STUDIES ON TRANSCRIPTION FACTORS INVOLVED IN ABIOTIC STRESS RESPONSES OF *Sorghum bicolor* (L.) MOENCH

Plants have to face major abiotic stresses during their growth and development which arise due to water limitation caused by inadequate rainfall, cold condition and salinity. Stress induced changes in the metabolism and development involves altered patterns of gene expression. Stress inducible genes code for either proteins which directly play a role in stress acclimation or those involved in signal transduction and regulation of gene expression. Transcription factors (TFs) are proteins that bind to the enhancer or promoter regions of genes and regulate their expression. The TFs act as master switches and trigger the simultaneous expression of a large number of stress-response genes that contribute to the stress phenotype. The ABA- responsive bZIP TFs (ABFs) are one such group of TFs which are known to regulate gene expression under abiotic stress conditions. *Sorghum bicolor* is a semi-arid crop and also a representative of tropical grasses having the C4 pathway of photosynthesis. Its ability to withstand desiccation stress as a result of many favorable morpho-physiological attributes makes it a good candidate to study plant responses to abiotic stresses.

In this thesis, attempts have been made to understand the probable regulatory (transcriptional, post-transcriptional and post-translational) mechanisms of the sorghum ABFs under abiotic stress situations.

The aspects dealt with include:

1. **Phylogenetic and conserved protein motif analysis of sorghum ABA-responsive bZIP transcription factors (ABFs) and 3D modeling of one sorghum ABF.**
2. **Expression analysis of ABA-responsive bZIP (ABF) transcription factors in sorghum plants exposed to abiotic stress.**
3. **Expression analysis of sorghum microRNA: sbi-miR156i having a target site in one sorghum ABF.**
1. Phylogenetic and conserved protein motif analysis of sorghum ABA-responsive bZIP transcription factors (ABFs) and 3D modeling of one sorghum ABF.

Thirteen orthologs of the Group A ABFs from Arabidopsis were identified in sorghum and rice from the Gramene database (http://www.gramene.org/). Phylogenetic analysis of all these ABF protein sequences revealed presence of four distinct clusters. Three clusters represented proteins of the ABI/AtDBF subfamily, which include ABFs playing a role in seed development, while one cluster represented the AREB/ABF subfamily which included genes expressed in vegetative tissues under drought stress. All ABFs showed the presence of three conserved protein motifs corresponding to a globular bZIP domain and two phosphorylation sites namely R/K-X-X-S/T and S/TXXE/D which are the target sites for serine/threonine protein kinases. The AREB/ABF cluster showed an additional motif that has been reported to play a role in phosphorylation-dependent interaction with 14–3-3 regulatory proteins.

3D modeling of one ABF (Sb04g034190) was carried out using ab initio methods and a satisfactory model was generated. The 3D structure of one 14-3-3 protein of sorghum was also obtained using homology modeling. In silico docking studies carried out between the ABF and 14-3-3 dimer revealed that successful docking occurred only when the ABF was phosphorylated, suggesting that phosphorylation could be a pre-requisite for the activation of the Sb04g034190 protein.

2. Expression analysis of ABA-responsive bZIP (ABF) transcription factors in sorghum plants exposed to abiotic stress.

Semi-quantitative RT-PCR was used to study the expression of ABF genes identified in the sorghum genome to understand their role in regulating abiotic stress responses. Seven sorghum ABF genes were seen to be expressed in sorghum seedlings, and the levels of their expression varied in plants exposed to desiccation stress, cold stress and in response to exogenous ABA application. Increased expression of Sb02g026570 was seen under cold stress condition, whereas Sb03g037740, Sb03g040970 and Sb09g023920 were seen to be down-regulated. Under drought conditions Sb04g034190, Sb08g003760 and Sb03g040970 showed increased expression, while Sb03g040970 was the only ABF found to be up regulated on exogenous ABA application.
Real time quantitative PCR was carried out to validate and quantify the expression of four sorghum ABFs (Sb04g034190, Sb02g026570, Sb08g003760 and Sb03g040970), which were seen to be differentially expressed in the semi-quantitative RT-PCR experiments. It was observed that under desiccation and three ABFs, Sb04g034190, Sb08g003760 and Sb03g040970 showed 9-, 4- and 2.5-fold increase in expression respectively under desiccation stress. Sb04g034190 and Sb02g026570 showed 70% and 40% higher expression under cold stress while Sb03g040970 showed 90% lower expression under cold stress conditions. These results provided a more precise estimation of differential gene expression than the comparisons made using band intensities.

