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Oryza sativa, C3HC4-type E3 ligase, OsRFPHC-4 is positive a role to salt stress

Jong Ho Kim[1], Cheol Seong Jang[1]*

[1]Plant Genomics Laboratory Interdisciplinary Program in Smart Agriculture, Kangwon National Univ, Chuncheon-si 24341, Republic of Korea

[Introduction]

Soil salinity accumulation is negatively affected major crops growth, productivity, and metabolism. Especially, among them, rice is higher susceptible to salinity stress than other crops. However, rice have developed diversity strategies to survival upon high salinity condition. Ubiquitins-mediate E3 ligase is closely relation for enhancing salt insensitive. So, in this paper, we demonstrate for Oryza sativa RING finger protein HC-4 (OsRFPHC-4) using overexpression and T-DNA knock-out plant under salt stress.

[Materials and Methods]

Plant growth condition: Rice seeds (Oryza sativa L. cv Dongjinbyeo) were germinated and grown in half-strength Kimura B solution in a growth chamber (16/8-h light/dark at 30/28℃ with 70% humidity).

Plant analysis: WT, OX-OsRFPHC-4 7 day-old seedling were treated with 100mM NaCl for 7day, that measure plant length, fresh weight, H_2O_2, Chlorophyll. qRT-PCR analysis for Na^+/K^+ gene and ICP.

In vitro ubiquitination: MBP-OsRFPHC-4, mutation MBP-OsRFPHC-4, empty vector were incubating with E1, E2 (UBC10) and ubiquitin for 30℃, 3h.

Subcellular localization: To identified OsRFPHC-4 localization in rice, we fused OsRFPHC-4 GFP vector and then transfected rice protoplast.

[Results and Discussion]

The overexpression OsRFPHC-4 plant were shown enhancing salt insensitive upon salt condition by measuring of fresh weight, chlorophyll content compare to WT. In contrast, knock-out plants were showed the opposite result. Additionally, GFP-OsRFPHC-4 protein was localized at cytoplasm in rice protoplast and we confirmed that not merged with transmembrane marker gene and HC-4 GFP protein. In vitro ubiquitin assay confirmed that OsRFPHC-4 possessed E3-ligase activity, however not shown mutation of E3 ligase. Analysis of qRT-PCR for Na^+/K^+ marker genes were shown interesting expression pattern which were identified significant different expression levels under 100 mM NaCl. These results suggest that OsRFPHC-4 were positive roles under salt stress.

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*Corresponding author: E-mail. csjang@kangwon.ac.kr Tel. *** - **** - ****