

ASSESSMENT OF RICE SORTS AND HYBRIDS ON THE SALT TOLERANCE USING LABORATORY METHODS

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Abstract

The article presents the results of the analysis of salt tolerance in rice samples by the laboratory methods at a concentration of NaCl – 1,5 %. The plants were grown in an incubator at temperature 26 °C. After 15 days, we measured the length of roots and shoots; the germination of seeds was counted. For the analysis of hybrids sensitive to salt Krasnodar varieties Novator with lines IR 52713-2B-8-2B-1-2, IR 74099-3R-3-3, NSIC Rc 106 – donor locus salt tolerance Saltol, using markers RM 493 and RM 7075. 90 plants were selected from the F₂ plants which are analyzed by PCR, and the results of DNA analysis identified 6 of them as salt tolerant samples homozygous for the desired locus. From their offspring was selected the promising form to create salt-tolerant rice sorts.

Keywords: rice, sort, sample, salt tolerance, DNA markers, PCR, Saltol.

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1. Introduction

Rice is the most important food crop for more than half of the world population. Million of hectares in the humid districts of Asia and other continents are technically suitable for the rice production but still untreated or give the very low seed harvests because of salt and problem soils. The fifth part of irrigable soils in the world suffers from the unfavorable influence of high salinity of soil and from the problems of salination [1]. The soil salination significantly limits the productivity of cultivated rice. In Russian Federation rice is cultivated on an area near 200 thousands of hectares. Near 80 thousands of hectares of the rice fields on South of Russia are incurred to salination to some extent [2].

The decrease of the crop capacity on the saline soils can be overcome by the improvement of the rice salt tolerance. That is why creation and introduction in agricultural production of the new productive sorts with the high tolerance to the salt stress is one of topical problems of this crop selection.

In last years was achieved the significant progress in elaboration of the molecular marking technologies and their use in the control of complicated agrotechnical signs by the marker selection [3]. Technologies of molecular marking of the loci of salt tolerance allows select the plant forms with target genes and create sorts with required properties without using provocative backgrounds [4–6]. The tolerance to salination at the different stages of growth is provided by the several independent genes and the main of them is **Saltol** placed in chromosome 1 [7]. Its main function is the control of balance of Na⁺/K⁺ ions in rice plants [8]. The base of the breeding strategy for transfer of the concrete allele from the donor line to the recipient one is hybridization and use of molecular markers that allow control the transfer of genes to the posterity in every generation that increases the speed and reliability of the process of selection.

2. Aim of the work

Assessment of the rice hybrid forms F₂–F₃ with the help of rolls and salt solution and also DNA-markers and PCR-analysis.

3. Materials and methods

There were used the sorts, collection samples and hybrids of *Research Institute of grain crops of I. G. Kalinenko*, Research institute of rice and agro-genetic institute (Hanoi, Vietnam). Hybrids of three combinations were received from the crossbreeding of salt tolerant donors IR 52713-2B-8-2B-1-2, IR 74099-3R-3-3 and NSIC Rc 106 with rareripe Krasnodar sort Novator.

The assessment of samples in the salt was carried out on the rolls of filtrated paper and polyethylene that were placed in the special trays then were added the salt solution in concentration 1,5 % NaCl [9]. The control seeds were germinated on distilled water. Plants were grown in thermostat at temperature 26 °C, humidity 70 %. In 15 days was measured the length of roots and sprouts, the rate of seeds germination was calculated (**Fig. 1**).



Fig. 1. The rice sprouts in 2 weeks on the salt solution (above) and on water (below)

The rice plants were grown on checks of FSUE “Proletarskoe” in Rostov region. From the rice leaves selected on the breeding plots in laboratory conditions of the institute of biology SFI was separated the genomic DNA. At SSR analysis 2 pairs of primers chained with salt tolerance locus were used: RM493 and RM7075 [10]. The statistical processing of experimental data was carried out using Excel program of Microsoft Office package.

4. Results of research

At the study of two Indian donors of the salt tolerance NSIC Rc 106 and FL 478 comparing with Krasnodar sort Novator and Rostov sorts Kuboyar, Svetly, Magnat, Boyarin, Yujanin and also with the collection sample of Chili 45-1-1 were established the significant differences in the length of roots and sprouts that varied on sorts and experiment variants (**Table 1**).

