Abiotic stress imposed by drought, salinity and extreme temperatures acts as major impediment and pose serious threat to the growth and productivity of crop plants. Research into the plant response to water stress is becoming increasingly important, as most climate-change scenarios suggest an increase in aridity in many areas of the globe (Petit et al., 1999). On a global basis, drought (assumed to be soil and/or atmospheric deficits), in conjugation with coincident temperature and radiation, pose the most important environmental constraints to plant survival and to crop productivity (Boyer, 1982). Agriculture is a major user of water resources in many regions of the world. With increasing aridity and growing population, water will become an even scarcer commodity in near future. Even though in viable agriculture severe water deficit should be a rare (but catastrophic) event (Passioura, 2002), a better understanding of the effects of drought on plants is vital for improved
management practices and breeding efforts in agriculture and for predicting the fate of natural vegetation under climate change.

Foxtail millet (*Setaria italica* L.) is a dry-land grain crop cultivated as intercrop along with groundnut, red gram, cowpea and *Pennecitum etc.*, in Rayalasema region of Andhra Pradesh and North Karnataka. Foxtail millet is also known as Italian millet, German or Hungarian or Siberian millet, and is said to be the origin of Eastern Asia (China). This crop sustains the lives of the poorest rural people and will continue to do so in the foreseeable future. Therefore, and because this crop is mostly consumed by disadvantaged groups, they are often referred to as “Coarse grain” or “poor people’s crop”. Improvements in production, availability, storage, utilization and consumption of this food crop will significantly contribute to the household food security and nutrition of the rural poor people. Foxtail millet grows in harsh environments where other crops fail to grow. In India foxtail millet is cultivated in 2.5 million hectares and the total annual production is 1.5 million metric tons.

Drought tolerance of plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. Genotypic variation exists among the various crops with in the same 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.
Seeds of foxtail millet (*Setaria italica* L.) (cv. Prasad, Lepakshi, Chitra and Krishnadevaraya) procured from Regional Agricultural station, Nandyal, Andhra Pradesh, India. Seeds were surface sterilized with 0.1% (w/v) sodium hypochlorite solution for 5 minutes, thoroughly rinsed with distilled water, germinated in plastic pots containing 2 kg soil:sand (2:1) mixture. The pots were maintained in the departmental botanic garden under natural photoperiod (10-12 hours; temperature 28 ± 4°C). Plants were maintained at 100% soil moisture level and allowed to grow for 21 days.

Twenty one day-old plants were subjected to a progressive water stress by withholding the water. Plant soil water status (percent soil moisture level) was measured at regular intervals by gravimetric method to obtain required soil moisture levels (100, 75, 50 and 25%). The leaf samples were collected from the plants once the soil moisture reached to 75, 50 and 25% and from respective controls (100% soil moisture level) and analyzed physiological and biochemical traits in four foxtail millet cultivars (Prasad, Lepakshi, Chitra and Krishnadevaraya) for their drought tolerance.

**Screening foxtail millets to identify tolerant cultivar**

To identify the drought tolerant cultivar, four cultivars were screened, based on biometry and biochemical traits, cultivar Prasad found to be relatively tolerant than other cultivars (cv. Lepakshi, Chitra and Krishnadevaraya).
To evaluate further the drought tolerant potentials of four foxtail millet cultivars, we analyzed leaf biomass, relative water content, cell membrane stability, total chlorophyll content and proline levels in four cultivars (21 day-old pot grown plants). Plants showed significant variations in the biomass levels, relative water content (RWC), cell membrane stability (CMS), total chlorophyll content (TCC) and proline levels. The cultivar Prasad showed relatively higher leaf biomass when compared to other three cultivars studied. Similarly, cultivar Prasad maintained high RWC, CMS, TCC and proline levels during drought stress conditions.

**Isolation, cloning and characterization of CAX1 gene**

The major objective of the present study was cloning and characterization of a novel stress responsive gene *i.e* CAX1 (calcium antiporter) from a drought tolerant foxtail millet cultivar Prasad. So in order to clone and characterize calcium antiporter (*CAX1*) gene from a drought tolerant foxtail millet cultivar Prasad, we isolated total leaf RNA, purified mRNA from RNA and converted into first strand cDNA by reverse transcription. The cDNA generated used for PCR using gene specific primers. The amplified PCR product analyzed on 1% agarose gel electrophoresis along with known molecular weight markers showed a clear single band with ~1300 bp in size without any non-specific reaction indicating the amplification of the gene. The amplified PCR product was purified by GenElute™ PCR clean-up kit (Sigma, USA). Further the purified product ligated into TOPO TA cloning vector (pCR 2.1 TOPO, 3.9 Kb in size) and transferred into *E.coli* DH5α™-T1R (Invitrogen, Germany). These
were plated on LB medium containing antibiotic (kanamycin), allowed for over night incubation and the white colonies were picked from the LB agar medium plates in the morning.

**Cloning and analysis of CAX1 gene**

The transformed white colonies were screened for the presence of insert by M13 primers in a colony PCR reaction. The colonies which showed the positive amplification of desired fragment and plasmids of these positive colonies were isolated. Further, recombinant PCR product was purified and sequenced. The nucleotide sequence of the full-length cDNA encoding for foxtail millet CAX1 was 1356 bp in length and deduced protein was composed of 451 amino acids with an apparent molecular weight of 44 kDa. The deduced amino acid sequence of foxtail millet CAX1 showed an overall 100% amino acid sequence identity with CAX1 isolated from rice (AB112656), 72% amino acids in maize (NP_001104999), 62% of amino acid sequences in mung bean (BAA25753), 79% similarities in wheat (ABO32802) and 66% similarities with Arabidopsis (AAF91349). However, the overall size of the protein was conserved among the reported CAX1 gene that suggested a strong conservation of protein architecture. *In silico* analysis revealed the presence of sodium/calcium exchanger domain, Ca\(^{2+}/H^+\) domain and Ca\(^{2+}/Na^+\) antiporter domains. These domains are similar to the other calcium exchanger proteins. The nucleotide sequence of the full-length cDNA encoding for foxtail millet CAX1 was 1356 bp in length and deduced protein was composed of 451 amino acids.
Summary

acids with an apparent molecular weight of 44 kDa.

Conclusions:

• Four foxtail millet cultivars studied for their drought tolerance and cultivar Prasad found to be relatively more tolerant than cultivars Chitra, Lepakshi and Krishnadevaraya.

• CAX1 gene cloned and characterized from a drought tolerant foxtail millet cultivar Prasad.

• The nucleotide sequence of the full-length cDNA encoding for foxtail millet CAX1 was 1356 bp in length and deduced protein was composed of 451 amino acids with an apparent molecular weight of 44 kDa.

• Structural analysis of the primary amino acid sequence of foxtail millet CAX1 indicates the presence of eleven trans-membrane domains at position spanning amino acid residues 50-72, 82-104, 132-154, 158-180, 190-212, 230-252, 278-300, 313-335, 348-370, 381-403, and 405-427 respectively.