

## CHAPTER 47

### PLANT MOLECULAR BIOLOGY

#### Doctoral Theses

01. GHOSH (Soma)  
**Molecular characterization of calcium and potassium transporters regulating stress responses in plants.**  
Supervisor: Prof. Girdhar K. Pandey  
Th 28467

#### *Abstract*

Plants have an extremely complex network of signaling pathways, and calcium (Ca<sup>2+</sup>) plays a vital role in regulating signaling events. In addition to being an essential macro-nutrient, Ca<sup>2+</sup> takes part in a number of physiological, biochemical, and developmental processes. The Ca<sup>2+</sup> signaling pathways are characterized by four phases: stimulus-specific signaling that mobilizes Ca<sup>2+</sup> signals, Ca<sup>2+</sup> influx towards the cytoplasm, stimulation of Ca<sup>2+</sup> signaling pathway and the restoration of [Ca<sup>2+</sup>]<sub>cyt</sub> to resting levels through the Ca<sup>2+</sup> efflux system. These phases utilize a multi-component Ca<sup>2+</sup> signaling toolkit. Transporters are the critical components that maintain the Ca<sup>2+</sup> homeostasis and participate in plants' various biotic and abiotic stress responses. In addition to Ca<sup>2+</sup>, Potassium (K<sup>+</sup>) plays a vital role as a macro-nutrient, governing a range of essential physiological processes such as maintaining cellular turgor pressure, regulating stomatal movements and serving as a cofactor for various enzymatic activities etc. This study comprises four objectives that illustrate the potential role of Ca<sup>2+</sup> and K<sup>+</sup> transporters under stress responses in plants. The function of Cation/ Ca<sup>2+</sup> exchangers (CCXs), namely CCX3 and CCX5, in the heavy metal stress response has been illustrated in the first two objectives, and a thorough analysis of how the Ca<sup>2+</sup> signaling kinase protein, CIPK9 controls the activity of exchangers has been conducted. The function of another gene belonging to the same family, CCX1, in boosting the plant immune response against *Pseudomonas syringae*, is described in the third objective. The final objective explores the significance of a K<sup>+</sup> transporter KT12, a member of KUP/HAK/KT family and describes the molecular mechanism underlying CIPK9-KT12 complex regulation of K<sup>+</sup> uptake and homeostasis in plants. Overall, the research incorporated in this thesis illustrates the potential of the Ca<sup>2+</sup> and K<sup>+</sup> transporters and how they control the plant's ability to tolerate or resist stress conditions.

#### *Contents*

1. Thesis organization 2. Review of literature 3. CIPK9 interacts with CCX3 and regulates heavy metal stress responses in *Arabidopsis thaliana* 4. CIPK9-CCX5/CCX3 modules work together under heavy metal stress responses in *Arabidopsis thaliana* 5. CCX1, a cation/calcium exchanger, positively regulates *Pseudomonas syringae* infection responses in *Arabidopsis thaliana* 6. Functional analysis of *Arabidopsis* CIPK9- KT12 module in response to K<sup>+</sup> starvation condition 7. Material and Methods 8. Summary and Conclusion. References. Appendices.

02. Saini (Lokesh Kumar)  
**Functional Investigation of Protein Phosphatases and CIPK3-ABR1 Module During Various Stress Conditions in Plants.**  
 Supervisor: Prof. Girdhar K. Pandey  
Th 28468

*Abstract*

Due to their sessile nature, plants encounter a variety of abiotic and biotic stresses throughout their lifespan. These harsh environmental conditions have a negative impact on the growth and development of plants. To combat these stresses, plants have evolved intricate signaling mechanisms to bring about an adaptive response. Despite extensive research on plant adaptation to various stresses, there remains a lack of understanding regarding the delicate balance between a plant's stress and growth response. To enhance crop productivity, a detailed mechanism of signaling pathways under adverse environmental conditions needs to be elaborated. These signaling mechanisms are tightly regulated at different levels, like the transcriptional, post-transcriptional, translational, and post-translational levels. Post-translation modifications, such as reversible phosphorylation, have been shown to be very critical in various signaling mechanisms in plants. Reversible phosphorylation is governed by two types of enzymes: protein kinases and protein phosphatases. Therefore, this study focuses on comprehending the role of protein phosphatases and kinases under diverse stress conditions in Arabidopsis. In the first objective, a detailed functional analysis of a PP2C type phosphatase, AP2C1, suggested that it negatively regulates the K<sup>+</sup> deficiency response by inhibiting the function of Calcineurin B-like protein-interacting protein kinase, CIPK9. The second objective examines the role of two ABA-responsive protein phosphatases, AIP1 and PP2CA, in the negative regulation of the cadmium stress response. The third objective deciphers the role of two rice dual specificity phosphatases, OsPP42 and OsPP117, as positive regulators of the methyl jasmonate-induced oxidative stress response and the plant immune response to *Pseudomonas syringae* in Arabidopsis. The fourth objective investigates the role of the calcium-responsive kinase-transcription factor module, CIPK3-ABR1, in the plant immune response to *Pseudomonas syringae*. Overall, this study suggests that these genes play critical role in stress mitigation and these can be targeted to generate stress-resilient crops in the near future.

