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Expression of CsRCI2s by NaCl stress reduces water and sodium ion permeation through CsPIP2;1 in *Camelina sativa* L.

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Abstract

Camelina (*Camelina sativa* L.) is a potential bio-energy crop that has short life cycle about 90 days and contains high amount of unsaturated fatty acid which is adequate to bio-diesel production. Enhancing environmental stress tolerance is a main issue to increase not only crop productivity but also big mass production. CsRCI2s (Rare Cold Inducible 2) are cold and salt stress related protein that localized at plasma membrane (PM) and assume to be membrane potential regulation factor. These proteins can be divide into C-terminal tail (CsRCI2D/E/F/G) or no-tail group (CsRCI2A/B/C/H). However, function of CsRCI2s are less understood. In this study, physiological responses and functional characterization of CsRCI2s of Camelina under salt stress were analyzed. Full-length *CsRCI2s* (A/B/E/F) and *CsPIP2;1* sequences were confirmed from Camelina genome browser. Physiological investigations were carried out using one- or four-week-old Camelina under NaCl stress with dose and time dependent manner. Transcriptional changes of *CsRCI2A/B/E/F* and *CsPIP2;1* were determined using qRT-PCR in one-week-old Camelina seedlings treated with NaCl. Translational changes of CsRCI2E and CsPIP2;1 were confirmed with western-blot using the antibodies. Water transport activity and membrane potential measurement were observed by cRNA injected *Xenopus laevis* oocyte. As results, root growth rate and physiological parameters such as stomatal conductance, chlorophyll fluorescence, and electrolyte leakage showed significant inhibition in 100 and 150 mM NaCl. Transcriptional level of *CsPIP2;1* did not changed but CsRCI2s were significantly increased by NaCl concentration, however, no-tail type CsRCI2A and CsRCI2B increased earlier than tail type CsRCI2E and CsRCI2F. Translational changes of CsPIP2;1 was constitutively maintained under NaCl stress. But, accumulation of CsRCI2E significantly increased by NaCl stress. CsPIP2;1 and CsRCI2A/B/E/F co-expressed *Xenopus laevis* oocyte showed decreased water transport activity as 61.84, 60.30, 62.91 and 76.51 % at CsRCI2A, CsRCI2B, CsRCI2E and CsRCI2F co-expression when compare with single expression of CsPIP2;1, respectively. Moreover, oocyte membrane potential was significantly hyperpolarized by co-expression of CsRCI2s. However, higher hyperpolarized level was observed in tail-type CsRCI2E and CsRCI2F than others, especially, CsRCI2E showed highest level. It means transport of Na^+ ion into cell is negatively regulated by expression of CsRCI2s, and, function of C-terminal tail is might be related with Na^+ ion influx. In conclusion, accumulation of NaCl-induced CsRCI2 proteins are related with Na^+ ion exclusion and prevent water loss by CsPIP2;1 under NaCl stress.

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