50 40 30 20 7% 6% 10 3% 2% 1% 1% 0 20 0 10 30 40 50 60 70 80 90 100 Salt tolerance, %

C = 213\*10\*normal(x; 43,1536; 14,6565)

Figure 2 – Distribution of F4 rice lines by salt tolerance, 2016

The bulk of the samples (86%) had low and average salt tolerance - from 20 to 60%. About 3% of the samples were completely unstable, 44% were poorly resistant, 42% were moderately resistant, 7% were stable, 3% (7 samples) showed high resistance to salinity - the ratio of the mass of seedlings in salt and fresh water exceeded 80%.

The best resistant and highly resistant samples are presented in table 1. In the standard variety Novator, the salt tolerance, determined by the ratio of the mass of one seedling in the experience to control, was 41.9%, whereas in the best samples it reached 97.8%. At the same time, the ranks of salt tolerance, determined by seed germination and seedling mass, did not always coincide.

No. 2016	Germination, %			Mass of one seedling, mg			
	Experience	Control	Ratio, %	Experience	Control	Ratio, %	
Novator,	10	100	10.0	24	0.6	41.0	
standard	18	100	18,0	36	86	41,9	
7328	90	100	90,0	109	112	97,8	
7340	22	68	32,4	44	47	92,2	
7343	22	98	22,4	78	86	91,2	
7322	46	98	46,9	79	91	86,8	
7337	46	98	46,9	72	83	86,4	
7356	44	94	46,8	94	114	82,3	
7108	20	92	21,7	82	102	80,8	
7156	34	100	34,0	69	90	77,0	
7085	60	100	60,0	47	64	74,0	
7097	60	98	61,2	35	50	69,4	

Table 1 - Released rice samples for salt tolerance, 2016

7285	26	82	31,7	49	71	68,9
7370	58	100	58,0	61	90	68,0
7363	72	90	80,0	50	74	67,4
7101	82	100	82,0	41	62	66,5
7268	22	100	22,0	95	144	66,3
7149	74	92	80,4	51	79	64,6
Standard deviation	26,2	9,2	26,7	12,9	21,1	14,8

Continuation of the Table 1

Comparison of data on salt tolerance and the presence of Saltol genes, detected by PCR analysis, allowed us to establish a positive relationship between them and a tendency to increase tolerance to salinity with an increase in the number of dominant alleles (Figure 3).

Recessive homozygotes showed an average salt tolerance of 41.5%, heterozygotes - slightly higher - 42.4%, and dominant homozygotes - 47.5%. Thus, the presence of the Saltol gene increased salt tolerance by 6%. Small differences between the three groups of samples indicate the presence of other mechanisms of resistance to salinization, controlled by different polygens.

The study in 2017 of the best salt-resistant lines of the three hybrid combinations showed that the most productive of them was sample 7328 (IR 52713-2B-8-2B-1-2 x Novator), which formed 6.85 t / ha of grain, at 0, 75 t / ha more standard (Table 2). In the control nursery of 2018, the study of these lines was continued. Of these, by productivity, 2 lines from the hybrid IR 52713-2B-8-2B-1-2 x Novator: 7328 and 7322 significantly exceeded the standard grade Uzhanin by 1.80 and 1.02 t / ha. For an average of 2 years, they formed a yield of 6.82-7.53 t / ha (in a standard of 6.25 t / ha) (Table 2).



Figure 3 - Relation of salt tolerance to the number of alleles of the Saltol gene

$\mathcal{N}_{\underline{0}}$	Name		Increase to standard, t / ha		
		2017	2018	average	
Standard	Uzhanin	6,10	6,40	6,25	-
7328	IR 52713-2B-8-2B-1-2 x Novator	6,85	8,20	7,53	1,28
7322	IR 52713-2B-8-2B-1-2 x Novator	6,22	7,42	6,82	0,57
7343	IR 74099-3R-3-3 x Novator	6,53	5,93	6,23	-0,02
7337	IR 74099-3R-3-3 x Novator	6,00	6,58	6,29	0,04
7340	IR 74099-3R-3-3 x Novator	6,55	6,04	6,30	0,05
7356	NSIC Rc 106 x Novator	5,75	6,65	6,20	-0,05
	Least significant difference	0,34	0,33		

Table 2 – The yield of rice lines in the control nursery

The remaining lines of the hybrids IR 74099-3R-3-3 x Novator and NSIC Rc 106 x Novator were at the standard level, although one of them (7340) matured 4 days earlier than Uzhanin.

Thus, combining classical breeding methods with growing seedlings on a saline background and PCR analysis of the presence of the salt tolerance gene, we managed to create high-yield lines of rice with a set of economically valuable traits. In their morphotypes, they differ from the Southerner standard in a lower height, shorter panicle, and a smaller mass of 1000 seeds (Table 3).

Varieties, sample	Plant height, cm	Panicle length, cm	Number of spikelets, pieces	Number of grains, pieces	Weight 1000 grains, g	Number of productive stems / m2
Uzhanin	110	18,8	110,0	97,3	30,0	228
7328	100	12,8	97,3	87,3	26,5	360
7322	89	14,5	99,0	87,0	26,0	328
7343	87	14,5	93,3	82,0	27,5	264
7337	92	16,3	97,0	86,8	26,8	284
7340	86	14,0	77,0	69,0	30,0	308
7356	85	14,5	100,8	87,0	24,3	316
σ	8,0	1,4	12,7	10,4	2,3	43,6

Table 3 - Biometric characteristics of control nursery samples, 2018

Due to the greater density of the stand (328-360 pcs./m2) under normal growing conditions, they form grain yield at the level and above the standard. On saline lands, they will have a more significant advantage over regular varieties. In 2019, it is planned to sow them on checks with a salted background.

## 4. Conclusion

1. Obtained hybrids of the Novator variety with three Asian lines donated to the salt-tolerant Saltol gene.

2. Early maturity lines with a complex of economically valuable traits were selected from fissile hybrid populations.

3. Using PCR analysis revealed rice forms with the dominant allele of the Saltol gene.

4. The method of germination of seeds in 1.5% solution of table salt allocated 7 samples with salt tolerance of more than 80%.

5. In the control nursery of 6 samples, two significantly exceeded the standard of the Uzhanin in grain yield.

**Conflict of Interest** 

Конфликт интересов

None declared.

Не указан.

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