1. INTRODUCTION
Plant productivity is adversely affected by a variety of abiotic stress factors such as salinity, cold, drought, floods, metal toxicity and high temperature (Boyer, 1982). Continued breeding exercises in the past few decades resulted in the enhanced genetic potential and performance of crop plants under such conditions (Acevado and Fereres, 1993). However, much more is desired to be achieved in ensuring a sustainable food production with the available land and water resources. Encroachment of cultivable land by the exploding urban populations has particularly posed a major threat to crop production, necessitating the development of stress tolerant cultivars suitable for the hitherto unused saline and drought-prone soils.

One of the main hurdles in reaching such a goal has been the limited knowledge on stress tolerance process, known to be governed by many diverse genes. Such Quantitative Trait Loci (QTL) have been uncovered by recent techniques like Restriction Fragment Length Polymorphism (RFLP) and Randomly Amplified Polymorphic DNA markers (RAPD). However, such techniques merely identify the DNA fragments associated with stress tolerance traits, the knowledge of which is of limited utility without deciphering the physiological and molecular basis of marker-phenotype relationship. Further, the new advancements in genetic engineering can not be applied to the development of abiotic stress tolerant crops without the identification of key molecules/components that confer tolerance to diverse stress conditions. For instance, the available recombinant DNA methods and the transgenic plant production technology can identify and transfer key genetic elements across the species and generic barrier, which could be exploited for the production of stress tolerant crops. To this end, extensive studies by a large number of groups are now beginning to focus and identify the central components that operate in divergent stress conditions.

Abscisic acid (ABA) is one such key component that attracted quite an attention in the recent past. Initially thought to be involved in the leaf abscission and stomatal movement, it was soon recognized to play a central role in many of the developmental events in a plant's life cycle. ABA has been convincingly proved to mediate various developmental and physiological processes that affect the agronomic performance of crop plants including the embryomorphogenesis, seed protein synthesis, seed maturation and
onset of dormancy. Most importantly, ABA has been implicated in many of the fundamental abiotic stress responses both at transcriptional and post-transcriptional level (Chandler and Robertson, 1994). ABA levels are known to increase in maturing embryos just before the onset of desiccation tolerance, as a programmed developmental event and in the vegetative tissues during abiotic stresses, resulting in the activation of similar set of genes and pathways (Skriver and Mundy, 1990).

Interestingly, the vegetative tissues in response to ABA and abiotic stress conditions start to mimic some of the molecular and biochemical events exclusive to the maturing embryo, probably by recruiting a similar molecular machinery for the induction of related sets of genes. Identification of similar ABA responsive elements (ABREs) in the regulatory regions of many ABA/stress-induced genes confirms such a possibility. These gene products were presumed to be of paramount importance in conferring desiccation tolerance to plants. The widespread utilization of a conserved stress responsive machinery controlled by a central component like ABA, therefore, supports the existence of common candidates controlling complex traits such as stress tolerance in diverse plants. Hectic efforts are on by various groups to identify the signal transduction cascade starting from the signal perception to the transcriptional activation of stress responsive genes mediated by ABA. Apart from the induction of such stress responsive proteins whose functions are still speculatory, ABA is also widely known to activate a set of anti-oxidant and anti-stress enzymes. Therefore, unraveling the role of ABA would certainly pave the way for a belter understanding of stress tolerance process that could eventually contribute to crop improvement.

In cereal crops, exogenous ABA application has been found to mediate several biochemical events involving the induction of many gene families (Chandler and Robertson, 1994). Particularly, ABA was found to induce several proteins belonging to the Rab family, lea family and other gene products like SalT and dehydrins in rice (Skriver and Mundy, 1990; Moons et al., 1995; Claes et al., 1990; Caplan et al., 1990). Further, many of the ABA responsive enzymes were found to be involved in the anti-oxidative functions in the cells as the active free radical species pose a major threat to the cellular components under abiotic stress condition. However, there were not many attempts to
understand the role of ABA in the salinity and chilling tolerance processes in the rice plant. In rice, ABA has been found to induce many stress responsive enzymes like superoxide dismutase and ascorbate peroxidase (Karunasree, 1998). Further, two polypeptides of molecular weight 23 kDa and 15 kDa were found responsive to ABA and other abiotic stresses in rice (Rao et al., 1993; Rao, 1993; Reddy, et al., 1993; Karunasree, 1992). A striking feature of all these polypeptides is their high boiling stability which indicates their high hydrophilic nature that has been a characteristic property of many stress responsive proteins reported earlier. Further characterization of these proteins and an analysis of their possible role could lead to a better understanding of stress tolerance processes in rice.

