INTRODUCTION

In order to survive in a dynamic environment all living cells must be able to identify and respond to variation in specific extracellular signals. In contrast to many animals that can seek protection from environmental stresses, plants are unable to change their location, thus are required to adjust to the changes and stresses of their environment to survive. This adjustment is mainly through the modulation of their gene expression at the transcriptional level. Target cells recognise signals through specific receptor on the outer surface of their plasma membrane. The binding of signals to receptors initiates a series of rapid events which eventually translate this external signals into a specific cellular response mediated by a selective alteration of the intra-cellular metabolism. Calcium plays a major role in transduction of various types of external stimuli into cellular responses. Calcium does not act in its free ionic form but, rather requires a binding protein, called calmodulin. The protein calmodulin is implicated in multitude of enzymatic systems known to be calcium regulated.

Calmodulin and calmodulin related proteins have been shown to regulates a number of fundamental cellular activities such as cyclic nucleotide and glycogen metabolism, intra-cellular motility (micro-tubules and micro-filaments), Ca^{2+} transport, Ca^{2+}-dependent protein-kinases and phosphorylation.
Calmodulin is ubiquitously present in the cells of all the eukaryotes. It is phylogenetically conserved and is composed of 148 amino-acids. Physical and chemical properties of calmodulin from all the sources examined so far are similar. The protein, calmodulin is stable to heat and acid treatment. Calmodulin contains four similar calcium binding domains, each consisting of 12 amino-acids. Out of these amino acids, the first and last show conservation in all the organisms studied.

The high resolution crystal structure of calmodulin reveals a dumb-bell shaped protein with two domains separated by a long central helix. Each domain contains two Ca\(^{2+}\) binding loops (Babu et al., 1988).

In plants, calmodulin has been thought to play an important role in signal transduction in various molecular and biochemical events induced by a variety of factors such as light, hormones and plant-pathogen interaction. The precise role of calmodulin in various signals transduction pathways in plants is yet to be elucidated. Though the protein is ubiquitously present in all the eukaryotic cells, its level varies between cells and also during cell cycle (Rasmussen et al., 1990). Recent studies in the case of *Arabidopsis* indicated that genes for calmodulin/calcium binding proteins could be induced and regulated in response to different environmental factors including rain, wind and touch (Braam and Davis, 1990). It apparently indicates that CaM gene system is inducible, though the presence of certain basic level
of the CaM protein suggests certain degree of constitutive expression of the gene. From animal system and also from multiple distinct cDNA clones of calmodulin in plants indicated that it belongs to a small multigene family. Whether each member of the gene family shows both constitutive and inducible or some are under constitutive control and others are inducible is an open question.

Regulation of gene expression in eukaryotes is mediated by specific interaction of trans-acting proteins components to DNA elements present in the upstream region of the gene. Since the most important portion of the gene in terms of regulation of its expression is its 5'-upstream sequences, it is imperative that the gene with its adjoining sequences be isolated, characterized and sequenced. The characterization and sequence determination is crucial in understanding the underlying molecular mechanism in activation of plant calmodulin gene (s) in response to various factors. If a single gene shows both constitutive as well as inducible expression, it would also contain regulatory elements that interact with trans-acting factors through probably some way different than that of the gene which is only under inducible control.

Therefore, the basic aim of the work embodied in this thesis was to clone, characterise and determining the sequence of the calmodulin gene with a view to gain some insight into its sequence organization and the existence of regulatory elements.
The plant species selected was *Arabidopsis thaliana* which is an excellent experimental model system for the study of plant molecular biology owing to its small genomic size, short life cycle, near absence of dispersed middle repetitive DNA and a well established classical genetics. The haploid genome size of *Arabidopsis* is only 70,000 Kb that allows a relatively small number of plaques to screened even for a single copy gene.

The main objectives of the work described in this study are:

1. Isolation of the calmodulin gene from a genomic library of *Arabidopsis thaliana* constructed in λ-EMBL-4 vector using a heterologous cDNA probe,

2. Restriction mapping of the clone and localization of calmodulin complementary sequences within the inserts,

3. Determination of nucleotide sequences of the CaM gene including its flanking regions,

4. Analysis of sequence data with respect to the amino acids of the protein and