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STUDY ON APPROACHES TO IMPROVE SALT TOLERANT IN RICE

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Abstract: The availability of whole genome sequence of rice has contributed to the rapid development in the area of functional genomics of salt tolerance in rice, which has paved a way for identifying new genes and pathways. Transgenic and breeding crop varieties with in-built salt tolerance are the most economical, less resource consuming and socially acceptable approach. Extensive germplasm collection provides a useful source of genetic diversity for the studied traits.

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Introduction:

Choice of germplasm is the most crucial for the success of breeding. Ideally germplasm should differ as much as possible for the traits to be improved or introgressed. Other traits should not vary too much, otherwise, keeping all the desirable traits into one superior agronomic background become very difficult. The best way would be to identify salt-tolerant germplasm lines, which can help transfer genes for tolerance into crop cultivars. The salt tolerance is a complex phenomenon because it may require the combination of different independent and/or interdependent mechanisms and pathways. A tolerant genotype can be expected to maintain lower Na$^+$ concentrations besides maintaining K$^+$ concentrations under high salt concentration, lower Na$^+$/K$^+$ rate (considered as desirable trait as it maintains the ion balance) and high biomass.

Reliable and repeatable screening techniques are the mainstay of any successful breeding programme, and they should be rapid, reproducible, easy and affordable. Conventional breeding is accelerated by the use of molecular markers linked to the tolerance trait being introgressed, which is known as MAS (marker-assisted selection). This allelic variation in the gene of interest can provide both a novel source of genetic material for MAS, and can also be used to design an easy, cheap molecular marker, thus providing a perfect marker for salt tolerance or, at least, for a trait contributing to salt tolerance. *Saltol*, a major QTL for salt tolerance, is being transferred into seven popular locally adapted rice varieties, namely, ADT45, CR1009, Gayatri, MTU1010, PR114, Pusa 44 and Sarjoo 52 (Singh et al., 2016). Tiwari et al (2016) identified a method for rapid identification of salt tolerance QTLs at reproductive stage in rice using bulked segregant analysis of bi-parental recombinant inbred lines (RIL). Rahman M A et al (2016) performed diversity analysis on 107 germplasm accessions using a genome-wide set of 376 single nucleotide polymorphism (SNP) markers, along with characterization of allelic diversity at the major QTL *Saltol*.

The results show that seven landraces (Akundi, Ashfal, Capsule, Chikirampatnai, Jatai Balam, Kalarata and Kutipatnai) accumulate less Na$^+$ and relatively more K$^+$, maintaining a lower Na$^+$/K$^+$ ratio in leaves. These tolerant genotypes have significantly lower shoot Na$^+$ concentration, and they effectively limit sodium transport to the shoots. These landraces will constitute alternative sources of salt tolerance genes in rice and could help in developing varieties combining functional alleles of several tolerance traits. Mutation breeding has a significant contribution toward production of high-yielding and salt tolerant rice varieties (Das et al., 2014). Deng et al (2015) isolated and characterized a salt-tolerant rice mutant *rst1*, which is tolerant to salinity and shows significantly higher shoot biomass and chlorophyll content, but lower lipid peroxidation and electrolyte leakage under NaCl stress. Microarray analysis of *rice d1* (*RGA1*) mutant revealed its role in regulating multiple abiotic stress such as drought, salinity, heat and cold (Jangam et al., 2016).

System biology

There is a need for different tools such as phenomics, transcriptomics, proteomics and metabolic study for understanding and dissecting out the mechanism of salt tolerance in rice, which can help in developing salt tolerant germplasms.

Phenomics is an area of biology concerned with the measurement of physical and biochemical traits of organisms in response to genetic mutation and environmental influences (wiki.ic4r.org/index.php/ Phenomics). Phenomic analysis is the key to functional genomics. A number of phenotyping platforms have been used in basic research, e.g. non- invasive methods that employ visible or fluorescence imaging. Some of the high-throughput phenotyping platforms used are visible red-green-blue imaging, chlorophyll fluorescence imaging, thermoimaging and hyperspectral imaging (Humplik et al., 2015). High- throughput platforms allow analysis of the plants on a higher scale. The platforms employ non-destructive methods and measurements like plant growth, morphology and physiology, which help to understand plant vigour etc. on a high-throughput scale repeatedly during plant life span, which enables users to directly link the phenotyping data obtained to the particular conditions. Hairmansis et al (2014) used image analysis to discriminate the different aspects of salinity (shoot ion independent stress and shoot ion dependent stress), which is a useful tool for genetic and physiological studies to elucidate processes that contribute to salt tolerance in rice.