Accumulation of free proline in the cytoplasm is another widespread event observed in crop plants in response to abiotic stresses and ABA application. Intra-cellular hyper-accumulation of osmolytes is governed by traits that are evolutionarily conserved spanning across the prokaryotes and eukaryotes, presumably conferring tolerance to the respective organisms. Many mutants were isolated in plants and bacteria that accumulate high amounts of the osmolytes and withstand the abiotic stress conditions (Dix, 1993; Csonka, 1989; 1981). Although plant species differ in their sensitivity and response to unfavorable growth conditions like decrease in water potential, all plants are inherently capable of adjusting to such stresses with an increase in concentration of some osmolyte or the other in the cytoplasm.

Osmolytes seem to perform diverse functions such as osmoregulation, osmoprotection, carbon and nitrogen storage, protection of cellular structures and lipid bilayers (by scavenging active oxygen). Further, the term “compatible solutes” aptly describes their non-interfere with the biological functions of the cell even at their highest accumulated concentrations. Osmolytes are also known to stabilize the proteins and membrane structures under diverse stress conditions, which have been widely described. There were many reports suggesting the protective role of osmolytes under stress conditions (Xin and Lee, 1993; Le Rudulier et al., 1984). However, the biophysical and molecular basis of protection offered by osmolytes is very scantily studied, particularly with reference to proline.
The present work was undertaken to explore the biochemical and biophysical basis of the role of ABA and proline in stress tolerance process in rice. Rice, being one of the most important crops of the developing world, was selected as a model plant to carry out these studies. About 60% of the world population consume rice as their staple food with most of its production and consumption being restricted to developing world. Further, the low yield of rice production in India that are mainly attributed to the divergent geoclimatic conditions, require the development of efficient stress adaptable cultivars. Attempts were made here to analyze the biochemical basis of stress tolerance processes with a focus on stress responsive proteins induced by ABA using SDS-PAGE and Western analysis. A relatively easier and reproducible screening method was established that makes use of the etiolated seedlings to score the tolerance mediated by ABA. Further, changes in in vitro phosphorylation status of proteins upon the application of ABA was investigated in the present work that could possibly lead to the identification of components involved in the signal transduction pathway mediated by ABA.

The work also includes in vitro assays that were necessary to analyze the biophysical interaction of osmolytes proline and betaine, with major biomacromolecules such as proteins and DNA. These in vitro studies were primarily undertaken to mimic the sub-cellular interactions between osmoprotectants and biomacromolecules whose association may be transient, but vital due to the abundance of osmolytes under the stress adapted conditions. The structural and functional analysis of the interaction could eventually lead to the establishment of vital clues in understanding stress tolerance processes in the cells. Further, the implications of in vitro assays performed do not just confine to rice plant but to plants in general as they tend to explore the basis of stress adaptation in vivo.

Objectives of this thesis are

- To study the major biochemical changes in rice in response to ABA application and correlate such changes with salinity and chilling tolerance processes
- To standardize a rapid and reliable system to screen the rice seedlings for salinity and chilling tolerance processes mediated by ABA.
• To analyze the role of stress/ABA responsive proteins in salinity and chilling tolerance processes in rice using various inhibitors.
• To understand proline-protein interactions in vitro to analyze the protective role of proline under various stress conditions
• To study the proline/betaine-DNA interactions to unravel the mechanisms of tolerance mediates by these osmolytes under salinity stress conditions.