In silico analysis of promoters of the ABF genes from sorghum and rice was carried out to identify the over-represented / co-localized motifs that may play a regulatory role in the expression of ABFs. Over-represented motifs were identified in the -1000 base sequences upstream to the sorghum and rice ABF coding sequences. In all, 14 over-represented motifs were identified, which included those related to hormone signaling, calcium signaling, light signaling and sugar signaling. Some of the motifs were similar to those over-expressed in another class of abiotic stress-induced TFs, namely the DREB (drought responsive element binding) TFs, which were studied previously in the laboratory. This was suggestive of an induction of abiotic stress related TFs by similar, redundant stress signaling pathways.

Promoters (2000 base upstream region from the coding sequence) of the 4 sorghum ABF genes (that were found to be differentially expressed) and their corresponding rice orthologs were analyzed with respect to other stress-related motifs besides the ones already shown to be over-expressed. While all the rice ABFs showed presence of ABRE motifs in their promoters, one of the sorghum ABFs (Sb08g003760) lacked ABREs in its promoter, suggestive of ABA-independent mechanisms for its activation.

Expression analysis of the ABFs was also carried out in the stems of four genotypes of sorghum when the plants were exposed to drought stress in the field at early reproductive stage. The aim was to study stage-specific expression of the TFs and to correlate their expression to the differences observed in water retention ability of the sorghum genotypes on exposure to drought stress. The same ABFs expressed in seedlings were
also expressed in sorghum stems. Four ABFs, \textit{Sb04g034190}, \textit{Sb08g003760}, \textit{Sb02g026570} and \textit{Sb09g023920} showed higher expression in drought stressed sorghum genotype ICSV25280 but not in M35-1, where down-regulation of three of these ABFs was observed. Both these genotypes failed to retain water in their stems on drought stress application. SPV1411, which showed water retention in its stem on drought stress exposure also showed an increased expression of two of these ABFs. Hence it was not possible to correlate drought tolerance of the sorghum genotypes to the expression of specific ABFs.

3. Expression analysis of sorghum microRNA: \textit{sbi-miR156i} having a target site in one sorghum ABF.

MicroRNAs (miRNA) having a target site in the 4 differentially expressed ABFs were identified using the psRNATarget server (http://plantgrn.noble.org/psRNATarget/). A miRNA from \textit{Bruguiera cylindrica} bcy-miR156 was found to have a target in the ABF \textit{Sb03g040970}. The sorghum miRNA, \textit{sbi-miR156i} showed 90% similarity to the bcy-miR156. The precursor of \textit{sbi-miR156i (sbi-MIR156i)} was amplified and its expression was analyzed by semi quantitative RT-PCR in the same RNA samples that were used earlier for studies on expression of ABFs under desiccation, cold and ABA stress. Expression of the miRNA was seen to decrease in desiccation and ABA stressed samples that showed an increase in ABF expression whereas under cold stress the miRNA expression was induced when its target ABF expression was down-regulated, indicating that ABF expression may be regulated through miRNA besides showing transcriptional regulation.

In conclusion, ABF expression in sorghum appeared to be regulated at various levels – transcriptional, post-transcriptional (mediated by miRNA) and post-translational (mediated by phosphorylation and through interaction with 14-3-3 protein), indicating that multiple and redundant pathways are used by plants to fine tune their stress responses.

Kshitija Sawant                     Sujata Bhargava
(Ph.D. Candidate)                    (Research Guide)