Hairmansis et al (2014) used a non-destructive image-based phenotyping protocol to assess salt tolerance traits of two rice varieties (IR64 and Fatmawati), and the response of rice to salinity (50, 75 and 100 mmol/L NaCl) could be clearly distinguished from the control as indicated by the reduced increase of shoot area. The study of plant physiology can contribute to evaluate the function of target genes as well as germplasm for salt tolerance which will bridge gap between genotype and phenotype (Fig. 3). Sexcion et al (2009) observed that the morpho-physiological traits are associated with salinity tolerance in rice varieties and classified Pokkali, Cheriviroppu, FL478 and IR651 as salt tolerant varieties due to consistent expression of high vigour, low standard evaluation score, high shoot/root biomass, lower shoot Na^+ accumulation and lower shoot Na^+/K^+ ratio compared to sensitive genotypes. Dr. KWON (unpublished data) suggested that Pokkali has a higher water flux than IR29, especially in the night. Also, Pokkali has a higher ability of water uptake and transpiration than IR29.

Stomatal transpiration is the most dominant form of transpiration being responsible for most of the water loss in plants. Salt tolerance could be achieved through higher capacity of water uptake during night even when transpiration is low, indicating the ability of roots to uptake water is important. Plant phenomics has the potential for identifying the genetic basis of salt tolerance mechanisms and assist in pyramiding different tolerance mechanisms into breeding lines (Hairmansis et al., 2014).

Transcriptomics is examination

Transcriptomics is examination of whole transcriptome changes across a variety of biological conditions (Thompson and Goggin, 2006). Microarrays were used as the standard tools for the analysis of genome wide transcriptional response by studying the expression of all the expressed genes in a single experiment (Zhou et al., 2007). Salinity inducible transcripts in rice were identified by using microarray technology (Kumari et al., 2009). Illumina HiSeq 2000 platform was used to analyze the transcriptome profiles of the leaves and roots at the seedling stage under salinity compared with those under normal conditions in Dongxiang wild rice (Zhou et al., 2016). Pandit et al (2010) used differential transcriptome analysis to identify candidate genes for salt tolerance using genome-wide microarray using bulked segregant analysis.

Proteomics is the study of proteins, in particular their structures and functions. The study of a protein is the shortest and direct way to describe the role of the gene linked with the particular protein (Lee et al., 2009). Liu et al (2014) found the differentially expressed proteins in rice roots and leaves after salt treatment, and these proteins may be served as marker group of rice resistant to salt stress. Zhang et al (2009) reported OsRMC to be one of the 10 root-secreted proteins identified using 2-DE approach and highly responsive to salinity. Knocking down the expression level of *OsRMC* in transgenic rice led to insensitive seed germination, enhanced growth inhibition, and improved salt-stress tolerance than in non-transgenic plants. These results indicate that *OsRMC* negatively regulates the salt tolerance of rice plants. Proteome profiling also confirms higher expressions of SOD, peroxidase and plastidic cystathionine-β synthase and other proteins in the tolerant lines, whose genes are co-located in the QTL intervals for salt tolerance mapped in the RIL population (Mishra et al., 2016).

Metabolites are the final product of cellular reactions which reflect the reaction of biological systems to environmental fluctuations (Royuela et al., 2000). Mishra et al (2016) used salt-tolerant CSR27, salt-sensitive MI48 and their extreme tolerant and sensitive RIL progenies for the elucidation of salinity tolerance metabolic pathways. Differentially expressed antioxidant enzymes, amino acids and proteins in contrasting rice genotypes and co-location of their genes in the QTLs mapped using bi-parental population indicate their role in salt tolerance. Nam et al (2015) used discriminatory nuclear magnetic resonance based metabolomics to study salt sensitive and salt tolerant varieties, and found that the major factor for discriminating salt tolerant and salt sensitive varieties is the change of carbohydrate and aliphatic regions. Nam et al (2015) found a positive correlation of metabolite changes with growth potential and salt tolerance of rice genotypes. Dr. KWON (unpublished data) suggested that partial least squares discriminant analysis enabled the comparison of salt tolerance and sensitive rice varieties, though it is less apparent, probably due to the effect of genetic variations between rice varieties.