Among the studied sorts the maximal sprout length in the salt solution was in sorts Boyarin (7,33 cm) and Svetly (7,06 cm). The shortest sprouts were formed in sorts Novator (3,79 cm) and NSIC Rc 106 (3,91 cm). The most root length was noted in control in sorts Boyarin (10,57 cm) and Novator (10,52 cm), and in experiment – in Boyarin (5,24 cm), FL 478 (5,53) and NSIC Rc 106 (5,17).

Table 1
Sizes of 15-days rice sprouts on the salt solution and water

Sort	Sprout length, cm		Root length, cm	
	Experiment	control	experiment	control
Novator	3,79	6,10	4,44	10,52
NSIC Rc 106	3,91	6,22	5,17	6,77
FL 478	4,81	5,77	5,53	6,20
Chili 45-1-1	5,53	8,52	5,14	10,26
Kuboyar	5,97	10,64	4,70	10,15
Svetly	7,06	9,64	5,01	10,01
Magnat	4,15	7,46	4,87	9,99
Boyarin	7,33	10,83	5,24	10,57
Yujanin	5,30	7,75	4,64	9,31

In this case two salt resistant samples demonstrated their advantage. The ratio of root length in experiment to the control one that characterizes the degree of tolerance turned out the most one just in them. In FL 478 this value is 89,2 %, in NSIC Rc 106–76,4 % (Fig. 2).

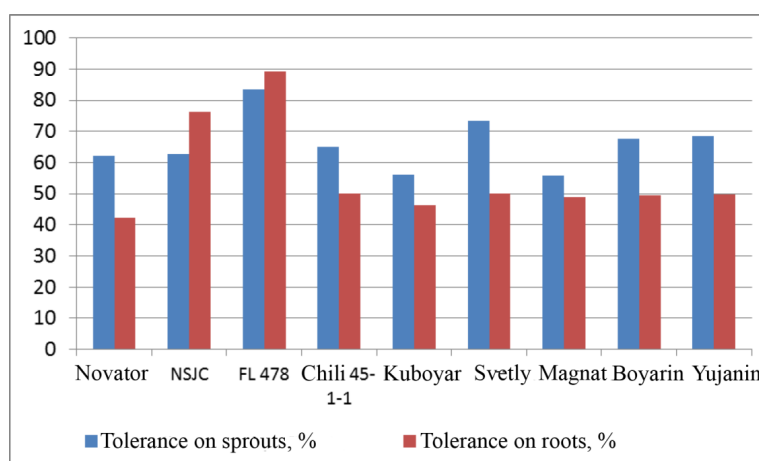


Fig. 2. Salt tolerance of the rice sorts, %

At the same time the tolerance to salination in Novator was minimal – 42,2 %, little more in Kuboyar – 46,3 %. In other samples the salt tolerance of roots was near 50 %. That is why FL 478 is most important for breeding.

Salt tolerance defined by the sprout length ratio differed from the one by roots. At the first place in rating was also FL 478 (83,4 %), then Svetly (73,3 %), Yujanin (68,4 %) and Boyarin (67,7 %). Sort NSIC Rc 106 (62,8 %) was only at the fifth place. The minimal values were in Magnat and Kuboyar (56 %).

Indian sorts were very late-maturing, blossomed in September and cannot mature in time in the field conditions of Rostov region. For transferring the tolerance genes to the native sorts was realized hybridization of donors NSIC Rc 106, IR 52713-2B-8-2B-1-2 and IR 74099-3R-3-3 with rareripe sort Novator [9].

From the plants of second generation were selected 90 rareripe well seeded samples (30 in each combination), that were analyzed by PCR method for the presence of introduced *Saltol* alleles.

In hybrid combination IR 52713-2B-8-2B-1-2×Novator were revealed two plants, homozygous on *Saltol* gene. 17 plants were heterozygous, 9 – homozygous on recessive allele. Analogous results were received also on two other combinations of crossbreeding. Thus, in hybrid

IR 74099-3R-3-3×Novator were detected 2 SS, 8 Ss and 14 ss; in hybrid NSIC Rc 106×Novator – 2 SS, 13 Ss and 15 ss. Deviations in the results were affected by artificial selection of the rareripe plants best on morphotype. In general at studying genotypes on SSR-markers in each hybrid combination were revealed 2 forms homozygous on *Saltol* allele. They were seeded in the field on F₃ from which were selected the best forms for breeding and further study.

