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Introgression the *SalTol* QTL into the Elite Rice Variety of Russia by Marker-Assisted Selection

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Abstract: The present work is devoted to investigation of the possibility to apply methods of Marker-Assisted Selection (MAS) to introgression the *SalTol* QTL into genotypes of elite Russian varieties of rice. It was shown that microsatellite markers Rm493, may be effectively used to control transfer the *SalTol* QTL genes into Russian populations of rice. Based on the highly productive variety "Novator", we obtained the lines Nov-129 and Nov-148 carrying loci *SalTol* in homozygous state. The lines Nov-129 and Nov-148 are used as an improved salt tolerance donor source to obtain hybrids tolerance to salinity.

Keywords: *SalTol*, DNA-Markers, Marker-Assisted Selection, Rice

Introduction

At the present time, new technologies that allow to reduce the period of selection process and to increase reliability of breeding material analysis is particularly important for breeding crops. One of these approaches is the technology of molecular markers (Hospital, 2009; Kumar *et al*., 2015). DNA marker analyses provide several significant advantages in comparison with analysis of morphological and physiological traits (Collard *et al*., 2008; Usatov *et al*., 2014a). Almost any gene or locus may be marked, suggesting the existence of a number of such specific markers (Hospital, 2009; Platten *et al*., 2013). In particular, DNA markers may be successfully used in breeding of rice with respect to its tolerance to salinity (Ashraf *et al*., 2012).

Soil salinity is one of the major problems in all over the World. Presently, a major QTL *SalTol* associated with the Na-K ratio in rice and seedling-stage salinity tolerance, are marked on chromosome 1 (Platten *et al*., 2013). Nevertheless, allelic variations of microsatellite loci associated with specific trait in related groups of genotypes do not always correlate with that trait in genotypes other origins (Senadheera *et al*., 2009; Mardani *et al*., 2014). This circumstance can lead to erroneous results when analyzing the presence of the recipient alleles in hybrid population (Ashraf *et al*., 2012; Mardani *et al*., 2014). In this connection, the study of the informational value of SSR markers associated with *SalTol* QTL for introgression the *SalTol* region into genotype of the elite Russian variety of rice was conducted.

Materials and Methods

Plant Materials

The highly productive elite variety "Novator", which was obtained from the I. G. Kalinenko All-Russian Institute for Crop Cultures (VNIIZK) (Russia) was used as the recipient. Line IR61920-3B-22-2-1 (NSIC Rc106) was used as a donor *SalTol* region.

Genotyping

SalTol region was identified during the selection process by closely linked microsatellite markers RM8094, RM493 (http://gramene.com).

To perform the molecular genetic analysis, genomic DNA was isolated from leaf tissue as described in (Boom *et al*., 1990), with our modification (Markin *et al*., 2015). Polymerase chain reaction was carried out in 25 µL reaction mixture of the following composition: 67 mm Tris-HCl buffer, pH 8.8, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgSO_4$, 0.1 mM mercaptoethanol, 0.25 mM of each dNTP (dATP, dCTP, dTTP and dGTP), 20 pM primers, 2.5 units of Taq-polymerase and 15 ng isolated DNA. Amplification was performed in the thermo cycler Palm Cycler (Corbett Research, Australia). Thermal regime of the reaction was

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chosen individually for each pair of primers on the basis of their sequences. For majority of reactions the optimal thermal regime was as follows: (1) denaturation at 94°C for 5 min, (2) 35 cycles at the following thermal and time regime: primer annealing at 55-60°C for 30 s, 30 s elongation at 72°C, denaturation at 94°C, 30 s, (3) 8 min final elongation at 72°C (Usatov *et al*., 2014b). Amplification products were analyzed by electrophoresis in 2% agarose gel supplemented with ethidium bromide in Tris-Borate buffer. The obtained gels were photographed with the Gel-Documenting system (GelDoc 2000, BioRad, United States). Gene Ruler 1 Kb DNA Ladder (Fermentas, Lithuania) was used as a molecular weight marker.

Evaluation of Salinity Tolerance

In a laboratory experiment, rice seeds were soaked in water for 12 hrs, then placed in special trays and saline added in concentrations of 1% NaCl (Thomson *et al*., 2010; Xiong and Choong, 2014). Control seeds were grown in distilled water. Seedlings were grown in a growth chamber (KBWF E5.2; Binder, GmbH) under the following controlled environmental conditions: 70% relative humidity, 26±2 C and a 14 h photoperiod. After 14 days, root length, shoot length and total germination percentage were measured (Ali *et al*., 2014; Kumar and Kumar, 2014).

Determining Concentrations of Sodium and Potassium

The Na+ and K+ content in shoots and roots were measured using a capillary electrophoresis system (Capel-105M; Lumex, Russia) according to the method of Platten *et al*. (2013).

Dried samples of plant material were treated with 10 mL deionized water at 100°C for 1 h and the extract used to determine the contents of free inorganic ions.

