Oryza sativa Heat- and Salt-induced RING Finger Protein 1 (OsHSRP1) Negatively Regulates Response to High Temperature and Salinity in Arabidopsis

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[Introduction]
Environmental stresses such as cold, drought, high temperature, and soil salinity, negatively affect seed germination, vegetative growth, and reproductive development of plants. Soil salinity and high temperature are the major agricultural problem because cause cellular dehydration and ionic toxicity and leads to a decrease in the productivity of crops. Here, we report on salt-induced RING finger E3 ligase, Oryza sativa Heat- and Salt-induced RING finger protein 1 (OsHSRP1).

[Materials and Methods]
Plant materials: The 14-day-old seeding were treated with abiotic stresses. To study the gene expression pattern, leaf and root tissues were harvested at different time points (0, 6, 12, and 24 h) after stress treatments.
In vitro ubiquitination assay: The purified MBP-OsHSRP1 as E3 was mixed: human E1, purified 6X His-tagged AtUBC10 as E2, and bovine ubiquitin, and then incubated in a ubiquitination reaction buffer for 3h at 30°C.
Subcellular localization and BiFC assay: To identify the subcellular localization of OsHSRP1 and two partner proteins were cloned into EYFP vector. In addition, the full-length clone of OsHSRP1 and partner genes were inserted into 35S:HA-SPYCE(M) and 35S:c-Myc-SPYNE(R) vectors for BiFC assay, respectively.

[Results and Discussion]
Transcript analysis of OsHSRP1 gene highly expressed at various abiotic and hormone stresses, such as ABA (100 mM), NaCl (200 mM), drought, and heat (45°C). In addition, in vitro ubiquitination assays demonstrated that OsHSRP1 shoed E3 ligase activity by RING C3HC4 type domain. The result of Yeast-Two hybridization and bimolecular fluorescence complementation (BiFC) support that OsHSRP1 interacting with two substrates, O. sativa Glyoxalase and O. sativa Cysteine proteinase 1, at cytosol. Heterogeneous overexpression of OsHSRP1 exhibited sensitive phenotype in compared to control plant under high salinity stress. Also, OsHSRP1-overexpressing Arabidopsis showed decreased tolerance to heat stress. These results suggest that OsHSRP1 play a negative regulator in salt and heat stress response.

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