Regulation of Auxin signaling in plants during stress response; A molecular genetics study on *Aux/IAA* transcription repressor gene family

A thesis submitted for the Degree of Doctor of Philosophy

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June 2018

Abstract

Plants cope with changing biotic and abiotic environment by acclimation of their growth and development to prevailing conditions. The developmental plasticity of the plants to cope with changing environmental conditions requires a diverse integration of growth performances with signal perception and transduction systems. The plant hormone auxin regulates virtually every aspect of plant growth and development in responses to environmental cues and pathogenic attacks. Plants interpret auxin signals through a short nuclear signaling pathway, in which auxin binding stabilizes the interaction between auxin receptors and a group of transcriptional repressors called Aux/IAAs. Among the four domains of Aux/IAAs, domain II is critical for the own degradation of protein, in response to auxin. Auxin is known to play a major role in stress tolerance mechanisms in plants along with Ca^{2+}. Calcium signal is transduced in cells through a number of Ca^{2+} sensing molecules including Calmodulin (CaM). CaM interacts with SAURs, which is an early auxin response gene. However, the lack of proper information on molecular biological mechanisms involved in auxin-dependent growth performance of plants during stress tolerance made it difficult to understand. Here, this thesis reports that Aux/IAA proteins interact with CaM in a Ca^{2+}-dependent manner.

The putative CaM binding domains (CaMBDs) were identified on all the available Aux/IAA proteins in Arabidopsis genome using Helical Wheel Projection program. The results revealed that most of the Aux/IAA proteins contain two putative CaMBDs on both domain I and II. In certain Aux/IAA proteins a single putative CaMBD was identified on either domain I or II, while IAA20 and IAA30 did not have any putative CaMBD. In most of the cases, the CaMBDs overlap with the EAR motif in domain I and auxin receptor binding motif or degron motif in domain II. Pull-down reactions and yeast two hybrid assays used during the study demonstrated that domain I and II of IAA7 interact with CaM in Ca^{2+}-dependent manner. Furthermore, the experiments carried out using *HS::AXR3-GUS* reporter construct proved that Aux/IAAs are stabilized in the presence of Ca^{2+} and degraded faster in the presence of Ca^{2+} chelator EGTA. The results of pull-down assays carried out using auxin receptor F-box proteins TIR1 and AFBs indicated that the interaction is less in the presence of Ca^{2+}/CaM and favored in the presence of CaM inhibitors and EGTA.

Taken together, the results indicate that most of the Aux/IAAs interact with CaM in Ca^{2+}-dependent manner and it stabilizes the Aux/IAA proteins. The interaction of CaM-Aux/IAA reduces the interaction of TIR1/AFBs-Aux/IAA and it might cause alterations in downstream gene expression. The results of this study provide novel insights to connect calcium, stress and auxin signaling stress tolerance mechanisms.

Key words: auxin, Aux/IAA transcription repressors, SAUR, calmodulin, calcium, domain I, domain II, biotic stress, abiotic stress,