The split was far from the Mendel one 1:2:1. The number of heterozygotes was close to statistically probable one but the number of *Saltol* homozygotes turned up essentially lower than expecting one, and *saltol* – on the contrary, significantly higher. Thus, only 6 plants of 90 are homozygous on *Saltol* gene [10].

In 2015 the families of these homozygous and heterozygous forms were grown in the field where they essentially continued to split by many morphological signs and vegetative period. Among them were selected 340 best plants with heightened bushiness and seeding for receiving the sufficient amount of seeds for analysis and sowing. They were studied for salt tolerance by the roll method. The best lines are presented in rank order of the sprout length in the **Table 2**.

Table 2Germination and length of the sprouts of rice hybrids F₃ in rolls on 0,5 % NaCl solution in 15 days

№ of sample 2015	Hybrid, sort	Mean germination, %	Root length, mm	Sprout length, mm	Seedling length, mm
standard	Novator	78	4,4	3,8	8,2
6916-1	NSIC Rc 106×Novator	90	6,3	5,5	11,9
7005-1	IR 52713-2B-8-2B-1-2×Novator	79	7,0	5,7	12,6
6978	IR 52713-2B-8-2B-1-2×Novator	85	9,9	3,5	13,4
7074	IR 74099-3R-3-3×Novator	95	9,1	5,1	14,2
7067-1	IR 74099-3R-3-3×Novator	93	10,2	4,1	14,3
6905-1	NSIC Rc 106 × Novator	85	7,8	6,6	14,5
6992-1	IR 52713-2B-8-2B-1-2×Novator	88	8,8	6,4	15,2
7038-1	IR 74099-3R-3-3×Novator	81	10,6	4,7	15,3
6977-1	IR 52713-2B-8-2B-1-2×Novator	81	7,9	7,5	15,5
6998-1	IR 52713-2B-8-2B-1-2×Novator	92	7,9	8,0	15,9
6922-1	NSIC Rc 106×Novator	91	4,9	11,5	16,4
7008-1	IR 52713-2B-8-2B-1-2×Novator	97	7,2	9,5	16,7
7053-1	IR 74099-3R-3-3×Novator	93	7,8	9,5	17,3
6901	NSIC Rc 106×Novator	95	11,2	6,7	17,9
6907-1	NSIC Rc 106×Novator	98	11,0	7,2	18,3
7046-1	IR 74099-3R-3-3×Novator	91	13,2	6,5	19,7
6975-1	IR 52713-2B-8-2B-1-2×Novator	99	12,3	13,0	25,3
	HCP 05	2,0	1,3	0,9	2,2

Germination of seeds varied from 78 % (Novator) to 99 % (6675-1). In parent sort Novator the sprout length was 3,8 cm, and in hybrid samples – 3,5–13,0 cm. Correspondingly, the root length in standard – 4,4 cm, in samples – 6,3–13,2 cm. The most strong, long seedlings were formed in lines 6907-1 (NSIC Rc 106×Novator), 7046-1 (IR 74099-3R-3-3×Novator) and 6975-1 (IR 52713-2B-8-2B-1-2×Novator) – up to 25,3 cm that is in 3 times more than in Novator.

They can be interesting for the marker rice selection for creation of the salt tolerant sorts.

5. Discussion of the results of research

The global change of climate to the warming leads to aridization and salination of soil causing the negative consequences for the production of rice that is the one of most important crops in the world. The climate change significantly worsens the unfavorable effects of abiotic stresses on the rice plants. The most part of rice lands is already affected by the increasing salinity level. But the problems of salt stress in plants can be effectively smoothed using the tolerant rice sorts.

In the result of previous studies we established the significant differences between the collection samples and sorts on the sprout and root length and separated 7 especially salt tolerant forms with the long sprouts and roots that must be recommended for breeding on tolerance to this stress-factor [11].

The earlier correlative analysis demonstrated that there is the mean positive correlation between the length of sprouts of the different rice sorts in the salt solution and salt tolerance $r=0,63\pm 0,16$, between the length of roots and salt tolerance $r=0,54\pm 0,19$ and strong positive correlation between the sprout and root length $r=0,84\pm 0,15$ [12].

It is necessary to create the salt tolerant rice sorts using the donors of this sign at hybridization. But selection on the provocative backgrounds of the needed recombinants is rather complicated. That is why it is necessary to brood the rice sorts with the high potential of the crop capacity and seeds quality using the modern instruments of biotechnology [13, 14].

