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Identification of Rice Accessions Associated with K^+/Na^+ Ratio and Salt Tolerance Based on Physiological and Molecular Responses

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Abstract: The key for rice plant survival under NaCl salt stress is maintaining a high K^+/Na^+ ratio in its cells. Selection for salt tolerance rice genotypes based on phenotypic performance alone will delay in progress in breeding. Use of molecular markers in tandem with physiological studies will help in better identification of salt tolerant rice accessions. Eight rice accessions along with the check Dongjin were screened using 1/2 Yoshida solution with 50 mmol/L NaCl at the seedling stage. The accessions IT001158, IT246674, IT260533 and IT291341 were classified as salt tolerant based on their K^+/Na^+ ratios. Seventeen SSR markers reported to be associated with K^+/Na^+ ratio were used to screen the accessions. Five SSR markers (RM8053, RM345, RM318, RM253 and RM7075) could differentiate accessions classified based on their K^+/Na^+ ratios. Banding pattern of the accessions was scored compared to the banding pattern of Dongjin. The study differentiated accessions based on their association of K^+/Na^+ ratio with molecular markers which are very reliable. These markers can play a significant role in screening large set of rice germplasms for salt tolerance and also help in identification of high-yielding varieties with better salt tolerance. The salt tolerant accessions can be taken forward into developing better varieties by conventional breeding and exploring genes for salt tolerance.

Key words: rice; salinity; K^+/Na^+ ratio; simple sequence repeat; salt tolerance

Rice (*Oryza sativa* L.), one of the most important crops globally, is the main cereal crop in Asian countries, which represents 50%–80% of people's daily calorie intake (Khush, 2005). Salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious limitation to increase rice production worldwide (Gregorio, 1997). There is tremendous variation for salt tolerance within the species of rice (Sabouri and Biabani, 2009), which needs to be explored. The response of rice to salinity varies with growth stage. Previous studies have indicated that rice is sensitive to salt stress during seedling and reproductive stages (Zeng et al, 2001). Screening of germplasm at the seedling stage is readily acceptable as it provides a rapid screening that is difficult at the vegetative and reproductive stages (Gregorio et al, 1997). Rice responds to salt stress using a number of strategies that include Na^+ exclusion, K^+ mining ability, high K^+/Na^+ ratio, tissue tolerance and higher growth vigour (Blumwald, 2000). There is a positive relationship between high K^+/Na^+ ratio and salinity tolerance (Gregorio and Senadhira, 1993), making it best indicator of growth and yield under salt stress (Gill and Singh, 1995). The ability of crops to maintain low cytosolic Na^+ in leaves is regarded as one of the key determinant factors for salt tolerance (Munns and Tester, 2008). Phenotyping for salt tolerance studies has been previously reported in rice (Kanawapee et al, 2012; Pires et al, 2015; Sakina et al, 2016).

Complex traits involved in salinity tolerance have been a hurdle for conventional breeding to make significant progress and have led to increased interest in molecular breeding methods (Gregorio et al, 2002; Thomson et al, 2010). Searching for DNA markers tightly linked to traits related to salt tolerance has become a major objective in most breeding programs and it

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is assumed that molecular markers will facilitate a fast and cost-effective screening of large populations (Munns and James, 2003). Simple sequence repeats (SSRs) are the genetic markers that have been widely used in rice diversity studies (Oliveria et al, 2006; Bhowmik et al, 2009; Soubir et al, 2009; Senguttuvel et al, 2010).

Pires et al (2015) suggested that in order to have a complete understanding of salinity tolerance in plants, we should focus on studying each tolerance mechanism independently by selecting appropriate species or specific germplasms that best exemplify a specific mechanism. The aim of the present study was to screen rice accessions for salinity response and to evaluate SSR markers for the identification of salt tolerant genotypes at the seedling stage based on their K^+/Na^+ ratio. Thus, the identification of these systems is of great use in understanding inter-cultivar differences and will serve as a source for molecular handles to improve rice salt tolerance via molecular breeding.

MATERIALS AND METHODS

Rice materials

Eight rice germplasm accessions along with one salt susceptible variety Dongjin used as a check were obtained from the National Agro-Biodiversity Center, Rural Development Administration (RDA), the Republic of Korea (Table 1). These accessions were selected based on Reddy et al (2017).

