Plants frequently encounter stresses that adversely affect growth, development, or productivity. Stresses can be biotic, imposed by other organisms, or abiotic, arising from an excess or deficit in the physical or chemical environment. Among the environmental conditions that cause damage is water logging, drought, high or low temperatures, excessive soil salinity, and inadequate mineral nutrients in the soil. Abiotic stresses are the principal cause of crop failure worldwide dipping average yield for most crops as by 50% (Boyer, 1982; Bray et al., 2000). Besides this, world’s population continues to expand day to day, leading to severe food scarcity in near future. To meet the demands of food production, if the crops can be redesigned to better cope up with these adverse conditions, agricultural production can be increased dramatically. Therefore, understanding of plant tolerance to different stresses is of fundamental importance.

Drought is one of the important abiotic stresses cause adverse effects on productivity of crops (Boyer, 1982). About one third of world’s arable land suffers from inadequate supplies of water for agriculture, and yields of rain-fed crops reduced
by drought. Plants experience drought stress either when the water supplies to root 
become difficult or when the transpiration rate becomes very high. These conditions 	en often coincide under arid and semi arid climates.

Plants respond to drought at physiological, cellular and molecular levels. The 
responses of plants to drought stress are highly complex, early responses of plants to 
drought stress usually help the plant to survive for some time, while the acclimation 
of the plant subjected to drought indicated by the accumulation of certain new 
metabolites associated with the structural capabilities to improve plant functioning 
under drought stress (Ramachandra Reddy et al., 2004). The morphological and 
physiological responses to drought stress include increase in root length and density, 
plant architecture, the reduction in leaf size, variation in leaf cuticle thickness, sunken 
stomata, specialized leaf surfaces to decrease the rate of transpiration, leaf rolling, 
hormonal regulation, desiccation tolerance (membrane and protein stability), 
maintenance of photosynthesis, timing events during reproduction (Bohnert et al., 
1995; Shinozaki and Yamaguchi-Shinozaki, 1997; Bray, et al., 2000). Cellular 
responses to water deficit includes loss of turgor, change in plasma membrane fluidity 
and composition, changes in water activity and/or solute concentration, protein-lipid 
interactions (Bray, 1997; Heide and Poolman, 2000).

The major molecular responses of the plants exhibit to drought stress is altered 
gene expression. There is up- and down-regulation of many genes in response to 
drought, thought to protect cells by the production of stress proteins and also by the 
regulation of genes for signal transduction (Shinozaki et al., 2003). Many genes that
were up- and down-regulated by drought stress have been isolated and reported in *Arabibopsis*, rice, barley, maize, sorghum and wheat (Oztur *et al.*, 2002; Seki *et al.*, 2002; Rabbani *et al.*, 2003; Zheng *et al.*, 2004; Way *et al.*, 2005). The drought induced genes were usually classified into two groups according to Shinozaki and Yamaguchi-Shinozaki (1997). The first group included the proteins that might directly protect plants cells from stress-related damages, such as water channel proteins, proteases and various detoxification enzymes, and they called as functional proteins or down stream genes. The second included the protein factors that regulate gene expression and signal transduction in stress response termed as regulatory or up-stream genes (Oono *et al.*, 2003). They include various transcription factors, protein kinases, protein phosphates, enzymes involved in phospholipid metabolism and other signaling molecules such as calmodulin binding proteins.

Plant cells are highly equipped with efficient mechanisms to perceive, transduce and respond to a wide range of signals via receptors results in generation or synthesis of non-proteinaceous molecules often termed as messengers. These messengers control diverse cellular processes through sensors (proteins/enzymes) (Bowler and Fluhr, 2000; Reddy, 2001; Rudd and Franklin-Tong, 2001; Yang and Poovaiah, 2003). The messengers include Ca$^{2+}$, small organic molecules such as cyclic nucleotide monophosphates and inorganic molecules such as hydrogen peroxide and nitric oxide (Levine *et al.*, 1994; Trewavas and Malho, 1997; Sanders *et al.*, 2002; Demidchik *et al.*, 2002b; Guo *et al.*, 2003; Scrase-Field and Knight, 2003; Talke *et al.*, 2003).
Calcium has many important structural and physiological roles in plants and make up the 5% of the dry weight to the plants (Broadley et al., 2003). It is important in maintaining the stability of the cell walls, membranes and membrane bound proteins, due to its ability to bridge chemical residues among these structures (Helper and Wayne, 1985). It is absorbed from the soil solution and transported to various sites in the plants through the xylem, like other minerals. It mediates several plant processes like cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell elongation, cell differentiation, cell polarity, photomorphogenesis (Bush, 1995).

