

THE CHANGING PARADIGMS OF AUXIN SIGNALING; INVOLVEMENT OF CALCIUM

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Auxin primarily regulates plant growth and development through the degradation of Aux/IAA repressor proteins by binding to TIR1/ AFB family of auxin receptor proteins through the domain II of the protein, thus releasing Auxin Response Factors (ARFs) and initializing the transcription of auxin response genes. During biotic and abiotic stress conditions the cellular Ca²⁺ level of plants increases. Ca²⁺, not only acts as a signaling molecule, but also important in regulating both biotic and abiotic stress tolerance in plants. In addition, it regulates many auxin related growth processes. We identified two putative binding sites of Calmodulin (CaM) in Aux/IAA7. In this study we hypothesized that, stress induced increased cellular Ca²⁺ may activate CaM and it might bind onto the Aux/IAAs and prevent degradation of the repressor and lower the transcription rate of early auxin responsive genes. The objective of this study was to identify the effect of Calcium or/ and stress factors on auxin signaling pathway.

A chimeric protein of Aux/IAA7 tagged with *-myc* was constructed and expressed in *E.coli* and interacted with purified GST-CaM expressed in *E.coli* with the presence of 1 mM CaCl₂ and 2 mM EGTA separately and pulled down using a Glutathione column and performed SDS-PAGE. The results of Western blot using anti- myc antibody showed that interaction of two proteins was high in the presence of Ca²⁺ and no interaction was observed with EGTA. The significance of Calcium ions on the interaction between Aux/IAA proteins and auxin receptors (AFB1) was studied using *Arabidopsis* plant proteins over expressing AFB1-*myc* and purified bacterially expressed GST-IAA7. These two proteins interact with each other in auxin dependent manner and were treated with 1 mM CaCl₂, 2 mM EGTA and 50 μM CPZ (CaM inhibitor) separately and SDS-PAGE was performed. The results of Western blot using anti –myc antibody showed that, interaction of two proteins was less in Ca²⁺ treated sample and it was high in EGTA and CPZ treated samples.

Our study shows that Aux/IAA proteins interact with CaM in Calcium dependent manner. The interaction of auxin receptors such as TIR1 and AFBs with the repressor proteins may be partially inhibited by elevated cell Ca²⁺ level and may prevent the transcription of early auxin responsive genes as a mechanism to stress tolerance in plants.

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