Phenotypic study of salinity tolerance

All the seeds were sterilized before proceeding to germination. Seeds were allowed to germinate on wet paper in petri dishes for 4 d. The germinated seeds were transferred into plastic tray filled with 1/2 strength Yoshida's solution (Yoshida et al, 1976) and placed in a growth chamber for 6 d. Seedlings with 3 or 4 foliar leaves were selected and transferred to a growth box with 1/2 strength Yoshida's solution for 4 d, and then were imposed with NaCl stress (50 mmol/L) for further 10 d. Salinized and non-salinized setups with three replications were maintained. At 10 d after salt treatment, individual plants were sampled, and upper and lower (roots) parts were separated. The lower parts were dried in an oven at 80 ºC for 48 h prior to measuring dry weight. The upper parts were put in a cylinder half-filled with distilled water at 28 ºC for 12 h to make a turgid status, after which they were frozen in liquid nitrogen and stored at -80 ºC. The frozen parts were thawed at room temperature for 30 min, and the thawed tissues were centrifuged with relative centrifugal force at 12 000 × *g* for 20 min at 4 ºC in a tailor-maid tube with 0.22 μm pore size filter, which allows the expressed sap to go through. After sap extraction, the sample was dried in an oven at 80 ºC for 48 h prior to measuring dry weight of the upper part. The expressed saps were used to determine ion concentration. Ion (K^+ and Na^+) concentrations of expressed saps were determined using Metrohm 882 Compact IC Plus, USA. Prior to analysis of 5 μL cell sap, samples were diluted in 5 mL distilled water (1:1000 dilution), and the diluted samples were injected into the IC system. XLSTAT and QI Macros softwares were used for statistical analysis of the data (Reddy et al, 2017).

Table 1. List of accessions used in this study.

Accession	Origin	Type	Sub-population
IT001158	India	Introduced cultivar	Indica
IT246674	Bangladesh	Introduced cultivar	Not available
IT260533	India	Not available	Indica
IT291341	Iran	Introduced cultivar	Indica
IT168699	South Korea	Weedy	Japonica
IT175218	South Korea	Weedy	Japonica
IT219993	China	Not available	Not available
IT227934	South Korea	Landrace	Japonica
Dongjin	South Korea	Breeding line	Japonica

Molecular analysis

DNA isolation was done from leaf tissues of 10-day-old seedlings. DNA was extracted using Inclone Genomic Plus DNA Prep Kit (Inclone, Yongin, Korea) as per the kit instructions. Totally, 17 SSR markers (Table 2) were selected from previously reported studies for salt tolerance with respect to K^+/Na^+ ratio (Koyama et al, 2001; Yao et al, 2005; Bhowmik et al, 2009; Senguttuvel et al, 2010). Each PCR was carried out with 10.0 μL reaction mixtures containing 1.0 μL of 10× buffer, 0.9 μL dNTPs, 1.0 μL forward primer, 1.0 μL reverse primer, 0.1 μL *Taq* polymerase, 5.0 μL ddH_2O and 1.0 μL (20 ng) of each template DNA. PCR profile was maintained as initial denaturation at 94 ºC for 5 min, followed by 36 cycles of denaturation at 94 ºC for 1 min, annealing for 1 min (specific for each primer) and extension at 72 ºC for 90 s, and final extension at 72 ºC for 10 min. The PCR products were run on 3% agarose gels. Banding patterns of the accessions were scored comparing with that of Dongjin.

RESULTS

Screening of genotypes based on their ion concentration in plant tissues

There were significant differences in the Na^+ and K^+ concentrations among accessions under NaCl stress (50 mmol/L NaCl), however, no significance for the control condition was found. K^+/Na^+ ratio under control condition is ignorable. K^+/Na^+ ratio at 50 mmol/L NaCl was significantly different among the eight rice accessions. Four accessions IT001158, IT246674, IT260533 and IT291341 exhibited higher K^+/Na^+ ratio whereas accessions IT168699, IT175218, IT219993 and IT227934 exhibited lower K^+/Na^+ ratio compared to the check Dongjin. The analysis of variance showed that K^+/Na^+ ratio of accessions was highly significant ($P \leq 0.05$) compared with Dongjin except for the accession IT219993 (Fig. 1). The accession IT260533 exhibited the highest K^+/Na^+ ratio among all the accessions while the