Statistical Analysis

The collected data were subjected to Analysis Of Variance (ANOVA). All data were represented by an average of the ten biological replicates and the Standard Deviations (SD).

Results

Parental SSR Polymorphism Screening

In this study, 21 SSR markers associated with the *SalTol*/QTL region were checked with two parent's varieties in order to find out polymorphic primers to further use for screening the *Saltol* loci of the crossing populations. The molecular analysis of rice samples by 2 microsatellite markers of the *SalTol* loci showed that only Rm493 provided reliable well-reproducible spectra and in formativeness for identification of the *SalTol* region in hybrid population.

Genotyping

We performed hybridization of the conventional variety Novator, subspecies *japonica* and sensitive to salt with the line NSIC Rc106, subspecies *indica*, which was used as a donor of the *SalTol* loci, in order to develop tolerant lines of rice on the basis of Russian varieties. First generation hybrids were used for obtainment of F_2 population.

Out of the F_2 generation plants by the traits of earliness, non-shattering of seeds and exaristate were selected 90 rice plants. Selected plants were analyzed by PCR for the presence of introduced alleles *SalTol*. Data of electrophoretic analysis of PCR product of the Rm493 marker, which is linked to the *SalTol*, shown on Fig. 1.

Donor tolerance allele of the parental line NSIC Rc106, which is marked on the Fig. 1 as 2.2, was found in homozygous state in two samples (129 and 148). Plants No. 62-64, 66, 67, 138-147, 149 and 150 carried both donor and maternal alleles, which were heterozygous by the *SalTol* locus. Other samples carried only the allele inherited from the variety "Novator" and thus, were rejected.

Evaluation of Salinity Tolerance

Evaluation of potential salt tolerance of the F_2 rice plants at seedling stage revealed significant variations of tolerance to salinity depending on the genotype (Table 1).

Sensitive to salt variety Novator was characterized by the highest decline seed germination –55, 6%. The line NSIC Rc106 and second generation plants which were homo-, heterozygous by locus *SalTol* showed the most stable by traits of germination (decrease germination less than 5%).

The lowest suppression of growth parameters was showen in the line NSIC Rc106 and homozygous by *SalTol* fragment of F_2 generation plants, while the largest decline roots length and shoots height under salt stress was found in the variety "Novator" and second generation plants that not inherited *SalTol* locus according to the data of molecular analysis (Table 1).

Potassium/Sodium Content Ratio

The K+/Na+ content ratio in shoot and root of the studied rice varieties is shown in the Table 2.

In general, it was found that by applying salinity, the K+/Na+ ratio in root was significantly ($p \leq 0.01$) reduced both in parental varieties and F_2 generation plants. On the other hand, the saline-susceptible variety Novator and F_2 generation plants without of the *SalTol* fragment had a K+/Na+ ratio in shoot significantly less than the line NSIC Rc106 and F_2 generation plants carrying the *SalTol* loci (tolerant).

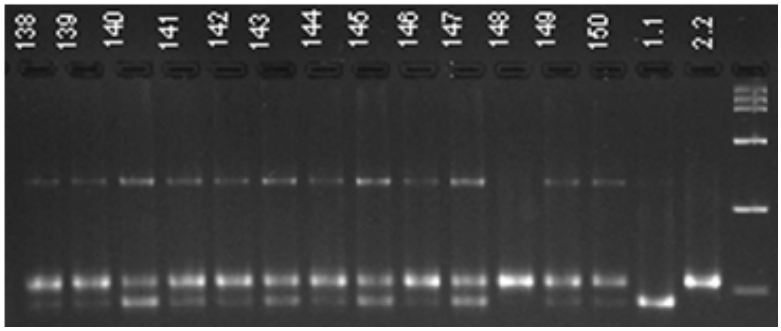

Fig. 1. The electrophoregram of amplification products of genomic DNA of rice with the primer Rm493. Molecular weight marker – 1 Kb

Table 1. The germination (%), shoot length (cm) and root length (cm) of 14-day-old seedlings of rice under control and salt stress conditions

	Germination (%)		Shoot length (cm)		Root length (cm)	
Samples	Control	1 % NaCl	Control	1 % NaCl	Control	1 % NaCl
Novator	99±1	52±2.5*	11.4±1.3	3.4±0.5*	9.3±1.0	2.2±0.7*
NSIC Rc106	87±1.5	85±1	13.6±1.6	7.2±1.1	11.5±1.7	7.7±1.0
129	85±2	85±1	14.3±1.5	7.5±0.9	10.1±0.8	6.7±0.9
148	90±3	85±2	14.9±1.5	8.1±1.2	10.5±0.5	6.3±.1
Hetero-zygotes	85±1	87±1.5	13.2±2.3	6.2±1.8	10.8±1.5	5.1±0.9
Homo-zygotes (without*SalTol*)	94±1.5	61±2*	12.2±1.2	4.6±0.6*	9.7±1.4	1.69±0.9*

