Preface

DNA methylation is the most important epigenetic phenomenon that governs gene regulation. There are two important functions of DNA methylation, protecting the genome from transposons and regulation of gene expression. Cytosine methylation is so abundant and it is often called as the fifth base of DNA. The DNA methylation in the cytosine residue is well studied in regulation of gene expression, silencing of transposons, genomic imprinting and self-incompatibility. The methylation mark on DNA is read by proteins of a class which represents methylated DNA binding proteins. These proteins contain a methyl CpG binding domain and are called methyl CpG binding domain (MBD) proteins. DNA methylation and MBD proteins play a major role in gene silencing mechanism. Gene silencing occurs at both transcriptional and post-transcriptional level. The promoter DNA methylation is mainly associated with transcriptional gene silencing whereas methylation in the gene body is associated with transcriptional as well as post-transcriptional gene silencing.

The interpretation of DNA methylation in the cellular environment, which leads to regulation of the gene expression is poorly understood. With the recent advancement in genome sequencing and emerging protein databases of different genomes, genes encoding MBD proteins have been found to be present in almost all eukaryotic systems. Several MBD proteins have been identified and characterized in plants and animals. The property of these proteins to bind to methylated DNA and their role in transcriptional gene silencing is known, but the exact mechanism how these proteins work is still in the dark. The biochemical functions and molecular characterization of these proteins are still unclear.

In the present study, an attempt was made to functionally characterize the role of MBD protein in the model system Arabidopsis thaliana in both transcriptional and post-transcriptional gene silencing. The work started with identification of AtMBD genes involved in gene silencing. A model system to study gene silencing was generated by expressing gus reporter gene in different mutants of AtMBDs and transgenic lines with down regulation of the expressions of different AtMBD genes. The silencing of the gus reporter gene was monitored after introducing an RNAi or
artificial microRNA construct specific to *gus* transcript. *AtMBD6* gene was found to be involved in post-transcriptional gene silencing. Functional characterization of its protein was then performed. Along with *AtMBD6* three other genes *AtMBD4*, *AtMBD10* and *AtMBD11* were also selected to find their roles in transcriptional gene silencing. These genes were over-expressed as well as down-regulated in *Arabidopsis* for their functional characterization. The genes that are regulated downstream to *AtMBDs* were identified using transcriptome analysis. The differentially expressed genes were annotated and the metabolic pathways that are affected by the mutation in *AtMBDs* were identified.

Further, to know the mode of action of these proteins the interacting partners were identified using yeast two hybrid library screening. Some novel interactions were identified using yeast two hybrid screening. These interactions were further confirmed by co-localization of interacting partners and florescence resonance energy transfer between them.