## Engineering of Salt-Stress Tolerance in Plants through Activation of Inositol Metabolic Pathway

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## Summary

One of the largest problem that should overcome in the 21st century is "food crisis and environmental degradation". The rapid environmental degradation, which exceeds the self-cleaning power of the earth, progresses. Development of the crop with a high yield performance and with a high environmental stress tolerance is urgent target of plant breeding. It is important for future food guarantee that we reveal the metabolic regulation of the crop under various environmental stresses and control it appropriately.

Inositol is one of the essential nutrients for the growth of plants and animals, and is the central substance of the divergent biochemical processes. Metabolites of *myo*-inositol have various biological roles and participate in several important cellular processes, that is, ascorbic acid works in active oxygen scavenging system, raffinose family oligosaccharides work for osmo-protection, phytic acid acts as antioxidant, inositol phosphates act as signal transduction factors, and glucuronic acid and galacturonic acid are components of the cell wall and cell membrane, respectively. Thus, inositol metabolism is crucial for stress tolerance, normal plant growth and development. The appropriate control of inositol metabolism may lead to the increase in biomass production and the improved stress tolerance. *Myo*-inositol-1-phosphate synthase (MIPS; EC 5.5.1.4) catalyzes the reaction from glucose-6-phosphate to *myo*-inositol-1-phosphate, the first step of inositol metabolism. We have found that overexpression of *MIPS* gene in transgenic rice plants (MIPSox) leads to improve salt-stress tolerance in rice. To clarify which metabolic change leads the improved salt stress tolerance in the MIPSox plants, we performed metabolome analysis.

Compared to non-transgenic rice, chlorophyll concentration of MIPSox was maintained and the growth of the fourth leaf did not stop after 250mM NaCl treatment. From the metabolome analysis, we found that both the increase of metabolites, which are responsible to ABA-dependent signaling pathway, and the damage to TCA cycle and glycolysis in MIPSox plants under salt stress conditions were significantly smaller as compared to non-transformants. Then, the improvement of salt stress tolerance in MIPSox has become clear from both sides of the metabolites and phenotypes. From a comparison of the metabolites at the time before salt stress treatment, MIPSox accumulated both raffinose and ascorbic acid that play an important role in stress tolerance. Additionally, the activation of glycolysis pathway, pentose phosphate pathway, and TCA cycle was suggested, because the increase in metabolites associated with these pathways was observed. Activation of the pentose phosphate pathway may lead to the accumulation of NADPH that is needed in the active oxygen scavenging system of ascorbate-glutathione redox cycle.

These results suggest that the stress-anticipatory preparedness in MIPSox plants under control conditions might be responsible for the improved salt tolerance.