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Subcellular Redistribution of CsRCI2D under NaCl Stress in *Camelina sativa* L.

Hyun-Sung Kim[1], Sehee Kim[1], Sung-Ju Ahn[1]*

[1]Department of Bioenergy Science and Technology, Collage of Agriculture and Life Science, Chonnam National University, Gwangju 61186, Republic of Korea

[Introduction]

Rare Cold Inducible 2 (RCI2A to H) proteins are known to be small size plasma membrane (PM) proteins. Expression of RCI2s are significantly induced by abiotic stresses such as cold, drought, and saline stress. In previous, we reported protein interaction of RCI2s response to NaCl stress which is regulate water transport activity of aquaporin PIP2. In this study, we investigated subcellular redistribution of CsRCI2D stress to understand function of RCI2s mediated remove, recycle, and degrade of PM proteins.

[Materials and Methods]

CsRCI2D gene was cloned into p35SFAST-eYFP vector for transient expression using agrobacterium GV3101 infiltration in tobacco. Subcellular localization and vesicle traffikcing was observed using confocal laser scanning microscope. Seven-day-old Camelina seedlings were grown on hydroponics. The seedlings were treated with 0 to 200 mM for 0 to 6 hours. The samples were grounded by ice-cold homogenization buffer, then, fractionated by 8 phase (15 to 50 %) sucrose density gradient system. Fourteen linear membrane fractions were collected for wastern-blot analysis using antibodies of CsRCI2D, PM-H^+-ATPase, V-ATPase, and binding immunoglobulin protein (BIP).

[Results and Discussion]

CsRCI2D protein is belong to C-terminal tail-type in RCI2s. Subcellular localization of eYFP-CsRCI2D fusion protein is not only observed at PM but also in endo-membrane vesicles adjacent to PM. This means CsRCI2D is localized at PM but also internalized into other membranes. In result of membrane fractionation using sucrose density gradient, expression of CsRCI2D was significantly shifted to lower density of membrane fractions by exposed to 50 to 200 mM NaCl. However, the expression of CsRCI2D at fractions 11 to 13 as higher density region partially increased in 100 and 200 mM NaCl. In time dependent expression of CsRCI2D at 150 mM NaCl showed that gradually shifted to lower fraction for 3 hours, but slightly moved to higher density at 6 hours. In addition, the expression of CsRCI2D was partially over-wrapped whithin marker proteins of PM (PM H^+-ATPAse), tonoplast (V-ATPase) and ER (BiP). These results indicated that CsRCI2D redistributed to other membrane from PM by NaCl stress. In conclusion, we found that CsRCI2D can internalize through membrane trafficking when exposed to NaCl stress. It is speculated that the trafficking of CsRCI2 is related to the internalization of the PM transport protein for ionic homeostasis. Information of CsRCI2s internalization may helpful to understand function of CsRCI2s in cell under abiotic stress.

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*Corresponding author: Tel. +82-62-530-2052, E-mail. asjsuse@jnu.ac.kr