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Positive Regulator, a Rice C3HC4-type RING Finger Protein HC-2 (OsRFPHC-2), in Response to Salt Stress

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[Introduction]

Rice is one of major crops for human life. However, environmental stresses such as salinity negatively affect in rice growth, seed germination and reproduction. In this study, we analyzed salt-induced RING finger C3HC4 type E3 ligase (OsRFPHC-2). To determine the biological functions of OsRFPHC-2, we examined E3 ligase activity to invitro ubiquitination assay. Additionally, overexpression OsRFPHC-2 plant performed more tolerance phenotype in 100mM salt stress than WT (Oryza sativa L. cv Dongjinbye). These results showed that OsRFPHC-2 may act as a positive regulator in response salt stress.

[Materials and Methods]

Plant growth condition: Rice seeds were grown half-strength Kimura B solution in a growth chamber (16/8-h, light/dark at 30°C with 70% humidity Plant analysis: 2week-old-seedling WT (Dongjinbye) and *OsRFPHC-2* overexpressing plants were treated with 100mM NaCl for 7day In vitro ubiquitination assay: The purified MBP-OsRFPHC-2as E3 ligase were mixed with E1, 6X His taqE2(UBC10), UB, ATP for 30°C, 3h. Subcellular localization: To identify localization of OsRFPHC-2 in rice, we fused OsRFPHC-2 in pGWB405(GFP) vector.

[Results and Discussion]

To identify plant phenotypic effects under salinity stress we measure plant length, weight and H_2O_2 of overexpressing plants and WT. To identified subcellular localization of OsRFPHC-2, we fused vector that include C-terminal GFP protein (35S;;OsRFPHC-2-GFP). OsRFPHC-2 was expressed in the cytosol in rice protoplasts. Some previous studies suggest that E3 ligase regulates molecular mechanisms in response to various abiotic stress. Therefore, we analyze the E3 ligase activity of the OsRFPHC-2. The immunoblotting results clearly showed the presence of poly-ubiquitinated chains. However, the mutated OsRFPHC-2 were not detected any chains. These results suggest that OsRFPHC-2 is a regulator in response to salinity stress.

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