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E3 Ligase, the *Oryza sativa* Membrane and Cytosol-localized RING Finger Protein 1 (OsMCRP1), Negatively Regulates Salt Stress Responses

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[Introduction]

Plants are sessile organisms that can be exposed to environmental stress. Plants alter their cellular processes to survive under potentially unfavorable conditions. Protein ubiquitination is an important post-translational modification that has a crucial role in various cellular signaling processes in abiotic stress response.

[Materials and Methods]

Rice (*Oryza sativa* 'Donganbye') seeds were grown in a growth chamber at 30 °C, 70% relative humidity, and a 12 h light/12 h dark cycle. Fourteen-day-old seedlings were treated with 150 mM NaCl, and half-strength Murashige and Skoog (MS) medium was added. Shoot and root tissues from these seedlings were harvested at different time points (6, 12, 24, and 48 h). For the salt stress tolerance assay, WT (35S:YFP) and overexpressing Arabidopsis plants were cultured on half MS medium under a 16 h (light)/8 h (dark) cycle at 22 °C for 3 d, and then the seedlings were transferred to fresh half MS medium supplied with 150 and 200 mM NaCl and grown vertically for 10 d.

[Results and Discussion]

In this study, we characterized *Oryza sativa* membrane and cytosol-localized RING finger protein 1, OsMCRP1, a membrane and cytosol-localized RING E3 ligase in rice. OsMCRP1 transcripts were highly induced under salt stress in rice. We found that OsMCRP1 possesses E3 ligase activity. The results of the yeast two hybrid system, *in vitro* pull-down assay, BiFC analysis, *in vitro* ubiquitination assay, and *in vitro* degradation assay indicate that OsMCRP1 regulates degradation of a substrate protein, OsPEX11-1 (*Oryza sativa* peroxisomal biogenesis factor 11-1) via the 26S proteasomal system. Phenotypic analysis of OsMCRP1-overexpressing plants demonstrated hypersensitivity to salt response compared to that of the wild type and mutated OsMCRP1^{C269A} plants. In addition, OsMCRP1-overexpressing plants exhibited significant low enzyme activities of superoxide dismutase, catalase, and peroxidase, and accumulation of proline and soluble sugar, but a high level of H_2O_2. Furthermore, qRT data on transgenic plants suggest that OsMCRP1 acted as a negative regulator of salt response by diminishing the expression of genes related to Na^+/K^+ homeostasis (*AtSOS1*, *AtAKT1*, *AtNHX1*, and *AtHKT1;1*) in transgenic plants under salt stress. These results suggest that OsMCRP1 plays a negative regulatory role in response to salt stress by modulating the target protein levels.

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