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Molecular analysis of gene *OsCRF1* induced by chilling stress: expression pattern, *in vitro* ubiquitination assay, and subcellular localization

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[Introduction]

Chilling (sub-optimal temperature) stress adversely affects plant growth and productivity during rice cultivation period, especially at seedling and reproductive stages. We selected the *Oryza Sativa* chilling-induced RING E3 ligase gene (*OsCRF1*) and examined its expression pattern in different tissues during vegetative and reproductive stages.

[Materials and Methods]

Rice seedlings (*O. sativa* L. Donganbyeo and *O. sativa* L. Satbyelbyeo) were grown in mesh supported plastic containers filled with a half-strength Murashige and Skoog (MS) solution or in pots filled with a commercial soil (Baroker, Seoul Agricultural Materials Co., Korea), in a growth chamber (16/8 h light/dark photoperiod at 25 °C with 70% relative humidity). Electrolyte leakage assay and subcellular localization were confirmed, and E3 ligase activity was confirmed by in vitro ubiquitination assay.

[Results and Discussions]

We assessed the phenotypic effect on two varieties and examined expression levels of *OsCRF1* under chilling stress. The degree of cell membrane injury caused by chilling is reflected by the intracellular electrolyte leakage rate; therefore, the evaluation of electrolyte leakage was employed to determine the metabolic activity of Donganbyeo and Satbyeolbyeo during chilling treatment. Higher electrolyte leakage was observed in Satbyelbyeo than Donanbyeo, suggesting that the metabolic activity of Donanbyeo is higher than that of Satbyeolbyeo under chilling stress. , we observed a high expression of *OsCRF1* under chilling stress, E3 ligase activity, and nucleus targeting, providing some important clues toward understanding the molecular mechanisms governing the response to chilling stress mediated by OsCRF1 pathways. Moreover, based on the results of expression pattern of *OsCRF1* in Donanbyeo and Satbyeolbyeo under chilling stress suggests that OsCRF1 exhibits its function in the weaning stage. However, further work is necessary to clearly understand the mechanism by which OsCRF1 acts against chilling stress in rice.

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