CHAPTER 44

PLANT MOLECULAR BIOLOGY

Doctoral Theses

01. BISHT (Diksha)
Delineating the Calcium-Dependent Potassium Signaling Network in Plants via Transcriptomic and Genetic Analyses.
Supervisor: Prof. Girdhar K. Pandey
Th 29368

Abstract

Potassium, K^+, plays an indispensable role in plant's growth and development. Plants have evolved a specific module comprising calcineurin B-like proteins (CBLs) and CBL-interacting protein kinases (CIPKs) for K+ sensing and uptake. CIPK9 and CIPK23 are the two known critical regulators of K+ deficiency responses in Arabidopsis. Loss-of-function mutants of the CIPK9 and CIPK23 genes are sensitive to K+ deficiency. Using publicly available DAP-seq and ChIP-seq data, a few transcription factors binding to CIPK9 and CIPK23 promoter regions were identified. KANADI1 was identified and characterized as one of the transcriptional regulators of CIPK9. The reduced expression of CIPK9 in kan1 mutants under K+ deficiency implicates that KAN1 acts as a positive regulator of CIPK9. In addition, KUP9 and KUP3 were validated as interactors of CIPK9 and the CIPK9-KUP3 module was functionally characterized for its regulatory role in maintaining cellular K+ homeostasis. Comparative root system architecture and transcriptomic analyses of cipk9, cipk23, and the cipk9/23 double mutant were conducted to elucidate their differential roles in mediating K+ deficiency responses. A detailed phenotypic analysis and microscopic studies showed that root growth in cipk9 and cipk23 mutants was impaired due to the decreased auxin and elevated ethylene levels under K+ deficiency. Comparative transcriptomic analysis identified several root-related candidate genes, including NAC1, EIN3, and SAUR15, as potential regulators of CIPK9 and CIPK23, which require more detailed exploration. Additionally, CIPK23 was functionally characterized from a halophyte, Sesuvium portulacastrum, for its role in conferring stress tolerance in Arabidopsis. Heterologous expression of SpCIPK23WT and SpCIPK23T183D in Arabidopsis generates less ROS and confers tolerance to K+ deficiency. Collectively, the present study illuminates the complexity of the K+ starvation gene regulatory network and identifies novel regulators of CIPK9 and CIPK23. Further, it provides a valuable resource for functional studies on K+ signaling components in plant development and stress conditions.

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under potassium deficiency conditions in Arabidopsis 7. Materials and methods 8. Summary and conclusion. References and annexures.

02. KAUR (Inderjit)
Tetraspanins Orchestrate Plant Stress Responses: Functional Dissection of AtTET2 in Arabidopsis Thaliana, OsTET2 and OsTET5 in Rice (Oryza Sativa).
Supervisor: Prof. Surekha Katiyar Agarwal
Th 29369

Abstract

Tetraspanins (TETs) are four-pass membrane proteins that organize specialized tetraspanins- enriched microdomains (TEMs) and regulate cellular signaling. However, their roles in plant stress adaptation remain poorly characterized. This study aims at functional characterization of AtTET2 in Arabidopsis thaliana and OsTET5 and OsTET2 in rice during abiotic and biotic stress conditions. OsTET5 acts as a positive regulator of drought and salinity tolerance through improved antioxidant capacity and proline accumulation. In contrast, OsTET5 negatively regulates blast resistance, as its over-expression increased susceptibility to Magnaporthe oryzae. Subcellular localization, mating-based split ubiquitin assays (mbSUS), and protein studies reveal targeting of OsTET5 to the plasma membrane (PM) and endoplasmic reticulum (ER), it undergoes homomeric self-association, and assembles into higher-order membrane complexes. Its C-terminal cytosolic tail is critical for proper PM localization and self-association. Yeast two-hybrid library screening with C-terminus tail linked OsTET5 to phosphoinositide-Ca^{2+} signaling, metabolic reprogramming, and stress adaptation pathways. Similarly, OsTET2 is a stress-inducible rice tetraspanin responsive to water-deficit and ABA treatment. It localizes to the ER, Golgi apparatus, and PM, with its C-terminal tail being essential for correct subcellular targeting. OsTET2 overexpression improves drought tolerance through antioxidant regulation, proline-mediated osmotic adjustment, and better control of stomatal movements. It also modulates ABA sensitivity during seed germination and enhances blast susceptibility, indicating a role in abiotic and biotic stress signaling. It associates into higher-order membrane complexes and interacts with proteins associated with redox regulation, defense, metabolism, and cellular homeostasis. AtTET2 is induced by ABA, osmotic and heat stress, localizes to the ER and PM and regulates ABA-dependent seed germination and acquired heat stress tolerance. Notably, it interacts with membrane proteins such as aquaporin and fluoride exporter. Overall, this study demonstrates that plant TETs are key regulators of stress responses and represent promising targets for improving stress resilience in crops.

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