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# Differentially regulated kinases and phosphatases in roots may contribute to inter-cultivar difference in rice salinity tolerance

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**Rice 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 divergence in tolerance between rice cultivars can be exploited to gain insights into mechanisms of salinity tolerance, for example by carrying out comparative transcriptomics studies. We recently showed that specific transporters in roots of the tolerant rice cultivar FL478 are differentially regulated compared to their counterparts in the more sensitive IR29 cultivar and that this may contribute to the observed lower $Na^+$ influx, reduced $Na^+$ translocation to the shoot, and lower $Na^+$:$K^+$ ratio observed in FL478. In this addendum we further evaluated some of the regulatory genes that are potentially important in the modulation of membrane transporters involved in rice cation homeostasis.**

**Key words:** phosphorylation, rice, transciptomics

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## Introduction

Soil salinity negatively impacts on agricultural production worldwide. Salinity exerts its adverse impact in many ways but predominantly suppresses growth through osmotic stress, ion toxicity in the form of excessive $Na^+$ and $Cl^-$ accumulation, and negative effects on nutrition, particularly of $K^+$ and $Ca^{2+}$.[1]

Plants have evolved many defense mechanisms against salt stress which in salt sensitive plants (glycophytes) include restriction of $Na^+$ and $Cl^-$ absorption at the root level, extrusion of surplus ions into the apoplast and sequestration of excess ions into intracellular compartments such as the vacuole.[1,2] At the whole plant level, many glycophytes (salt sensitive plants) limit the salt load to the shoot whereas halophytes (salt tolerant species), tend to adjust shoot osmolarity through accumulation of $Na^+$ and $Cl^-$ in green tissues.

## Different Cultivars as Tools to Study Salt Tolerance

Rice salt sensitivity varies considerably across species and cultivars. This can be exploited in comparative studies to discover genes and proteins that contribute to tolerance. We recently conducted studies at the physiological and transcriptomics levels to compare roots of two rice cultivars that differ greatly in salt tolerance, the sensitive IR29 (IR) and tolerant FL478 (FL).[3] This work focused on the role of specific membrane transporters on ion movement, particularly that of cations, during rice salt stress. The main outcomes are that net $Na^+$ influx is lower in FL roots compared to IR roots, that $Na^+$ translocation to the shoot in FL is limited and that FL maintained a higher level of $K^+$ in its shoots. The transcriptomics analyses identified a number of transporters that may underlie these differences including the cation exchanger OsCHX11 (upregulated in FL), which may contribute to increased $K^+$ uptake during salinity, the nonselective cation channel OsCNGC1, whose downregulation in FL would limit $Na^+$ uptake in this cultivar, and the $Ca^{2+}$ exchanger OsCAX and the vacuolar $Ca^{2+}$ permeable channel OsTPC1 which were both found to be downregulated in FL. The latter would potentially increase apoplastic

**Table 1.** Transcripts of kinases and phosphatases in roots of the rice cultivar FL 478 that are significantly changed (≥two-fold) after exposure to 12 days of salt treatment at 50 mM NaCl

| TIGRv4 locus | Annotation | Degree of expression |
|---|---|---|
| Os11g31540 | Brassinosteroid Insensitive 1 (OsBRI1) | 2.06 |
| Os02g05630 | Protein phosphatase 2C (OsPP2C10) | 0.20 |
| Os01g74530 | Protein phosphatase 2C containing protein | 0.50 |
| Os11g02240 | CBL-interacting serine/threonine-protein kinase 15 (OsCIPK15) | 0.48 |
| Os12g41090 | CBL-interacting serine/threonine-protein kinase (OsCIPK04) | 0.50 |
| Os09g19510 | Senescence-induced receptor-like serine/threonine-protein kinase | 0.49 |
| Os04g54190 | Serine/threonine-protein kinase receptor precursor | 2.11 |
| Os03g55620 | Serine/threonine-protein kinase 12 | 0.28 |
| Os01g02300 | Receptor kinase ORK10, putative | 0.48 |
| Os12g41520 | Receptor-like protein kinase | 0.39 |
| Os12g01510 | Protein kinase family protein | 0.43 |
| Os12g01510 | Protein kinase family protein | 0.40 |
| Os09g18150 | Protein kinase domain containing protein | 0.41 |
| Os07g48090 | Protein kinase domain containing protein | 0.41 |
| Os05g40270 | Leucine-rich repeat transmembrane protein kinase | 0.49 |
| Os04g57300 | Phosphatidylinositol 3- and 4-kinase family protein | 0.44 |
| Os03g18000 | Phosphoinositide-specific phospholipase C | 0.46 |
| Os02g57660 | Phosphatidylinositol-4-phosphate 5-kinase 9 | 0.47 |

