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Molecular Dissection of a Rice H2-type RING Finger Protein and Its Potential Role in Salt Stress

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[Introduction]

As sessile organisms, plants cannot avoid various biotic and abiotic stresses, such as high salinity, water deficits, and extreme temperatures. Salinity stress is one of the major adverse factors that adversely affect to plant growth and alters development. In this study, we have tried to retrieve RING E3 ligase gene, which is believed to regulate substrate proteins in high salinity stress via ubiquitin 26S proteasome pathway.

[Materials and Methods]

Rice seeds (*O. sativa* L. cv Donganbyeo) were grown with commercial soil in a growth chamber (16/8-h light/dark photoperiod at 25/23°C with 70% relative humidity) for 2 weeks. For salt treatment, the seedlings were treated at 200 mM and then harvested at various time points: 1, 3, 6, 12, and 24h. To study molecular characteristics of OsH2_14 protein, we perform the confocal imaging assay, BiFC, Yeast two Hybrid, pull-down assay, *in vitro* ubiquitination assay and *in vivo* protein degradation assay.

[Results and Discussions]

Based on previous study, we identified one gene, Oryza sativa H2-type RING Finger protein 14 (OsH2_14) that significantly up-regulated under high salinity stress. Results of subcellular localization showed that the *OsH2_14* protein mainly detected at the membrane and additionally both cytosol and Golgi in rice. In addition, Yeast two hybrid (Y2H) and bimolecular fluorescence complementation (BiFC) showed that OsH2_14 E3 ligase interaction with two cytosol substrates, i.e. OsSalT and OsPRF2, and one membrane Na+-selective transporter, OsHKT2;1. Interestingly, the low fluorescence intensity of OsHKT2;1 was detected in high salinity treated rice protoplasts as compared with non-treated normal protoplasts when OsHKT2;1 was co-transfected with OsH2_14. Overexpressing plants of *OsH2_14* in both rice and *Arabidopsis* showed insensitive phenotypes with respect to salt-responsive seedling growth. In addition, each of the transgenic plants showed lower sodium accumulation than control plants in shoot and root. These findings might support that the OsH2_14 E3 ligase might positively regulate the cellular functions under salt stress conditions in rice

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