World population is predicted to reach nine billion by 2050. According to the UN climatic report (http://www.solcomhouse.com/drought.htm) the Himalayan glaciers that feed to the Asia’s largest rivers (Ganges, Indus, Brahmaputra, Yangtze, Mekong, Salween and Yellow) may disappear by 2035 due to rise in temperature. In addition, if the present situation prevails over many years, it is expected that by 2025, 1.8 billion people will live in countries or regions with absolute water scarcity. Thus, the availability of fresh water is a major commodity to improve the economy of a country. However, on-going climatic changes, desertification, salinization, rapidly shrinking agricultural land for industrialization and/or habitat use are major threats to sustainable food production. Climate change is anticipated to have many negative impacts on agriculture due to elevated temperature, salinity, unpredictable rains and floods in some places, and prolonged drought in other parts of the world (Pachauri and Reisinger, 2007; Reynolds, 2010; Saha et al., 2010).

Over 35% of the world's land surface is considered to be arid or semiarid agricultural regions based on annual precipitation. Majority of world’s agricultural lands are exposed to various environmental stresses decline upto 50–70% major crop productivities (Mittler, 2006). Global need for plant derived products such as feed and food is increasing significantly because
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of rapid growth in world population. Crop plants play a pivotal role in supplying the ever-
increasing food and energy demands. In the coming decades, over three billion additional
inhabitants will have to be fed with an alarmingly decreasing arable land area (Morison et al.,
2008; Marshall et al., 2012). Hence, there is an obvious and urgent need to increase crop
productivity in the near future.

The long term practice of classical breeding for yield under well watered conditions
narrowed the genetic range of present cultivars ability to tolerate abiotic stress (Yang et al.,
2010). Advancement in plant molecular biology and genetic engineering approaches are being
explored not only in the identification of useful traits to improve sustainable crop yield with a
minimum input of water, fertilizers, and other agrochemicals but also modify the crop plants with
enhanced tolerance to abiotic stresses (Bartels and Sunkar, 2005; Vinocur and Altman, 2005;
Umezawa et al., 2006; Wang et al., 2009). With continually growing of global population, slowly
depleting water resources, and continued reduction of arable land is global concern for the
development of stress-tolerant cultivars and water-use-efficient crops for sustainable crop
productivity.

Stress is defined as environmental conditions that reduce growth and yield below
 optimum levels (Cramer et al., 2011). Plants are sessile, unavoidably encounter to various types
of abiotic stresses. In order to survive, plants have also evolved a myriad of defence strategies to
perceive and respond under stress conditions such responses are both elastic (reversible) and
plastic (irreversible) for acclimatization and adaptation of stress tolerance. Acclimatization
reflects the structural and physiological plasticity of the plant to cope with stress (Levitt, 1980).
Adaptation of stress tolerance includes the activation of molecular control mechanisms based on
the regulation of stress-related genes.
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Drought affects the plant productivity by inhibiting growth and photosynthesis (Chaves et al., 2009). Adaptation to drought is one of the most complex biological processes. Some apparent morphological changes under drought stress conditions are reduction of number and size of leaves and stomata, shedding of older leaves, thick cuticle and an increase in root to shoot ratio. Further, the physiological, biochemical and molecular changes under drought stress includes the reduced growth, osmotic adjustment, osmoprotection, transcriptional activation/inactivation of specific genes, transient increases in ABA levels, accumulation of compatible solutes and protective enzymes, increased levels of antioxidants and suppression of energy-consuming pathways. Such alteration of growth patterns in plants contributes to survival under water depletion conditions (Shao et al., 2008; Farooq et al., 2008; Hossain et al., 2009; Jaleel et al., 2009; Waseem et al., 2011).

Improvement of stress tolerant species by traditional breeding methods is slow, tedious, time consuming and unpredictable. Moreover, many quantitative traits are difficult to distinguish from environmentally-induced variations. The advent of new technologies in genetic engineering provides a faster and more reliable means for crop improvement, because it avoids the transfer of unwanted chromosomal regions. Moreover, through genetic engineering, multiple genes can be assembled and simultaneously introduced to the crop of interest (Hirayama and Shinozaki, 2010; Jain, 2015).

Drought tolerance is a multigenic trait involving participation of complex set of genes. With the advent of functional genomics approach, we can understand the functions of these genes. A large number of drought induced genes have been identified in a wide range of plant species (Seki et al., 2002; Shinozaki et al., 2003; Shinozaki and Yamaguchi-Shinozaki, 2007). These genes function to protect the cells from water deficit by producing important metabolic proteins and by regulating the genes which control signal transduction in drought stress response.
These stress related genes can be classified into two groups based on the functions of their encoding products. The first group of genes includes transcription factors, protein kinases, enzymes related to phospholipid metabolism and calcium signalling molecules such as calmodulin binding proteins and 14-3-3 proteins. Over-expression of some of these genes has also generated drought stress tolerant plants such as barley, rice, soybean and tobacco (Wang et al., 2005a). Most of these gene products may function in stress response and tolerance at the cellular level. The second group of genes, such as LEA proteins, dehydration inducible proteins, cold acclimation proteins, enzymes for metabolism of osmolytes, antioxidant proteins, ion transporters and channel proteins and chaperones or HSPs (Shinozaki and Yamaguchi-Shinozaki, 2007).