Calcium is reported to be participates in adaptation to various stress factors (Knight, 2000). Signals associated with chilling stress, heat shock, salinity and drought, anoxia, elicitors, osmotic shock, mechanical stimulation, as well as oxidative stress, induce a transient increase in cytosolic Ca$^{2+}$ level. Calcium is the principal component in the transduction of hormonal signals induced by gibberellic acid, ABA, IAA, and cytokinins.

The Ca$^{2+}$ concentration in the resting cells are the nanomolar range (100-200 nM) where as it is millimolar range (1-100 mM) in extracellular and intracellular Ca$^{2+}$ stores (Trewavas and Malho, 1997; Reddy, 2001; Rudd and Franklin-Tong, 2001). A large portion of the total Ca$^{2+}$ is bound to cell walls and anionic macro molecules inside the cell. And the Ca$^{2+}$ is compartmentalized into organells functioning as Ca$^{2+}$ stores, with the central vacuole containing most of the water-soluble Ca$^{2+}$.

During Ca$^{2+}$ mediated signal transduction there is elevation of cytosolic Ca$^{2+}$ levels by the channels, pumps and transporters in response to a wide variety of
signals. And the elevation is up to 3 µM concentration and the magnitude and
duration of Ca\textsuperscript{2+} changes vary depend on the signal or cell type. Since vacuole is the
largest Ca\textsuperscript{2+} pool in a typical plant cell, vacuolar Ca\textsuperscript{2+} channels play a critical role in
Ca\textsuperscript{2+}-mediated signal transduction as well as in Ca\textsuperscript{2+} homeostasis (Bush, 1995;
Hetherington and Brownlee, 2004) during stress conditions in the plants. The proton-
gradient force-driven Ca\textsuperscript{2+}/H\textsuperscript{+}antiporters and ATP-driven Ca\textsuperscript{2+}-ATPases act in
opposite way to Ca\textsuperscript{2+}-permeable channels by pumping elevated calcium into the
exterior and/or intracellular organells. The activities of pumps and transporters
terminate the Ca\textsuperscript{2+} signature as well replenish Ca\textsuperscript{2+} stores for generation of future Ca\textsuperscript{2+}
signatures.

Ca\textsuperscript{2+}/H\textsuperscript{+} antiporters can drive Ca\textsuperscript{2+} against its concentration gradient at the
expense of energy of the electrochemical proton gradient (Blackford \textit{et al.}, 1990). These are high-capacity, low-affinity transporters that have been physiologically
characterized from a variety of plants and appear to locate predominantly on the
vacuolar membrane, but also on the plasma membrane and chloroplast thylakoid
membrane (Luo \textit{et al.}, 2005; Qi \textit{et al.}, 2005). The Ca\textsuperscript{2+}/H\textsuperscript{+} antiporters are usually
activated upon drastic increase in the cytosolic calcium content. The first cloned gene
encoding Ca\textsuperscript{2+}/H\textsuperscript{+} antiporters from the plants were \textit{CAXI} (calcium antiporter/cation
exchanger) (Hirschi, 1996; Hirschi, 2001). The gene was identified in yeast and
demonstrated its ability to restore growth on high Ca\textsuperscript{2+} media of yeast mutant
defective in vacuolar transport.
Foxtail millet (*Setaria italica* L.), an important small millet belongs to family poaceae. Foxtail millet is known for its higher tolerance to drought and salinity. Genotypic variation exists among various crop species including foxtail millet. There are few reports in literature, concerning physiological and biochemical responses of foxtail millet to abiotic stress and very little is know about the genetic mechanism of stress tolerance in foxtail millet. In our earlier studies on the drought tolerance of foxtail millet cultivars, we have screened four local cultivars viz., Prasad, Chitra, Krishnadevaraya and Lepakshi and identified cultivar Prasad as drought tolerant and Lepakshi as a drought susceptible. Although some physiological mechanisms have been identified, which of these mechanisms are important and what mechanisms operate at biochemical and molecular level in these cultivars to impart stress tolerance is unclear.

Depending on its growth conditions and life cycle, there is high probability that this plant contains a large number of genes that can be used to provide stress tolerance to this crop. Due to high genetic similarity among millet crop genomes, comprehension of the underlying genetic mechanism of various stress related genes from this plant will be of great advantages to transform these genes into other cultivars as well as other crop plants to make them stress tolerant.

The present study therefore, aimed at cloning and characterization of a gene encoding a putative calcium antiporter (*CAX1*) from stress tolerant foxtail millet cultivar subjected to drought stress.
Objectives:

- To study stress marker traits such as Biomass, Relative water content (RWC), Cell membrane stability (CMS), Total chlorophyll content (TCC) and Proline levels in tolerant cultivar.

- To clone and characterize a gene encoding a putative calcium antiporter (CAX1) from tolerant cultivar.