**Table 2.** Transcripts of kinases and phosphatases in roots of the rice cultivar IR29 that are significantly changed (≥two-fold) after exposure to 12 days of salt treatment at 50 mM NaCl

| TIGRv4 locus | Annotation | Degree of expression |
|---|---|---|
| Os11g31530 | Brassinosteroid Insensitive 1 (OsBRI1) | 2.17 |
| Os02g05630 | Protein phosphatase 2C containing protein (OsPP2C10) | 2.27 |
| Os11g02300 | Mitogen-activated protein kinase | 2.35 |
| Os09g03620 | Protein kinase domain containing protein | 0.50 |
| Os05g48660 | Protein kinase domain containing protein | 2.07 |
| Os05g28520 | Serine/threonine-protein kinase | 2.08 |
| Os05g03450 | Protein kinase domain containing protein | 2.16 |
| Os01g19150 | Shaggy-related protein kinase NtK-1 | 2.07 |

$Ca^{2+}$ deposition, which has been shown to reduce apoplastic entry of $Na^{+}$.[4]

## Modulators of Ion Transport

The modulation of membrane transport is likely to be both post transcriptional and post translational and will strongly rely on adequate signaling processes that cover various stress response aspects such as re-establishment of ionic and osmotic balance, detoxification and damage repair and signaling related to relieve growth inhibition. How ionic levels and imbalances are sensed and regulated is largely unknown but perception of osmotic stress has been shown to involve transmembrane histidine protein kinases and possibly stretch activated channels.[5] Mitogen activated protein kinases (MAPKs) are involved in transducing signals for compatible osmolyte synthesis and detoxification by antioxidants.[6] Hormones and their biosynthesis are central players in growth related signaling.[7] We further analysed our data to identify potential regulators, focusing on proteins involved in the phosphorylation status of proteins since this is the predominant mechanism of posttranslational modification.[8]

## Kinases and Phosphatases that may Regulate Salinity Tolerance

**Tables 1 and 2** list kinases and phosphatases in IR and FL respectively, whose transcript levels were modulated at least two-fold by salinity. Only two transcripts occur in both FL and IR: a brassinosteroid insensitive-1 (BRI-1)-associated receptor kinase (Os11g31540) and a phosphatase 2C (Os2g05630). BRI-1 associated receptor kinases are essential for the perception of brassinosteroids. These hormones often act synergistically to others such as auxin and gibberellins and typically augment growth by promoting cell elongation in green tissues. However, in roots brassinosteroids usually inhibit growth.[9] The BRI-1 transcript is induced in both cultivars by a factor of around 2, possibly indicating an increased capacity to bind brassinosteroids and thus reduce root growth in response to salinity.[10] Protein phosphatase 2C (PP2C) type phosphatases function as regulators of various signal transduction pathways.[11] The best characterised PP2Cs are ABI1/ABI2 and related proteins that are central components in abscisic acid (ABA) signal transduction.[12] Interestingly, this transcript is moderately upregulated in IR (two-fold) but strongly downregulated in FL roots (five-fold). The significance of this can only be speculated upon in the absence of functional data for Os2g05630 (*OsPP2C10*). But, it is tempting to relate this transcriptional difference to the superior growth of FL roots during salinity stress[3] which possibly involves reduced PP2C activity and therefore limits ABA dependent growth reduction in this cultivar.

The other transcripts listed in **Table 1** are primarily protein kinases of unknown function. Os11g02240 is a 'CIPK' or CBL (Ca binding protein like) interacting kinase, many of which are known to function in abiotic stress.[13] Interestingly, Os11g02240 is a close homologue to SOS2, the AtCIPK24 kinase that phosphorylates

the $Na^+$:$H^+$ antiporter SOS3.[14] Intriguingly, this transcript is downregulated in the tolerant FL. However, root [$Na^+$] levels were comparable between FL and IR suggesting that either Os1102240 does not function as a SOS2 orthologue or alternatively, that a SOS3 based response is of minor significance in the roots of rice. Another CIPK (OsCIPK04) is also downregulated in FL but does not appear to be regulated in IR. No functional data are available for this transcript.

## Conclusions

Improvement of rice salt tolerance is urgently required but necessitates a detailed understanding of the involved processes, genes and proteins. Transcriptomics and other studies using inter-cultivar differences have identified ion transporters that play a role in ionic and osmotic homeostasis. Here we show that specific kinases and phosphatases may also contribute to inter-cultivar difference in salt tolerance. Clearly the exact function of many of these needs to be established in further studies and it will be of particular interest to see whether any directly modulate ion transport.

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