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Short Communication

Identification of genomic regions for salinity tolerance at germination stage of rice using doubled haploid mapping population

Umakanta Ngangkham[1], Sonali Nanda, Jawahar Lal Katara and Sanghamitra Samantaray*

ICAR-National Rice Research Institute, Cuttack 795 006, Odisha

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Abstract

Seed germination is the most sensitive stage of rice for salinity stress. To map the genomic regions, a set of 117 doubled haploid (DH) lines derived from the F_1s of salinity tolerant Pokkali and susceptible Savitri were used. Preliminary screening at 0, 4, 8, 12, 16 and 20 dsm^{-1} of NaCl solution, Pokkali and Savitri showed significant variation of 78 % and 100 %, respectively for seed germination. Based on this study, 117 DH lines were evaluated at 16 dsm^{-1} NaCl, which exhibited variation in germination percentage, shoot length and root length. Bulked segregant analysis (BSA) was conducted by taking ten each extreme DH lines with 79 polymorphic SSR markers from 12 chromosomes to identify the genomic regions. Three markers, RM247, RM324 and RM283 were identified and found to be linked to four candidate genes, LOC_Os12g06560, LOC_Os12g06570, LOC_Os01g09550 and LOC_Os01g09560. Further research work is suggested to understand the tolerant mechanism of salinity at germination stage in rice.

Key words: Rice, bulked segregant analysis, doubled haploid, salinity, germination

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world nourishing more than 50% of the world population (Ngangkham et al. 2018). However, there are a number of challenges to maintain global rice production to strengthen the vulnerable populations to adapt to the effects of climate change. Among them, salinity is the major crop production limiting factor globally which affects land cultivation, plant physiological processes and yield (Kumar et al 2008). Generally, salinity stress affects germination, seedling stage and plant growth through ionic imbalance, oxidative compounds, ion-specific effect, osmotic stress and reactive oxygen species that disrupts plant ion homeostasis and water potential, resulting in metabolic damage, growth arrest and even death (Pandit et al. 2011; Bor et al. 2003). Therefore, identification of tolerant genotypes and their molecular mechanism towards salinity tolerance is of paramount importance to understand the problems of salinity stress. It may also be very useful to understand physio-biochemical attributes which will be utilized in breeding rice with high yield for salt affected cultivated area including coastal areas. Therefore, the present study was aimed at the identification of the genomic regions associated with salinity tolerance during germination stage.

A salt tolerant genotype, Pokkali was crossed with salt susceptible line, Savitri (suitable for shallow lowland ecosystem). The F_1 plants were used to develop a set of 117 DH mapping population through anther culture following androgenic protocol (Naik et al. 2016; Rout et al. 2016) for identification of the genomic regions of the salt tolerance during germination stage using BSA approach.

Salt treatment and germination of rice grains

Healthy and uniform seeds of Pokkali and Savitri were selected for salt treatments. Seeds were surface sterilized with 1.0% sodium hypochlorite aqueous solution for 5 minutes to prevent the fungal growth followed by washing four times with autoclaved distilled

*Corresponding author's e-mail: smitraray@gmail.com
[1]Present address: ICAR Research Complex for NEH Region, Umiam, Meghalaya 793 103
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water. The seeds were allowed to germinate under dark condition on a filter paper moistened with different concentrations of salt solution (NaCl): 0 (sterile distilled water as control), 4, 8, 12, 16 and 20 dsm^{-1} salt solutions with five replications. The germination percentages were recorded on the 7^{th} days after incubation by counting the number of germinated seeds divided by total number of seeds sown and converted the value into percentage. The average shoot and root length measured in millimetre (mm) from randomly selected ten seeds from each treatments.

The results showed that seed germination was significantly affected at 16 and 20 dsm^{-1} salt solutions which is corresponded to 160mM and 200 mM NaCl concentrations, respectively. The germination percentage of Savitri was 78±11.525% and 14.25±4.349% at 16 and 20 dsm^{-1} salt solutions, respectively. On the other hand, germination percentage of Pokkali was recorded 100% at 16 dsm^{-1} and 58.75±6.292% at 20 dsm^{-1} NaCl. Moreover, there was significant reduction of seed germination rate in Savitri with 22.0% and 75.74 % at 16 and 20 dsm^{-1} salt solutions, respectively in comparison to Pokkali. In case of the shoot length, Pokkali produced longer shoot length with an average of 0.98 mm in all the treatments as compared to the Savitri. Similarly, the root length of germinated Pokkali was more in all the treatments with an average of 1.77 mm as compared to Savitri. These results indicated that the Pokkali produced more shoot and root biomass even in higher concentration of NaCl solution.

Localization of genomic regions responsible for salinity tolerance through BSA

All 117 DH lines along with the parental lines were screened for rice seed germination ability in 16 dsm-1 NaCl solutions as per the description above. The germination percentage showed continuous variation with normal distribution whereas shoot and root length showed skewed distribution. The mean germination percentage of 117 DH lines was 53.29 % with a range of 3.3 to 96%. The linear correlation coefficient (r) analyzed showed significant positive correlation (p = 0.01) among the above three parameters. In order to identify the genomic region(s) responsible for germination percentage in salinity condition, ten each extreme DH lines with germination percentage of <20% were considered for susceptible bulk and >80% tolerant lines were selected for bulk and used to conduct BSA using polymorphic SSR markers.

