Mechanisms of salinity tolerance in rice (*Oryza sativa* L.): A study on salt responsive physiology, transcriptomics and genetics

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ABSTRACT

Rice (*Oryza sativa* L.) is the second most important cereal crop in the world but its production suffers from saline environments in many areas since it is one of the most salt sensitive crops. However, the large variation in tolerance between rice cultivars can be exploited to gain insights into mechanisms of salinity tolerance. A multifaceted approach encompassing physiology, biochemistry, transcriptomics and genetics was followed to study the mechanisms of salt tolerance in indica rice varieties at the seedling stage. Salinity tolerance in rice, like in other glycophytes, is a function of cellular ion homeostasis. The large divergence in ion homeostasis between the salt tolerant and salt sensitive rice varieties indicate that salt tolerant variety shows lower Na^+ influx, reduced Na^+ translocation to the shoot, and maintains a lower Na^+:K^+ ratio due to a higher membrane stability index (MSI) that effectively exclude the Na^+. As such, tolerant cultivars showed relatively unaffected growth supplemented with higher rate of productivity and better control on reactive oxygen species (ROS) under salinity stress. Lower TBARS (thiobarbituric acid reactive substance) content in salt tolerant FL478 roots than its sensitive counterpart IR29 under stress indicated that its cellular membrane was relatively unaffected by ROS despite high H_2O_2 content recorded under salt stress. Comparatively higher SOD activity along with a parallel increase in transcript level of superoxide dismutase (Os07g46990) in FL478 indicates that this protein may make a vital contribution to salt stress tolerance. Although, the content of ascorbic acid remained unchanged in FL478, the activity of the ascorbic peroxidises (APOXs) was reduced comparably in both cultivars. There were several transcripts of peroxidase family that showed salt induction (Os03g25300, Os03g55410, Os04g55740, Os05g04490, Os05g06970, Os07g01410 and Os07g48020) and may be important in the rice antioxidant response. Genome-wide root transcriptomic analysis revealed several genes encoding membrane transporter proteins which showed differential regulation in tolerant (FL478) and sensitive (IR29) cultivars in response to salinity stress. Genes encoding aquaporins and N transporters are induced in both cultivars. Genes encoding monovalent ion channels (*OsTPC1, OsAKT1, OsCNGC*), monovalent ion transporters (*OsHAK7*), antiporters (*OsCHX11*), Si influx transpoters (*OsLsi1*) and several metal and sugar transporters showed differential regulation between the cultivars. Heterologous expression data indicated that rice cDNA encodes for *PROLM* (Os06g31070), a prolamin precursor, can rescue salt sensitive yeast phenotypes (G19 and Axt3K) from high salt stress. Comparative study of *in planta* partitioning of Na^+ ions showed that salt tolerant mechanism adopted by Nonabokra and Bw400 are significantly deviated from that of pokkali which relies on minimizing the apoplastic Na^+ content in leaf. Three types of salt tolerant mechanisms in rice P-type (of Pokkali), N-type (of Nonabokra) and B-type (of Bw400) were identifiable in this study. Besides, Bw400 also showed a positive cytoplasmic effect on salt tolerance. Significantly large hybrid vigour of the F1 plants derived from Bw400 and Pokkali suggests that these parents have high prospect in rice breeding programmes for salt tolerance. Estimation of genetic indices of salt associated Na^+ and K^+ homeostasis in F1 plants indicated that Na^+ and K^+ homeostasis in rice was controlled by different proportions of additive and dominance effects. Rice genome bears a considerable heterosis effect that can be harnessed in development of salt tolerant hybrids or varieties. On whole, this study revealed several physiological processes of salt tolerance and their underlying genomic and genetic information which are of crucial importance in future rice improvement programmes for salt tolerance.