1: Introduction
Plants are sessile organisms and often subjected to different environmental stresses, including drought, salt, and temperature extremes. These abiotic stresses pose a serious threat to agriculture and crop productivity. Plants have evolved different adaptive strategies to alleviate the adverse affects of harsh environments by altering their molecular and cellular functions, as well as their physiological and biochemical pathways. Majority of these responses originate from changes in the gene expression and subsequent action of their gene products. Recent research has identified several transcription factors, such as AP2/EREBP (apetala2/ethylene responsive element binding protein), bZIP (basic leucine zipper), NAC (no apical meristem (NAM), ATAF1-2, cup shaped cotyledon (CUC2)), MYB, MYC, WRKY and their associated signalling networks are regulating dynamic co-expression of several stress responsive genes together to alleviate stress induced cellular damage. Plant stress responses are traditionally classified as either ABA-dependent or ABA-independent regulatory pathways (Shinozaki and Yamaguchi-Shinozaki, 2000; Thomashow, 1999; Xiong et al., 2002, Zhu et al., 2005). Majority of transcription factors were found to function in either one of the pathways for subsequent co-expression of stress adaptive genes. The ABA dependent transcription factors specifically bind to ABRE (ABA-responsive) core cis-acting sequences in the promoters and trigger the expression of ABA dependent plant stress responsive genes. On the contrary, the AP2/EREBP super-family of transcription factors that exists extensively in plants, bind to DRE/C-repeat sequences and regulate the expression of stress-related genes in an ABA-independent manner (Shinozaki and Yamaguchi-Shinozaki, 2000; Riechmann et al., 2000; Nakano et al., 2006). Based on the presence of different conserved domains AP2/EREBP super-family is further subdivided into several subfamilies like AP2, ERF, RAV, DREB.

The transcription activation factors interact with cis-elements present in the promoter region of various abiotic stress-related genes and thus up-regulate the expression of many genes resulting in imparting tolerance to abiotic stresses. The dehydration responsive element binding (DREB) transcription factors and/or C-repeat (CRT) binding factor (CBF) transcription factors have an important role in regulating the expression of plant stress responsive genes by interacting with the DRE(A/GCCGAC)
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and/or the CRT (TGGCCGAC) core *cis*-acting sequence in their promoters. Many
DRE binding transcription factors (DREBs) and/or CRT-binding transcription factors
(CBFs) have been identified, isolated and characterized from several plant species
including Arabidopsis (Sakuma *et al*., 2002), Brassica (Jaglo *et al*., 2001; Zhao *et al*.,
2006), tomato (Zhou *et al*., 1997), tobacco (Leubner-Metzger *et al*., 1998), soybean
(Chen *et al*., 2007), cotton (Huang and Liu, 2006), Salicornia (Gupta *et al*., 2010)
maize (Qin *et al*., 2004), rice (Dubouzet *et al*., 2003; Tian *et al*., 2005; Ito *et al*.,
2006), wheat (Shen *et al*., 2003), barley (Choi *et al*., 2002; Xue, 2003) and rye (Jaglo
*et al*., 2001). The ectopic overexpression of DREB homologous or heterologous plant
systems resulted in improved stress tolerance indicating a fair degree of conserva-
tion of DRE/DREB regulon system across different plants (Hseih *et al*., 2002;
Pellegrincschi *et al*., 2004; Lee *et al*., 2004; Oh *et al*., 2005; Ito *et al*., 2006; Xiao

The availability of whole genome sequence information of *Arabidopsis* and rice
identified several genes encoding for putative DREB transcription factors in these
model crop plants (Nakano *et al*., 2006, Seki *et al*., 2001), and majority of them were
characterized earlier as either DREBs and/or CBFs and their role in stress adaptation
either in rice and *Arabidopsis* (Dubouzet *et al*., 2003; Ito *et al*., 2006) or even in
heterologous plant systems (Gao *et al*., 2009; Qin *et al*., 2004). The nomenclature of
these transcription factors in the published literature is very ambiguous. The DREB
transcription factor family is one of the largest and broadly divided into DREB1 and
DREB2 sub families and each sub family contain several paralogs. Initially it was
proposed that DREB1 and its homologs were induced by low-temperature, whereas
DREB2 and its homologs were induced by dehydration and high-salt stress (Liu *et al*.,
1998), however, later studies indicate that this distinction is very diffuse and not
holding true when several DREB1 and DREB2 orthologs and paralogs were analyzed
for their transcriptional regulation in different plant species (Nayak *et al*., 2009).