But often it is difficult to transfer the salt tolerance genes in the high-yield sorts by the usual selection methods because of undesirable adhesion of genes that affect productivity and seed quality and occurs in hybrid posterity [15]. We also observed the very low frequency of segregation from the hybrid population of homozygous forms with the dominant Saltol alleles – near 7 % instead of expected 25 %. It is connected with the fact that for analysis were selected the leaves from plants which seed is matured to the beginning of September, was beardless and did not fall. The sample could not be representative for the genetic analysis of split because the breeding selection was used for the hybrid populations. The single elite plants that carry Saltol found at PCR analysis appeared as a result of crossing over. Using this mechanism it is possible to separate the genes unfavorable for our conditions from the one of interest. In ideal, it would be better to transfer the one gene from the donor to the genetic base of commercial sort fit to the local conditions. It is possible with the help of backcrosses.

Last years the use of molecular selection allows create many rice sorts in different countries. In Vietnam with the use of MAS was improved the salt tolerance of widely cultivated rice sort OMCS2000 [16]. In the different institutes is used the strategy of the reverse crossing with the marker control of transfer of favorable alleles of salination tolerance to the elite rice lines that significantly diminishes the terms of selection and make the laborious screening useful [17].

Because markers are chained with the certain signs of interest they can be used as biotechnological instrument for transferring the value genes of rice tolerance to the salt stress without undesirable adhesion [18].

Using backcrosses and marker control Vietnamese researchers successfully transferred the Saltol gene from the FL478 donor line to the BT7 sort that was selected as a recipient for the high quality of polished rice. Using SSR (RM493 and RM3412b) markers Saltol gene were successfully transferred to the genotype of BT7 that demonstrated the familiar phenotypic features comparing with original [19]. They established that there is no essential difference on morphological signs between BT7 and separated lines with Saltol gene.

In our previous researches was established that in plants carrying the recessive and dominant Saltol alleles in homozygous state the height, length of panicle, the number of realized seeds, mass of seeds from panicle were almost at the same level. Analysis demonstrated that the plants with the salt tolerance gene have the development of quantitative signs at the level of unstable ones in the general growth conditions of Rostov region [20].

Their advantage in conditions of the real salination of soil can be revealed at the growing on the saline soils. It must be done after receiving homozygous lines F_6-F_7 and breeding of their seeds in the number sufficient for the sowing on the large area.

The limitation of our research is in the fact that the plants tolerance at the different stages of ontogenesis often differed. Tolerance to the salt stress at the germination stage not always coincides with such at the blossom stage and vice versa. That is why for more complete characteristic of sort it is necessary to study plants during their life using lysimeters, microchecks with saline soil or naturally saline fields. But the costs for such screening are significantly higher than the ones of laboratory methods.

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6. Conclusions

1. With the help of rolls and salt solutions NaCl (1,5 %) were revealed the essential differences on the salt tolerance of the rice sorts and hybrids.
2. The split on Saltol gene differs from the theoretically expected one 1:2:1 because of selection of forms best on morphotype.
3. The marker selection allows separate the promising hybrids that can be interesting for creation of the salt tolerant rice sorts.