Drought stress generates secondary stresses like oxidative and osmotic stresses in plants. Drought stress initiates the cellular dehydration that leads to osmotic changes. Osmotic adjustment is accomplished with the accumulation of compatible solutes in plants. But, when plants are exposed to high light intensity under drought, accelerates the production of reactive oxygen species (ROS) such as singlet oxygen (\(1^1O_2\)), superoxide (\(O_2^-\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxyl radicals (OH\(^-\)) (Foyer and Noctor, 2003). Consequently, subcellular localization of defence mechanisms is required for efficient removal of ROS at their generation sites. The predominant species singlet oxygen (\(1^1O_2\)), superoxide (\(O_2^-\)) are extremely short lived. Under normal conditions, singlet oxygen is removed by ascorbic acid (AsA), and superoxide is rapidly removed by dismutation with the help of SOD enzyme to less reactive H\(_2\)O\(_2\) (Carvalho, 2008).

Among all primary radicals H\(_2\)O\(_2\) is moderately reactive and relatively long lived molecule, capable of diffusing through membranes (Choudhury et al., 2013). More than 70% of the total H\(_2\)O\(_2\) produced under drought stress through photorespiration and it is capable of damaging cellular components by oxidation and inactivates the enzymes of Calvin cycle, Cu-Zn
SOD, Fe SOD (Kuruppanapandian et al., 2011). To prevent the \( \text{H}_2\text{O}_2 \) toxic effect antioxidant enzymes like ascorbate peroxidase (APX), catalase (CAT), and ferritin reduce \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \). Unless these ROS are scavenged, superoxide (\( \text{O}_2^- \)) and \( \text{H}_2\text{O}_2 \) produce the most reactive ROS hydroxyl radical (OH'). It is most stable and potentially reactive with all biomolecules, accelerate the lipid peroxidation and increase the cytotoxic effect (Gill and Tuteja, 2010). It leads to the generation of secondary ROS radicals like endoperoxides and hydroperoxides, etc., thus initiating lipid autooxidation. (lipid hydro peroxides, alkyl hydro peroxides, and peroxinitrite.) These radicals can also be scavenged by Glutaredoxin (Grx), Peroxiredoxin (Prx), and Glutathione peroxidase (GPX). They were abundant under environmental constraints i.e., fungal infection, water deficit and metal stress, but are decreasing during photo-oxidative stress. The subsequent reactions lead to generation of reactive carbonyl species (RCS) from oxidised/peroxidised carbohydrates, lipids and amino acids (Mano et al., 2005; Carvalho, 2008; Shafaq, 2012). Reduction of RCS into alcohols is catalysed by aldoketo reductases (Mano, 2012; Sengupta et al., 2015).

Aldoketo reductases (AKRs) act as stress regulators and play a central role in the detoxification of wide range of RCS and promotes the stress tolerance towards the osmotic and oxidative stresses (Jez et al., 1997, Jez and Penning, 2001; Hyndman et al., 2003; Mindnich and Penning, 2009). The first identified plant AKR is aldose reductase of barley (MsALR), expression of this gene accelerates the production osmolyte sorbitol in the maturation of embryos and represents its significant role in osmoregulation and acquisition of desiccation tolerance (Bartels et al., 1991). In addition to that AKRs are effective in the detoxification of RCCs such as lipid-peroxidation derived products (Acrolein, HNE) and glycolysis-derived reactive aldehydes (MG) (Simpson et al., 2009; Turóczy et al., 2011; Saito et al., 2013; Kanayama et al., 2014; Éva et al., 2014). Therefore, many AKRs have significant contribution in reducing the cellular toxicity effect caused by environmental stresses.
Molecular and genomic analyses have facilitated gene discovery, and enabled genetic engineering using several functional and regulatory genes to activate specific or broad pathways related to drought tolerance in plants. Use of modern molecular biology in elucidating the control mechanisms of abiotic stress tolerance, and engineering stress tolerant crops is based on the expression of stress-related genes. Several evidences indicate that molecular tailoring of genes has potential to overcome limitations in creating drought tolerant transgenic plants. Stress tolerance has been improved by developing transgenics by expressing several stress responsive genes. The potential option is to identify genotypes and adapt transgenic approach to pyramid desirable traits which can likely improve the field level tolerance and hence higher productivity under rainfed conditions.

