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Molecular switch to regulate salt tolerance in rice

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In Brief

Soil salinity affects crucial metabolic processes in plants and severely reduces crop yield. Factors such as land clearing, inadequate drainage of irrigated land, unsustainable irrigation practices, rising water tables, and global warming worsen the condition of salt-affected agricultural land (Munns and Gilliham 2015). Rice is particularly susceptible to soil salinity, with the potential for substantial declines in yield in saline soil (Molla et al. 2015). Enhancing salt tolerance in rice through breeding tolerant varieties is needed to mitigate the impact of soil salinity on global rice production.

In this issue, **Cong Liu and colleagues (Liu et al. 2023)** identified a molecular switch that negatively regulates salt tolerance and provided an attractive candidate for breeding salinity tolerance in rice. Salt stress induces osmotic stress, ionic stress, and oxidative stress. Salinity-induced formation of reactive oxygen species (ROS) causes oxidative stress. While excess ROS is toxic to cells, a low level of ROS is needed for stress signaling, growth, and plant development. Thus, maintaining a balance between ROS production and scavenging is crucial for plant growth and development. Catalase (CAT) is an enzyme that decomposes cellular H_2O_2, a nonradical ROS. CAT plays a key role in safeguarding plant cells by maintaining H_2O_2 homeostasis. Rice possesses 3 CAT isoforms: CatA, CatB, and CatC. Among these isoforms, CatC dismutates H_2O_2 and improves salt tolerance in rice (Zhou et al. 2018). Reversible protein phosphorylation, catalyzed by kinases and phosphatases, is a well-known molecular switch mechanism. While several kinases are known to activate CAT through phosphorylation, the process of CAT deactivation by phosphatase remains unknown.

Liu and colleagues conducted immunoprecipitation-mass spectrometry and identified a protein phosphatase in the CatC complex, Phosphatase of Catalase 1 (PC1), which was localized in the peroxisome. *PC1* expression was induced by NaCl and H_2O_2, suggesting its involvement in the response to oxidative stress and salinity in rice. The authors confirmed the physical interaction between PC1 and CatC in vitro, in rice protoplasts, and in rice seedlings. To examine whether PC1 dephosphorylates CatC, the authors incubated phosphorylated CATs with PC1 and observed a significant decrease in phospho-serine levels of CATs after incubation. However, phospho-threonine/-tyrosine levels of CATs remained unaffected, indicating that PC1 specifically dephosphorylates phospho-serine residues on CatC. Five phospho-serine residues have been identified in CatC: Ser-9, −10, −11, −18, and −205 (Zhou et al. 2018). Incubating recombinant PC1 with 5 synthetic phospho-peptides representing these 5 serine residues revealed that PC1 dephosphorylates only the phospho-Ser-9-CatC peptide. When recombinant PC1 was incubated with CatC, CAT activity diminished by 50%, indicating that dephosphorylation of CatC by PC1 negatively affects its enzymatic activity. A recombinant CatC with phosphomimetic mutation ($CatC^{S9D}$) exhibited the highest CAT activity even in the presence of PC1.

Gel filtration analysis revealed that $CatC^{S9D}$ predominantly forms a tetrameric structure, indicating that the phosphorylated version of CatC favors quaternary structure formation and exhibits higher CAT activity. These findings along with additional analysis establish that PC1 dephosphorylates CatC at Ser-9, leading to the dissociation of the tetramer and inhibition of its enzyme activity (see Fig.). Salt stress significantly inhibits PC1 activity and increases CAT activity and phospho-serine levels in rice plants. After stress recovery, opposite trends were observed for PC1 and CAT activities.

The authors generated *PC1* overexpression (OE) and CRISPR-knockout (*pc1*) lines to further investigate the role

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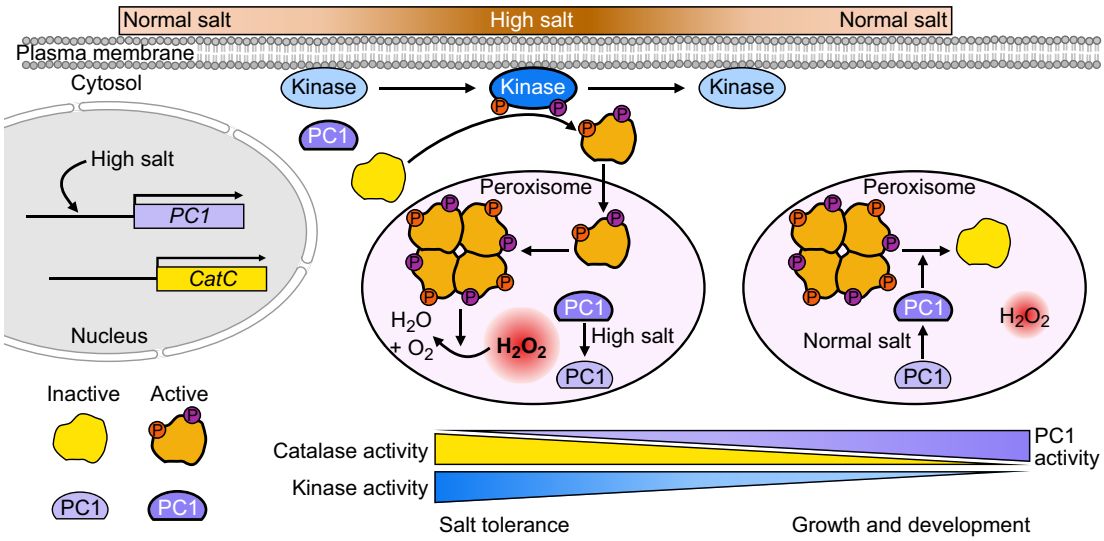