Genetic Approaches

Genomics approach involves identification of QTLs, molecular markers and high throughput genotyping. QTLs for salt tolerance have been identified in rice on chromosome 1, containing the major locus *Saltol* derived from Pokkali and *SKC1* (*OsHKT1;5*) from Nona Bokra. *Saltol* was reported to be involved in Na^+/K^+ homeostasis under salinity (Thomson et al., 2010, Platten et al., 2013). Both simple sequence repeat (SSR) and SNP marker analysis have been successfully used to discover salt tolerant varieties in rice (Dhar et al., 2011). Molla et al (2015) identified novel salt responsive candidate gene based SSRs from rice. Kurokawa et al (2016) reported a golden gate SNP array that targets specific genes controlling yield-related traits and biotic stress resistance in rice. A high-density rice SNP array with 51 478 markers has been developed on the Illumina Infinium platform (Chen H D et al., 2014). Tu et al (2014) suggested that genome duplication improves root resistance to salinity, and that enhanced proton transport to the root surface may play a role in reducing Na^+ entrance into the roots. He also found that Na^+ in tetraploid rice roots significantly decreases while root tip H^+ efflux in tetraploid rice significantly increases.

Transgenic approach

Transgenic approach uses recombinant DNA techniques to create plants with new and desirable characteristics. This approach has been successfully applied in producing rice salt tolerant plants by introducing new genes and testing against salinity. Transgenic plants overexpressing *OsPP1a* show enhanced tolerance to high salt treatment, and *SnRK1A*, *OsNAC5* and *OsNAC6* are up-regulated in these transgenic plants (Liao et al., 2016). Constitutive overexpression of the vacuolar Na^+/H^+ antiporter gene (*OsNHX1*) from the rice landrace (Pokkali) was used to enhance the level of salt tolerance in transgenic rice plants (Amin et al., 2016). Overexpression of *OsNHX1* in rice is also associated with altered shoot and root accumulations of Na^+ and K^+, improved biomass production and improved germination (Chen et al., 2007). Overexpression of *OsTPS1* associated with trehalose-6-phosphate synthase in rice is associated with increased compatible solute accumulation, improved plant survival, increased growth, reduced wilting and maintenance of photosynthetic efficiency (Li et al., 2011).

Ectopic expression of *PtCYP714A3* in rice leads to semi-dwarfed phenotype with promoted tillering and reduced seed size. Transgenic rice plants exhibit enhanced tolerance to salt and maintained more Na^+ in both shoot and root tissues under salinity stress. All these results not only suggest a crucial role of *PtCYP714A3* in shoot responses to salt toxicity in rice, but also provide a molecular basis for genetic engineering of salt-tolerant crops (Wang et al., 2016).

Li et al (2016) demonstrated that SIDP361 has high potential as a tool for genetically improving salt tolerance in rice. Sahoo et al (2014) reported the use of OsSUV3. Rice SUV3 functions as DNA and RNA helicase and provides salinity stress tolerance by maintaining photosynthesis and antioxidant machinery. Nath et al (2016) suggested that *PDH45* may be involved similar salt tolerance mechanism in rice transgenic plants in response to salinity stress, sustaining least Na^+ in shoot. Salinity induces the production of reactive oxygen species, which can be removed enzymatically, such as ascorbate peroxidase, catalase, SOD and glutathione reductase (Lin and Pu, 2010, Das et al., 2014).

Expression of *SOD* is significantly up-regulated by high-salt induction in *OsPP1a* inserted transgenic plants (Liao et al., 2016). Development of stress tolerant rice varieties with high yield is one of the mandates of the International Rice Research Institute and the national rice research programs in Asia. Care must therefore be taken in the choice of promoter used to drive the expression of salt tolerance transgenes, so as not to disrupt or negatively affect the plant phenotype.

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