Foxtail millet is one of the oldest crops cultivated for food grain, hay and pasture, mainly arid and semi-arid regions. It is an important grain crop in temperate, subtropical, tropical Asia and parts of southern Europe. China, India and Japan are the major foxtail millet growing countries in the world. In India, foxtail millet cultivation is confined to Andhra Pradesh, Karnataka and Tamil Nadu and some parts of Maharashtra. Foxtail millet is a small diploid (2n=18), self-pollinating, C4 panicoid short duration crop belongs to the family Poaceae. In addition to its economical uses, the physiological studies on foxtail millet remarkably considered that it is a good drought and salt tolerant crop including higher water use efficiency (WUE), small leaf area, arrangement of epidermal cells, thickening of the cell walls, and ability to form a dense root system. Its short life cycle and high WUE makes it a suitable crop for cultivation in semi-arid, dry and marginal lands.

Recently, the Joint Genome Institute of the Department of Energy, USA and Beijing Genome Initiative (BGI), China has sequenced the foxtail millet genome. Unlike major cereal crops, there are very few studies reported on functional validation of genes associated with
agronomic traits in foxtail millet (Doust et al., 2009). Foxtail millet is closely related to the major food and feed crops maize, wheat, sorghum and rice having small genome size ~515 Mb, with low amount of repetitive DNA and contains all the genes necessary for C₄ photosynthesis and primary metabolic processes (Li and Brutnell, 2011). Therefore, studies so far has been indicated that it is an excellent experimental model system and well known drought and salt stress tolerant crop, with an extensive germplasm collection, providing novel opportunities for underlying genetic mechanism of various stress related genes from this plant. Moreover, it provides great advantageous to transform these genes into other cultivars as well as other crop plants to make them stress tolerant (Puranik et al., 2011; Petti et al., 2013).

**Rationale of the present study**

The identification and characterization of stress responsive genes that are differentially expressed in response to drought stress is important to understand the genetic basis of plant adaptation to the stressed environment that is a complex process of altering the large number of developmental, biochemical, physiological and molecular changes. The identification and characterization of genes that are differentially expressed would be of greater importance in developing stress tolerant crops.

The classical approach to engineer plants for enhanced tolerance to abiotic stress consists in strengthening the endogenous systems by prevailing at different levels of the response, from sensors and signalling/regulatory elements (e.g., kinases, transcriptional factor), to direct action genes or effectors (e.g., antioxidant enzymes, heat shock proteins, enzymes for the synthesis of osmoprotectants)

ROS detoxification system is one of the strategies used for strengthening the endogenous systems of plants promoting stress tolerance. Several AKRs over-expression studies on many
plants including barley, alfalfa, rice and corn supports the detoxification of RCCs like Acrolein, HNE, methyl glyoxol and malondialdehyde (MDA) and promoting stress tolerance against a variety of oxidative stresses induced by methyl viologen, heavy metals, UV-B irradiation, osmotic and salt stress. The recently characterized AKR4C9 form *Arabidopsis thaliana* supports the importance of the enzymatic action directed towards oxidative stress-linked reactive carbonyls, such as MDA (Simpson *et al.*, 2009; Éva *et al.*, 2014). However, there were only few evidences linking with stress responsive *AKR* genes in the plant stress and the defence responses are not clearly understood.

Earlier in the parent lab, the contribution of aldose reductase enzyme activity was correlated with the sorbitol accumulation with the lowering of 4-hydroxynon-2-enal in foxtail millet cultivars was reported under salt stress (Veeranagamallaiah *et al.*, 2009). Based on this data, an attempt has been made to isolate, clone and characterize the stress responsive *AKR* gene from foxtail millet and validate its function in both prokaryotic and eukaryotic cells subjected to various abiotic stress conditions. Molecular characterization of the stress responsive *AKR* gene from foxtail millet would be a promising choice to understand various biological processes. The results we obtained would be useful in the near future to create multiple stress resistant crop genotype, therefore they could have agronomical benefit.

The major objectives of the present investigation are:

- Assessment of osmotic (PEG) and salt stress tolerance in foxtail millet seedlings by selective physiological and biochemical stress marker traits.
- Expression analysis of *aldoketo reductase1* (*AKR1*) gene in foxtail millet under PEG and salt stress by qRT-PCR.
- Cloning and characterization of stress responsive *AKR1* gene from foxtail millet (*Setaria italica* L. cv. Prasad)
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- Functional validation of SiAKR1 gene in bacteria (*Escherichia coli*) under different abiotic stresses.
- Functional validation of SiAKR1 gene in yeast (*Saccharomyces cerevisiae*) system under different abiotic stresses.
- Construction of binary vector for plant gene transformation (pCAMBIA2301: *SiAKR1*).