(05-1-18)

Direct interaction of a divergent CaM isoform and the transcription factor, MYB2, enhances salt tolerance in *Arabidopsis*

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Objectives

We have intended to show that a specific CaM isoform mediates salt-induced Ca^{2+} signaling through the activation of an MYB transcriptional activator, thereby resulting in salt tolerance in plants.

Materials and Methods

1. Material : *Arabidopsis*, *Agrobacterium tumerfaciens* GV3101, Soybean suspension cell,

2. Methods: cDNA expression library screening, site-directed mutagenesis, CaM binding assay, CaM mobility shift assay, Yeast split ubiquitin assay, Electrophoretic mobility shift assay, *Transient expression assay,* Proline measurement

Results and Discussion

We isolated a cDNA encoding a CaM binding transcription factor, MYB2, that regulates the expression of salt- and dehydration-responsive genes in *Arabidopsis*. This was achieved using a salt-inducible CaM isoform (GmCaM4) as a probe from a salt-treated *Arabidopsis* expression library. Using domain mapping, we identified a Ca^{2+}-dependent CaM binding domain (CaMBD) in MYB2. Specific binding of CaM to CaMBD was confirmed by site-directed mutagenesis, a gel mobility shift assay, split ubiquitin assay, and a competition assay using a Ca^{2+}/CaM-dependent enzyme. Interestingly, a specific CaM isoform (GmCaM4) enhances the DNA binding activity of AtMYB2, whereas this was inhibited by a closely related CaM isoform (GmCaM1). Overexpression of GmCaM4 in *Arabidopsis* up-regulates the transcription rate of AtMYB2-regulated genes, including the proline synthesizing enzyme, $\triangle^1$-pyrroline-5-carboxylate synthetase-1 (P5CS1), which confers salt tolerance by facilitating proline accumulation.

In this report we suggest that a specific CaM isoform mediates salt-induced Ca^{2+} signaling through the activation of an MYB transcriptional activator, thereby resulting in salt tolerance in plants.

Arabidopsis cDNA library 에서 대두의 divergent calmodulin isoform (GmCaM4)과 결합하는 전사조절 인자로서 고염와 탈수 스트레스 관련 유전자의 발현을 조절하는 MYB2 를 분리하였다. Domain mapping 을 통해서 AtMYB2 에서 하나의 CaM 결합부위를 동정하였고, 이들 CaM 과 AtMYB2 간의 특이적인 상호작용을 여러 생화학적 방법으로 증명하였다. divergent CaM 인 GmCaM4 는 AtMYB2 의 DNA binding activity 를 증가시키고, 반면에 conserved CaM 인 GmCaM1 은 AtMYB2 의 DNA binding activity 를 저해시켰다. GmCaM4 과발현 애기장대 식물체는 proline 생합성 관련 유전자인 P5CS1 을 포함하는 AtMYB2-regulated genes 발현 증가의 유도를 통해서 증가된 고염 저항성을 보였다.

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