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Functional characterization of salt-stress induced rare cold inducible gene from *Camelina sativa* (*CsRCI2D*)

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[Introduction]

Rare-cold-inducible 2 (*RCI2*) genes are involved in plant response to abiotic stresses. The possible functional roles of RCI2s in salt adaptation or tolerance in plants, our current knowledge on the roles of RCI2 in crops remains limited. Here, we report the functional role of a *Camelina* RCI2, CsRCI2D, in plant salt stress response. The expression pattern of *CsRCI2D* was analyzed under salt stress, and the function of CsRCI2D in salt stress response was determined using transgenic *Camelina* plants.

[Materials and methods]

- Confocal analysis: Tobacco leaves were infiltrated with *Agrobacterium* expressing the YFP-CsRCI2D fusion protein, and the YFP fluorescence signals were visualized under a laser scanning confocal microscope.
- Yeast pmp3 mutant complementation: BY4741 (WT) and the transformed *△pmp3* cells harboring CsRCI2D were cultured onto SG agar plates supplemented with NaCl.
- Phenotypic analysis of transgenic plants: The surface-sterilized seeds were sown on Hoagland medium supplemented with 200 mM or 300 mM NaCl, and the germination rate and seedling growth were investigated.
- Determination of stomatal conductance, H_2O_2, MDA, and ion contents: Stomatal conductance was determined from the adaxial part of the phyllodes from 21d-old plants, and 10-d-old plants were used for H_2O_2, and MDA according to previous methods. NaCl treated plants were washed with water and then dried at 60 °C. Dried plant materials were used for the determination of Na^+, K^+, and Ca^{2+} content by ICP-OES.
- Transcript analysis of antioxidant enzymes: Transcripts of antioxidant enzymes (*CsCuSODs, CsFeSOD, CsMnSOD, CsCATs,, CsAPX1,* and *CsGR*) were analyzed by real time RT-PCR.

[Results and discussion]

The YFP-CsRCI2D fusion protein was localized in the plasma and intracellular membranes similar to that for unidentified punctate structures in tobacco leaves. The *CsRCI2D* expression was significantly increased by salt stress but could not complement the salt sensitivity of *△pmp3*; however, C-terminal tail truncated CsRCI2D increased salt tolerance. *CsRCI2D*-overexpressing *Camelina* showed better germination rate and seedling growth. Under salt stress, Na^+ content in both roots and shoots of transgenic plants was obviously lower than that in WT, and K^+ content in transgenic plants was higher in the shoots compared to that in WT. Furthermore, *CsRCI2D*-overexpressing *Camelina* displayed higher stomatal conductance and lower H_2O_2 and malondialdehyde (MDA) upon salt stress. Under normal growth condition, *CsRCI2D*-overexpressing *Camelina* showed higher transcript levels of all antioxidant genes (*CsCuSODs, CsMnSOD1, CsFeSODs, CsCATs, CsAPX1,* and *CsGR*), whereas salt stress significantly induced all antioxidant genes in wild type. These results indicate that CsRCI2D contributes positively to salt tolerance during seed germination and seedling growth by protecting the plant cells through the regulation of ion homeostasis and oxidative damage.

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