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Molecular Analysis of a Rice C4H3-type RING Finger Protein (OsRFPv6) and its Overexpression Plant Suggest Salt Stress Tolerance.

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[Introduction]

Environmental stresses, such as salinity, drought, cold, high temperature are negatively affected in plants. Among salinity stress is one of the major abiotic stress, its adverse factor that reduce plant growth and reproduction product. In this study, we experiment RING E3 ligase (OsRFPv6), which is believed to plant positively regulated in salinity stress via ubiquitin mechanism.

[Materials and Methods]

Rice seed (Oryza sativa L. cv Dongjinbyeo) were grown nutrient solution in a growth chamber (16/8-h light/dark at 30/28 with 70% humidity) for 12 days. Rice seedling was treated 150mM NaCl and various abiotic stress with time point (1,6,12, and 24h). WT (Dongjinbyeo) and overexpression OsRFPv6 plant phenotype analysis treated 100 mM NaCl for 8 day and 24 h treated plant various salt marker gene qRT-PCR, Coro-Na staining, xylem sap assay. To study molecular characteristics of OsRFPv6, we performed confocal imaging assay, in vitro ubiquitination assay.

[Results and Discussions]

In this study, we indentified gene O. sativa C4H3-type RING finger protein (OsRFPv6) that significantly up-regulated under salinity, drought stress. Subcellular localization showed in OsRFPv6 that mainly detected plasma membrane and cytoplasm and in vitro ubiquitination assay confirmed E3 ligase function in OsRFPv6. Next, we performed with salinity stress (100 mM NaCl) response between WT and overexpressing OsRFPv6 plant. As a results transgenic plant indicated more insensitive phenotype including fresh weight, length that leaf, leaf sheath and root, in comparison WT. As well as under salinity stress in overexpressing seedling were high chlorophyll, soluble sugar, proline content and low level H2O2 content. The xylem sap and Coro-Na green staining analysis proceeded. interestingly, overexpressing OsRFPv6 seedling showed uptake Na+ and root tip tissue Coro-Na staining low fluorescence intensity under 100 mM salinity stress for 24h.This result means that OsRFPv6 regulated Na+ absorption in high salinity condition. Additionally various salt marker gene qRT-PCR analysis performed with treated WT, overexpression line 100mM NaCl for 24h. Among them, Na+ transporter was more down-regulated in the OX-OsRFPv6 than WT in 0,100 mM NaCl. this result supported xylem sap, Coro-Na staining result. These findings might support that the OsRFPv6 E3 ligase might positively regulate the function under salinity stress in rice.

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