References

- [1] Negrão, S., Courtois, B., Ahmadi, N., Abreu, I., Saibo, N., Oliveira, M. M. (2011). Recent Updates on Salinity Stress in Rice: From Physiological to Molecular Responses. *Critical Reviews in Plant Sciences*, 30 (4), 329–377. doi: 10.1080/07352689.2011.587725
- [2] Ladatko, N. A. (2006). Morpho-physiological features varieties rice, conditional on their resistance to soil salinity, in connection with the development of methods for evaluating the salt tolerance breeding samples. Krasnodar, 190.
- [3] Singh, D. (2011). Marker assisted selection and crop management for salt tolerance: A review. *African journal of biotechnology*, 10 (66). doi: 10.5897/ajb11.049
- [4] Islam, M. R., Salam, M. A., Hassan, L. et. al (2011). QTL mapping for salinity tolerance at seedling stage in rice. *J. Agric. Food Technol.*, 23, 137–146.
- [5] Ren, Z.-H., Gao, J.-P., Li, L.-G., Cai, X.-L., Huang, W., Chao, D.-Y. et. al (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics*, 37 (10), 1141–1146. doi: 10.1038/ng1643
- [6] Sabouri, H., Rezai, A. M., Moumeni, A., Kavousi, A., Katouzi, M., Sabouri, A. (2009). QTLs mapping of physiological traits related to salt tolerance in young rice seedlings. *Biologia Plantarum*, 53 (4), 657–662. doi: 10.1007/s10535-009-0119-7
- [7] Takehisa, H., Shimodate, T., Fukuta, Y., Ueda, T., Yano, M., Yamaya, T. et. al (2004). Identification of quantitative trait loci for plant growth of rice in paddy field flooded with salt water. *Field Crops Research*, 89 (1), 85–95. doi: 10.1016/j.fcr.2004.01.026
- [8] Platten, J. D., Egdane, J. A., Ismail, A. M. (2013). Salinity tolerance, Na⁺ exclusion and allele mining of HKT1;5 in *Oryza sativa* and *O. glaberrima*: many sources, many genes, one mechanism? *BMC Plant Biology*, 13 (1), 32. doi: 10.1186/1471-2229-13-32
- [9] Kostylev, P. I. (2011). Methods of breeding, seed production and varietal rice farming techniques. Rostov-on-Don: JSC “Book”, 288.
- [10] Thomson, M. J., Ocampo, M., Egdane, J., Rahman, M. A., Sajise, A. G., Adorada, D. L. et. al (2010). Characterizing the Saltol Quantitative Trait Locus for Salinity Tolerance in Rice. *Rice*, 3 (2-3), 148–160. doi: 10.1007/s12284-010-9053-8
- [11] Kudashkina, E. B., Kostylev, P. I. (2015). The study of salt tolerance of rice laboratory methods. The role of botanic gardens in the conservation and monitoring of biodiversity. Rostov-on-Don: Publishing House of the SFU, 401–404.
- [12] Kudashkina, E. B., Kostylev, P. I. (2015). Screening of rice accessions to salt tolerance. Contribution Vavilov society of geneticists and breeders in the innovative development of the Russian Federation. Cuban SAU, 70–72.

- [13] Usatov, A. V., Alabushev, A. V., Kostylev, P. I., Azarin, K. V., Makarenko, M. S., Usatova, O. A. (2015). Introgression the SalTol QTL into the Elite Rice Variety of Russia by Marker-Assisted Selection. *American Journal of Agricultural and Biological Sciences*, 10 (4), 165–169. doi: 10.3844/ajabssp.2015.165.169
- [14] Azarin, K. V., Alabushev, A. V., Usatov, A. V., Kostylev, P. I., Kolokolova, N. S., Usatova, O. A. (2016). Effects of Salt Stress on Ion Balance at Vegetative Stage in Rice (*Oryza sativa* L.). *OnLine Journal of Biological Sciences*, 16 (1), 76–81. doi: 10.3844/ojbsci.2016.76.81
- [15] Jeung, J. U., Hwang, H. G., Moon, H. P., Jena, K. K. (2005). Fingerprinting temperate japonica and tropical indica rice genotypes by comparative analysis of DNA markers. *Euphytica*, 146 (3), 239–251. doi: 10.1007/s10681-005-9022-2
- [16] Lang, N. T., Buu, B. C., Ismail, A. M. (2008). Molecular mapping and marker assisted selection for salt tolerance in rice (*Oryza sativa* L.). *Omonrice*, 16, 50–56.
- [17] Elahi, C. M. F., Seraj, Z. I., Rasul, N. M. et. al; Islam, A. S. (Ed.) (2004). “Breeding rice for salinity tolerance using the Pokkali allele: finding a linked marker,” in *In Vitro Culture, Transformation and Molecular Markers for Crop Improvement*. Science Publisher, NH, USA, 157–169.
- [18] Mackill, D. J. (2007). Molecular markers and marker-assisted selection in rice. Vol. 2: *Genomics Applications in Crops*. Springer, New York, NY, USA, 147–168. doi: 10.1007/978-1-4020-6297-1_7
- [19] Linh, L. H., Linh, T. H., Xuan, T. D., Ham, L. H., Ismail, A. M., Khanh, T. D. (2012). Molecular Breeding to Improve Salt Tolerance of Rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *International Journal of Plant Genomics*, 2012, 1–9. doi: 10.1155/2012/949038
- [20] Kostylev, P. I., Red'kin, A. A., Krasnova, E. V., Usatov, A. V., Makarenko, M. S. (2015). Effect of salt tolerance genes on quantitative characters of rice under the Rostov region. *Actual problems of biology, nanotechnology and medicine*. Rostov-on-Don, 92–94.