*Indicate significant differences from control at p<0.001

Table 2. K+/Na+ ratio in shoots and roots of 14-day-old seedlings of rice under control and salt stress conditions

	Shoot		Root	
Samples	Control	1 % NaCl	Control	1 % NaCl
Novator	0.9±0.13	0.20±0.05*	0.46±0.05	0.21±0.07*
NSIC Rc106	1.77±0.17	1.62±0.13	1.60±0.23	0.19±0.02*
129	1.73±0.15	1.63±0.21	1.82±0.20	0.66±0.08
148	2.01±0.19	1.80±0.10	1.5±0.17	0.75±0.04*
Hetero-zygotes	1.57±0.13	1.45±0.16	1.71±0.15	0.69±0.09*
Homo-zygotes (without*SalTol*)	1.18±0.03	0.34±0.15*	0.39±0.03	0.18±0.11*

*Indicate significant differences from control at p<0.01

Discussion

The F_1 progeny obtained from the crossbreeding "Novator" × NSIC Rc106 may be use in a series of backcrosses, which provided introduction of donor resistance alleles into the genotype of the recurrent parental form (the variety Novator) (Wang *et al*., 2011). Presence of the transferred alleles should be assessed by the method of MAS as described above. In the present work we selected perspective lines, which resembled the parental recipient phenotype, from the F_2 population obtained by self-pollination.

PCR analysis of linked markers allowed us to identify F_2 plants that carried different allelic variants of the *SalTol* loci. Samples 129 (Novator × NSIC Rc106) and 148 (Novator × NSIC Rc106) were homozygous by *SalTol* loci. Moreover, we identified fifteen lines that carried *SalTol* loci in heterozygous.

Laboratory testing of the breeding material of rice showed that germination, root length and shoot length at seedling stage decreased under salt stress. The lowest inhibition of growth processes under salinity was observed in the line NSIC Rc106 which was used as donor of the *SalTol* loci and in the F_2 plants having been inherited *SalTol* locus according to the SSR analysis. Determining concentrations of sodium and potassium in root and shoot revealed shoot K+/Na+ homeostasis in these lines, that is the evidence of working *Saltol* fragment. The data presented here is similar to the results of other studies (Mardani *et al*., 2014; Negrão *et al*., 2011).

Newly developed lines 129 (Novator ×NSIC Rc106) and 148 (Novator ×NSIC Rc106), carrying *SalTol* loci in

homozygous state, are used as the improved donor source of salt tolerance in order to obtain heterosis hybrids tolerant to the salinity.

It was previously shown that the salt tolerance of rice is the genetics of quantitative traits, which is controlled by multiple genes (Negrão *et al*., 2011; Mekawy *et al*., 2011). It had mapped more than 70 QTLs which were linked to the salt tolerance and two salt tolerance genes of rice (SKC1 and DST) have already been cloned (Hu *et al*., 2012; Cotsaftis *et al*., 2012; Kumar *et al*., 2015). Introduction of the tolerance QTLs into the elite varieties adapted to certain agro-climatic conditions, as well as pyramiding of several tolerance QTLs in one genotype are considered to be the most perspective ways of selection of varieties tolerant to the salt stress (Ashraf *et al*., 2012; Das *et al*., 2015; Hoang *et al*., 2015).

Marker Assisted Selection (MAS) as a high technology tool is presently increasingly used for real selection programs (Collard *et al*., 2008; Markin *et al*., 2015). Application of MAS provides clear understanding of the targeted gene inheritance from parental lines (Kumar *et al*., 2015). Therefore, it may be effectively used to correct the breeding of plants in order to obtain of new varieties less cost and time consuming (Wang *et al*., 2011; Kumar *et al*., 2015).

Nevertheless, only integration of molecular genotyping data with classical complex analysis of morpho-physiological traits of plants both in laboratory model experiments and in field are required to solve the problems of genetic marking of agronomic characters of crops.

Conclusion

The present study aimed at the application of marker assisted selection to introgression of the salinity tolerance QTLs *SalTol* into the genotypes of elite Russian varieties of rice. It was shown that Rm493 microsatellite markers may be effectively used to control the transfer of the *SalTol* fragment into the Russian populations of rice. Individual selection by morphological traits and in combination with MAS allowed us to simplify the selection scheme and obtain samples, carrying *SalTol* locus in homozygous condition. These samples were characterized by a complex of traits that corresponded to the agro-climatic conditions for rice breeding in Russia. This selection material was used as the improved source of tolerance to salinity in order to obtain heterosis hybrids tolerance to salt stress.

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Author's Contributions

All the six authors participated in the laboratory study, data analysis and the entire process of the article preparation.

Ethics

The authors state that this article conforms to the ethical standards specified by the American Journal of Agricultural and Biological Sciences.

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