The ectopic expression of rice DREB1A gene in transgenic *Arabidopsis* provided over
all drought, high-salt and freezing stress tolerance phenotype that is functionally
similar to *Arabidopsis* DREB1A, however, the rice DREB1A protein preferentially
interacting with DRE core *cis*-acting sequence GCCGAC compared to ACCGAC,
whereas, *Arabidopsis* DREB1A protein bind to both the sequences efficiently. The
difference in the DNA-binding specificity between Arabidopsis DREB1A and rice DREB1A proteins may be due to change from glutamic acid to valine at the 19th amino acid position in the DNA binding domain. Similar preferential interaction was also observed in other monocot plants like barley, wheat and rye DREB1-type transcription factors (Sakuma et al., 2002). Both cis-acting DNA sequences and trans-acting transcription factor amino acid sequences are co-evolved to regulate the coordinated expression of several stress adaptive genes. Similarly the architecture of promoter sequences of these stress-related genes are not simply colinear, but they are multifunctional and modular, and interact with multiple independently regulated transcription factors and display a transcriptional complexity to cope up the cross-talk response to various environmental stresses.

Expression of DREB1 genes was extensively investigated in various crops with regard to its role in plant adaptation under various abiotic stresses. However, only a limited number of plant species have been studied for DREB2 expression and their possible role in stress adaptation. Compared to DREB1, the DREB2 genes require complex post-transcriptional and/or post-translational modifications required in different plant systems to transactivate the stress adaptive genes. In case of Arabidopsis, DREB2A has a negative regulatory domain that contains serine/threonine-rich sequence, probably involved in 26S proteasome mediated protein degradation under non-stress condition (Rechsteiner and Rogers, 1996; Qin et al., 2008), Whereas, in case of rice DREB2B or its homologs in other monocots were reported to regulated by alternative splicing and produce non-functional transcript during non-stress condition and functional transcript during stress condition (Xue and Loveridge, 2004; Egawa et al., 2006; Qin et al., 2007). However, the mechanism of stress-responsive alternative splicing of these DREB2 transcripts is still unclear. In case of Pennisetum the functioning of DREB2A could be regulated by post-translational modification, the phosphorylated Pennisetum DREB2A did not bind to the DRE sequences, only the non-phosphorylated form of DREB2A interacting with DRE (A/GCCGAC) sequences in the promoters of target genes (Agarwal et al., 2007).

Whole genome analysis identifies six different putative DREB2 transcription factor-encoding genes in rice, out of which only OsDREB2A and OsDREB2B genes showed abiotic stress inducible gene expression. The ectopic expression of OsDREB2B in
both transgenic rice and Arabidopsis plants trans-activated its target stress adaptive genes (Matsukura et al., 2010), however, the ectopic heterologous expression of OsDREB2A in transgenic Arabidopsis did not trans-activate any of the target stress adaptive genes (Dubouzet et al., 2003). This may be due to the lack of required post transcriptional and/or post translational machinery in Arabidopsis heterologous system that may be required to processes OsDREB2A transcript or its encoded protein.

Rice (Oryza sativa L.) is an economically important food crop and more than half of the world’s population depends on rice; by 2025 about 60% more rice must be produced to meet the needs of the growing population. Compared to other crops, much more water is required for the paddy cultivation throughout its lifecycle. It has been estimated that 70% of the crop yield loss can be attributed to abiotic stresses, especially drought. Therefore, improvement of drought tolerance rice crop is desirable. In this study we have developed transgenic rice plants overexpressing OsDREB2A to study its role in stress adaptation.

Objectives:

- Cloning and in-silico analysis of cDNAs encoding for DREBs (1A, 1B, and 2A) from Oryza sativa cv pokkali
- Transcriptome analysis of DREB (1A and 2A) genes
- Expression and purification of OsDREB2A protein using recombinant expression system in E.coli.
- Cloning of OsDREB2A in plant transformation pGreen0179 vector under stress inducible promoter rd29A
- Developing transgenic rice plants carrying DREB2A gene by Agrobacterium mediated transformation.
- Molecular confirmation of transgenic plants
- Functional characterization of OsDREB2A rice plants (T1) under osmotic stress conditions