Introduction
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Plants have evolved remarkable strategies to co-ordinate their growth and development in response to internal and external signals in varied and changing environments. Although plant response to the physical and chemical signals have been known for some time, the mechanisms that couple signal perception to the response have taken longer to identify. By mid-1980's calcium was recognized as a potential intracellular messenger in plants, modulating responses through changes in its cellular concentration (Hepler and Wayne, 1985).

Calcium is thought to act as an intracellular second messenger within plant cells in response to a wide range of hormonal and environmental stimuli (Poovaiah and Reddy, 1993). Studies have shown that touch and wind elevate cytosolic Ca\(^{2+}\) (Knight et al., 1992). Various other stimuli like light, cold, pathogen infection and stress etc. are known to induce changes in [Ca]. Direct measurement of cytosolic free calcium, [Ca\(^{2+}\)], have indicated that specific cells respond to a range of stimuli by increasing [Ca\(^{2+}\)] prior to physiological effect.

Calcium interacts with a whole range of binding protein, including calmodulin, which in turn act as the biochemical switch to alter the activity of specific target proteins. The hallmark of calmodulin mechanism of action is that it transduces second messenger Ca\(^{2+}\) signal by binding to and altering the activity of a variety of other proteins. Upon binding to calcium, calmodulin interacts with several key enzymes and structural proteins and sequentially regulate their activity or function.

The calmodulin gene of *Arabidopsis thaliana* belongs to a multigene family consisting of at least six members. A member of this family, *AtCaM5* has been cloned and sequenced in our laboratory (Chandra and Upadhyaya, 1993). This gene consists of two exons and a single intron. The first and second exon are 76 bp and 371 bp respectively and intron is 364 bp. The role of CaM has been implicated in controlling a large number of physiological processes and much of this is based on the use of CaM antagonists (Roberts et al., 1986). Traditionally, the pharmacological compounds have been used in the form of CaM antagonists to inhibit CaM function. However, this approach suffers serious drawbacks that it inhibits all the forms of CaM proteins and since CaM being an
essential protein the plants are not able to survive under these conditions. Therefore transgenic approach using antisense RNA technology to down regulate gene expression offers advantage since the down regulation will vary almost from 0% to almost 100%. Hence, it will not completely block CaM and the transgenic plants can survive, which will help to study the CaM function. Calmodulin shows high degree of conservation at the nucleotide and amino acid level in various domains of the protein hence one can modulate CaM gene expression by using sense and antisense RNA technology. Recent advances in molecular techniques have offered new approaches to understand the role of CaM in plant growth and development by over expressing or blocking expression of CaM. Antisense and sense RNA strategy have become a major tool in plants to study the effect of down and up regulation of a gene or a set of gene(s). In this study transgenic approach has been utilized to study the consequences of altered levels of CaM on plant growth and development using sense and antisense constructs of AtCaM3 cDNA under the control of a strong constitutive promoter i.e. CaMV 35S.

A number of CaM cDNAs were isolated by screening Arabidopsis cDNA library using AtCaM5 as a probe (Sharma and Upadhyaya, unpublished). The cDNA clones isolated were AtCaM3 (~800 bp), a partial clone containing only one Ca\(^{2+}\) binding domain i.e. in the first exon and a 22 kDa calcium binding protein. From which we have used at AtCaM3 for further studies. Till date it has been shown that AtCaM3 is expressed in aerial parts of the plants except in the floral stalks (Perera and Zielinski, 1992). It is known that CaM gene is responsive to many external signals like cold shock (Polisensky and Braam, 1996), wounding (Bergey and Ryan, 1999), Touch (Ito et al., 1995). Calmodulin from different plants show high sequence conservation (more than 92%) at the amino acid level. This conservation is also seen among plants and animals. In Arabidopsis there seems to be multiple CaM genes. Till date six CaM genes and four isoforms are known in Arabidopsis. Among the Arabidopsis CaM gene family the exonic sequences of various gene members showed high degree of conservation but the untranslated 5' upstream and 3' downstream coding region show sequence divergence confirming the distinctintness of each member of the gene family. In Arabidopsis AtCaM5, cloned and sequenced in the laboratory, the coding region is interrupted at the 25\(^{th}\) amino acid by a single 491 bp intron.
Studies showed that in most of the CaM sequences studied, the intron position is constant but the length of the intron is variable. Based on this knowledge it was concluded that different members of the gene family could be identified on the basis of variable intron length.

The main objectives of our study were:

1. Generation of transgenic plants containing sense and antisense constructs of *AtCaM3* cDNA
2. Production of polyclonal antibodies for screening of the transgenic plants.
3. Study of response of transgenic and wild type plants to salt stress.
4. Study of the calmodulin gene family in *Arabidopsis* and *Brassicas*. 
