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Rare Cold Inducible 2 Proteins are Internalized from Plasma Membrane to Endomembranes under NaCl Stress in *Camelina sativa* L.

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[Introduction]

Rare Cold Inducible 2 (RCI2A to H) proteins are small size hydrophobic protein known to be localized at plasma membrane (PM). Expression of RCI2s are significantly induced by abiotic stresses such as cold, drought, and saline stress. Accumulation or over-expression of RCI2s contributes abiotic stresses tolerance but a cellular function remains unclear. In previous, we reported novel protein interaction between CsRCI2E/F and aquaporin CsPIP2;1 which is reduces water transport activity. Endocytic trafficking of aquaporin PIP2;1 under NaCl or osmotic stress have been reported in several species but not in CsRCI2s. In this study, we observed vesicle trafficking using fluorescence protein fusion at N-terminal of CsRCI2s. In addition, changes of protein accumulation in different membrane fraction such as PM and tonoplast. From these results we would like to describe novel function of CsRCI2s internalization under NaCl stress.

[Materials and Methods]:

CsRCI2D/E/F/G genes were cloned into p35SFAST-eYFP vector for transient expression using agrobacterium GV3101 infiltration. Subcellular localization and vesicle traffikcing was observed using confocal laser scanning microscope. One-week-old Camelina seedlings are grown on hydroponics and treated with 150 mM NaCl for 24 h. Then, the plants sampled for qRT-PCR and membrane protein extraction. Total membrane fractions were carefully loaded on 4 phase (16, 24, 34, 40%) sucrose density gradient. After ultracentrifugation, protein accumulation of each fractions (FR) were determined through western-blot analysis using against anti CsRCI2D/E/F/G, PM-H^+ATPase (PM marker), and V-ATPase (tonoplast marker).

[Results and Discussion]:

CsRCI2D/E/F/G proteins are belong to C-terminal tail-type in RCI2s. Expression of CsRCI2E and CsRCI2G significantly increased to 22.5 and 38.7 fold by NaCl exposure, respectively. But, CsRCI2D and CsRCI2F increased to 2.8 and 6.1 fold than control. This result indicates that expression of CsRCI2E/G showed higher fold changes than CsRCI2D/F. Subcellular localization of eYFP-CsRCI2D/E/F/G fusion protein observed at PM in tobacco leaves. However, eYFP-CsRCI2s signals also observed in endo-membrane vesicles moving from PM to intracellular region. This means CsRCI2s are not only localized at PM but also internalized into other membranes through vesicle trafficking under NaCl stress. In result of fractionation of membrane proteins using sucrose density gradient, accumulation of marker protein such as PM H^+-ATPase (PM, FR4, 5) and V-ATPase (tonoplast, FR3, 4) did not significantly changes by NaCl stress. In western-blot analysis, accumulation of CsRCI2D/E/F/G are observed at FR3 and FR4 under normal condition. However, increased accumulation of CsRCI2D/E/F/G at FR3 were observed at NaCl treated samples. This result means CsRCI2s shifted from PM to other endo-membrane. In conclusion, we found that CsRCI2D/E/F/G can internalize through endocytic vesicle trafficking when exposed to NaCl stress. Information of CsRCI2s internalization may helpful to understand function of CsRCI2s in cell under abiotic stress.

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