Genomic DNA was isolated from young leaf tissues using the cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). PCR amplification was performed in a 10 ml reaction mixture containing 1X Taq buffer (10 mMTris-HCl, 50 mMKCl, pH 8.3), 0.2 mM of each forward and reverse primers, 1.5 mM $MgCl_2$, 0.2 mM of each of dNTP, 20 ng template DNA, and 1U of Taq DNA polymerase (Dream Taq, Thermo Scientific, USA). The PCR programme was set up as follows: 94°C of 4 min for initial denaturation followed by 35 cycles of 94°C for 30 sec, primers annealing for 30 sec at 55°C and elongation for 1 min

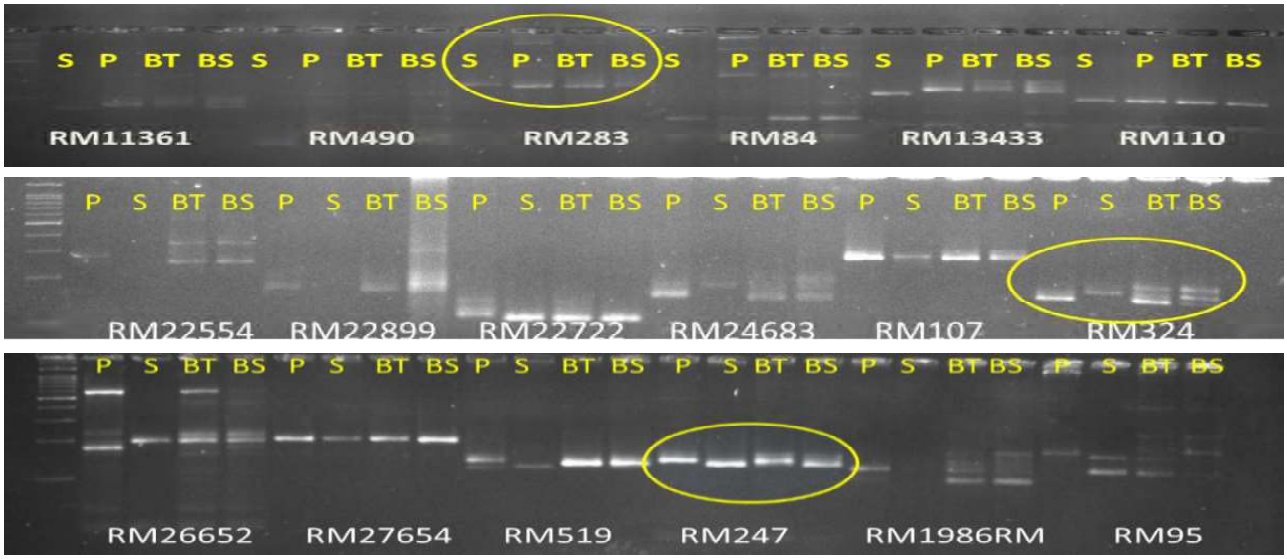

Fig. 1. Gel electrophoresis of BSA. P, S, BT and BS denoted genomic DNA of Pokkali, Savitri, tolerant bulk and susceptible bulk. Circle indicates the genetic association of markers with the traits of interest

at 72°C, followed by a final elongation at 72°C for 10 min. The PCR products were separated 3.5% Metaphor agarose gels (Lonza, USA) stained with ethidium bromide and documented using a gel documentation system (AlphaImager, USA). Out of the 600 SSR markers, 79 SSR (13.16%) distributing on 12 chromosomes were found to be polymorphic between Savitri and Pokkali. Out of the 79 polymorphic markers, only three markers were found to be linked/ associated with the salinity tolerance.

Three markers, RM247, RM324 and RM283 (Fig. 1) were used to study salinity stress. The RM247 from the chromosome 12 with the physical position of 3.18 Mb was mapped between the two genes i.e., LOC_Os12g06560 encoding putative protein and LOC_Os12g06570, a gene encoding cyclic nucleotide-gated ion channel. Such genes are involved in ion transport across the cell membrane. The RM324 from chromosome 2 at physical position of 11.38 Mb was localized within the retrotransposon genes. The marker, RM283 is localized on chromosome 1 with physical position of 4.88 Mb in between the two protein coding genes i.e., LOC_Os01g09550 encoding no apical meristem protein and LOC_Os01g09560 encoding mitochondrial-processing peptidase subunit alpha and mitochondrial precursor. The locus LOC_Os01g09550, known as *SNAC3*is, a stress responsive gene was recently identified and characterised as a responsive gene for drought and salinity tolerance in rice (Fang et al 2015). In transgenic rice, differential level of expression of *SNAC3* was strongly related to tolerance and sensitive to high temperature, drought and oxidative stress. The *SNAC3* gene had also induced a large number of ROS-scavenging gene transcription thereby conferring tolerant to abiotic stresses (Fang et al. 2015). The another flanking gene, LOC_Os01g09560 encoding mitochondrial-processing peptidase subunit alpha also equally important as per the reports of significant change in abundance of several mitochondrial proteins under salinity treatment in *Lupine* (Wojtyla et al 2013). The genes identified in the present study require further functional characterization and validation to understand the tolerant mechanism of salinity at germination stage in Pokkali genotype.

Authors' contribution

Conceptualization of research (UN, SS); Designing of the experiments (UN, SS); Contribution of experimental materials (SS, JK); Execution of field/lab experiments and data collection (SS, JK); Analysis of data and interpretation (UN, SN, SS); Preparation of manuscript (UN, SN, SS).

Declaration

The authors declare no conflict of interest.

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