Conclusions
The work described in this dissertation was undertaken as part of a multi faceted approach towards probing into the molecular mechanism of the unique stress response behavior of cultured Arachis cells that are maintained in our laboratory. In brief, an auxin autotrophic *Arachis hypogea* culture was sensitive to stress treatments leading to water loss and ABA treatments. Transient exposure to auxin for as less as an hour in the post stress state was sufficient for these cells to restore its growth potential under auxin autotrophic conditions. Anticipating that CDPKs would have an active role in determining the stress response of the Arachis cells the objectives were to clone and characterize a CDPK from stressed Arachis cells and then understand its role in the stress response behavior of the Arachis cells. The salient observations made are as follows:

**Chapter I**

- A cDNA clone for a CDPK was developed through RTPCR with total RNA collected from *Arachis cells* that were subjected to osmotic stress (0.4M sucrose 4 days). The nucleotide sequence of the Arabidopsis CDPK named AhCPK2 have been lodged with the GenBank ([gi:67479988](https://www.ncbi.nlm.nih.gov/nuccore/67479988)). The predicted amino acid sequence contains a kinase catalytic domain with subdomains I to XI of Ser/Thr kinases, a junction domain and a calmodulin like domain (CaMLD) with four calcium binding motifs. In addition to having the core domain arrangement of a canonical CDPK, AhCPK2 contains a bipartite nuclear localization signal (NLS) sequence (PROSITE code no PS00015) in its junction domain and the sequence of the second calcium binding EF hand loop (PROSITE code no PS00018; in its CaMLD was a deviation from the consensus.

- BLAST search and subsequent domain analysis revealed that several other CDPKs from different species showing strong homology with AhCPK2 also have a similar domain composition. They have bipartite NLS in their junction domains and have nonconsensus calcium binding EF hand loops in their respective CaMLDs. In essence we found that in the entire CDPK family there is an obligatory coupling between presence of NLS in their junction domain and non consensus Ca binding EF hands in their respective CaMLDs. Thus this subgroup of noncanonical CDPKs represents a unique and discrete case where interdomain compensatory changes are accompanied with a domain evolution.
Heterologous expression and attempts to characterize the expressed polypeptide revealed that this kinase failed to phosphorylate any known exogenous substrate. Interestingly, AtCPK10 which is a member of our target group, also failed to phosphorylate any exogenous substrate. These evidences suggest this subgroup of noncanonical CDPKs to be active against unique substrates that are yet to be identified.

The calcium binding property of the Arachis CDPK significantly deviated from those of the canonical CDPKs and is in strong consistence with the PROSITE prediction of a nonconsensus calcium binding EF hand loop in its CaMLD region. The binding capacity of the Arachis JD-CaMLD was determined to be 3.1 mol of calcium per mole of protein at saturation and the $K_v$ value was determined to be 392 $\mu$M. Under identical conditions the JD-CaMLD of a canonical CDPK with four consensus EF hands could bind 3.8 mol of calcium per mole of the polypeptide with a $K_v$ value of 0.19 $\mu$M. In spite of having wide differences in calcium binding properties, at saturating concentrations of calcium, the global fold (intramolecular structural changes accompanied with calcium binding), of the regulatory apparatus in the Arachis CDPK remain similar to its canonical counterpart. The activation principle of the Arachis CDPK thus appeared to follow the known activation principles for CDPKs.

Attesting to the presence of NLS in the JD, in vitro binding assays indicate the JD-CaMLD polypeptide of AhCPK2 to interact with nuclear transport factors of the importin family. Interaction of importin with the Arachis CDPK could only be detected at subsaturating concentrations of calcium indicating that under such conditions interdomain rearrangements allow functional exposure of the NLS sequence in the JD and its subsequent interaction with the nuclear transport factors. Absence of binding under saturating conditions suggested that such conditions bury the JD in the CaMLD in such a manner that the NLS becomes inaccessible to importins. The discrete difference in importin binding with the Arachis CDPK at saturating and subsaturating calcium concentrations suggest the kinase to have an intermediate structure at low calcium signature that is capable of interacting with importin. Homology modeling also indicated the feasibility of interaction of importins with the NLS present in the JD of such CDPKs in their activated form.

It appears that by virtue of having atypical calcium binding properties, our target group of CDPKs can sense a specific low calcium signature which helps in functional exposition of the NLS in the JD due to noncanonical interaction between
• the JD and its CaMLD. In response to the same signature canonical CDPKs with four consensus calcium binding sites are expected to have successful intramolecular interaction of JD with their respective CaMLDs and this would prevent access of importin to its JD. This probably justifies the coupling between a nonconsensus calcium binding site in a CDPK with the presence of an NLS in its JD.

Chapter II

• AhCPK2 encodes for a 58 kD polypeptide which was localized both in the soluble and the microsomal fractions in the normal Arachis cells.

• AhCPK2 is constitutively expressed in the Arachis cells both transcriptionally and post transcriptionally, and osmotic stress (0.4M sucrose 4 days) or simple ABA treatments (5 mg/L 1 day) that lead these cells to quiescence do not affect its expression. Auxin treatments also had no effect on AhCPK2 expression. AhCPK2 shows 88% homology with a stress or ABA induced CDPK AtCPK10 from Arabidopsis but unlike AtCPK10, AhCPK2 fails to respond to stress treatments by changing its level of expression.

• AhCPK2 in its soluble as well as its microsome associated state remains phosphorylated in normal cells with phosphorylations in threonine residues. The state of phosphorylation of soluble AhCPK2 differed under normal and stressed conditions. While it was phosphorylated in the normal cells, it dephosphorylated under osmotically stressed conditions. Immunoprecipitated AhCPK2 got phosphorylated from both normal and stressed cells suggesting that unphosphorylated AhCPK2 in the stressed cells was generated by stress induced dephosphorylation. Under prolonged stress AhCPK2 regained its state of phosphorylation and was dephosphorylated again when put back in normal conditions clearly showing that dephosphorylation of AhCPK2 is not a response to water loss per se but to a change of osmotic potential in the environment. No such change was noted in presence of ABA treatments indicating the noted oscillation of phosphorylation of this kinase to be an upstream event.

151
Subcellular fractionation revealed that only dephosphorylated form of AhCPK2 in osmotically stressed cells was localized in the nucleus. In normal cells or in ABA treated cells AhCPK2 do not dephosphorylate in its soluble or cytoplasmic form and was not detected in the nucleus. Thus an interesting correlation between the state of phosphorylation of AhCPK2 with its nuclear localization was noted. It is suggested that dephosphorylation of AhCPK2 upon perception of change in osmotic potential is an upstream event that is necessary for the nuclear localization of the kinase and initiation of downstream signaling.