*Contents*

1. Organization of the thesis 2. Review of Literature 3. Functional analysis of AP2C1 in CIPK9-mediated K<sup>+</sup> deficiency response in Arabidopsis 4. Functional analysis of ABA signaling components, AIP1 and PP2CA protein phosphatases in Cd stress response in Arabidopsis 5. Functional characterization of rice dual-specificity phosphatases, OsPP42 and OsPP117 in methyl jasmonate and biotic stress responses in Arabidopsis 6. Functional role of CIPK3-ABR1 module in plant immune response to *Pseudomonas syringae* in Arabidopsis 7. Materials and methods 8. Summary and conclusions 9. References. Annexures.

03. TONU ANGAILA CHITHUNG  
**Interplay of CAMTAs and miRNAs in Regulation of Abiotic Stress Response and Ionic Homeostasis.**  
 Supervisor: Prof. Saurabh Raghuvanshi  
Th 28744

*Abstract*

MiRNAs are 21–24 nt long non-coding small RNAs that play a regulatory role in diverse gene expression in plants and animals. In an attempt to understand the dynamics of miRNome considering the crucial role of miRNAs in various aspects of plants, we explored genes involved in miRNA biogenesis machinery. Manual curation of the miRNA biogenesis genes revealed that the number of genes involved in the processing step is much more than those of transcription and maturation & functioning step and are also more conserved across plant kingdom. Orthologs of some of the biogenesis genes in rice were found to be differentially expressed and epigenetically methylated in drought stress condition. Moreover, QTL trait analysis revealed their role in various yield and development traits. Preliminary link between calcium signalling and miRNA expression in rice was earlier established in the lab, attempt was made for similar study in Arabidopsis. In general, CAMTAs (Calmodulin binding transcription activators) are important molecules in calcium signalling known to interact with Ca<sup>2+</sup> sensors CaM or CMLs. The *camta3*, *camta4* and *camta6* mutants displayed reduced germination efficiency indicating its role in germination and possibly via mediating ABA signalling. The *camta3* single mutant displayed reduced plant stature, delayed phase transitions and flowering. The *camta4-+/6-+* double mutants displayed increase leaf area and plant height indicating their role in plant architecture. The *camta* mutants were tolerant to salt stress and sensitive to dehydration stress compared to WT. Subsequently, in an attempt to understand how CAMTAs regulate miRNAs, sRNA-seq was done for the *camta* mutant lines. Members of miR169, miR395 and miR399 were found to be DEMs. The *camta* mutants were sensitive to phosphorus starvation. The Pi transporters like PHO1 and PHO2 along with PHR1 were also significantly upregulated in *camta* mutants suggesting that CAMTAs regulate phosphate metabolic pathway via regulating the key phosphate transporters. Under control condition, there was significant difference in the levels of sulphur content in *camta* mutants w.r.t WT and the anti-correlation expression of miR395::targets were also observed. Additionally, the loss of function of CAMTAs displayed tolerant phenotype compared to WT under sulphur starvation. Thus, indicating that CAMTAs are involved in the regulation of sulphur uptake and assimilation via miR395 mediated pathway. Moreover, important macro and micro nutrient elemental profiling revealed differential elemental content in *camta* mutants w.r.t WT. These findings revealed that CAMTAs regulate ionic homeostasis via miRNA mediated pathway.

*Contents*

1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion 6. Conclusions. Publications and conferences. References and annexures.