Figure. A proposed working model of PC1-mediated salt response and growth in rice. *PC1* and *CatC* are upregulated under salt stress. Protein kinases phosphorylate and activate CatC at the plasma membrane. Phosphorylated CatC moves to the peroxisome for tetramerization for eliminating excess H_2O_2, thereby improving salinity tolerance. PC1 is inhibited under salt stress to maintain CatC phosphorylation. Upon salt stress recovery, PC1 becomes activated while the kinases are inhibited. Active PC1 dephosphorylates CatC at Ser-9, promoting its dissociation, and ensuring an appropriate level of H_2O_2 for normal growth and development. Adapted from Liu et al. (2023), Fig. 7.

of PC1 in salt and oxidative stress. *PC1* OE lines showed hypersensitivity to salt stress (3%–5% survival rate), while *pc1* knockout lines exhibited higher tolerance and survival rates (37.5%–52.5%) after salt treatment. The wild-type (WT) control plants grown under the same saline conditions displayed a 20% survival rate. Under normal conditions, the transgenic lines and WT plants were phenotypically similar. Compared with WT under salt stress, *pc1* mutants accumulated lower levels of H_2O_2, whereas *PC1* OE lines exhibited higher levels of H_2O_2 accumulation. These results indicate that PC1 negatively regulates salt tolerance and the H_2O_2-scavenging capacity in rice under salt stress.

Rice seminal root growth is hypersensitive to salt stress. *PC1* OE lines showed lower seminal root growth velocities compared with WT under salt stress, while *pc1* mutants exhibited higher seminal root growth. Upon salt stress recovery, *PC1* OE lines showed the highest seminal root growth velocities among the genotypes tested. These results indicate that PC1 plays a vital role in transitioning from salt stress to normal growth conditions. Interestingly, after 26 days of salt treatment and 10 days of recovery, *pc1* mutants displayed more green leaves than WT and *PC1* OE lines. Similarly, after 10 days of recovery, *pc1* mutants yielded 60% more grain per plant compared with WT, while *PC1* OE lines showed a sharp decrease in grain number. Under normal conditions, no difference was found in growth vigor and agronomic traits of WT, *PC1* OE, and *pc1* mutant lines. These results indicate that *PC1* deficiency increases salt tolerance in rice without noticeable growth and yield penalties. Hence, the study conducted by Liu and colleagues delineated the switch-off mechanism of CAT and identified PC1 as a critical moderator in balancing salt stress tolerance and growth in rice. This work provides a compelling strategy to improve rice plants for salinity tolerance with minimum yield penalties. The negative regulator identified in this study could be knocked-out through genome editing in diverse germplasm across the globe to breed region-specific salinity-tolerant rice varieties, contributing to food security in the era of climate change.

References

Liu C, Lin J, Wang Y, Tian Y, Zheng H-P, Zhou Z-K, Zhou Y-B, Tang X-D, Zhao X-H, Wu T, et al. The protein phosphatase PC1 dephosphorylates and deactivates CatC to negatively regulate H_2O_2 homeostasis and salt tolerance in rice. Plant Cell. 2023:**35**(9):3604–3625. https://doi.org/10.1093/plcell/koad167

Molla KA, Debnath AB, Ganie SA, Mondal TK. Identification and analysis of novel salt responsive candidate gene based SSRs (cgSSRs) from rice (*Oryza sativa* L.). BMC Plant Biol. 2015:**15**(1):1–11. https://doi.org/10.1186/s12870-015-0498-1

Munns R, Gilliham M. Salinity tolerance of crops—what is the cost? New Phytol. 2015:**208**(3):668–673. https://doi.org/10.1111/nph.13519

Zhou YB, Liu C, Tang DY, Yan L, Wang D, Yang Y-Z, Gui J-S, Zhao X-Y, Li L-G, Tang X-D, et al. The receptor-like cytoplasmic kinase STRK1 phosphorylates and activates CatC, thereby regulating H_2O_2 homeostasis and improving salt tolerance in rice. Plant Cell. 2018:**30**(5):1100–1118. https://doi.org/10.1105/tpc.17.01000