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Prediction of DNA Methylation Marks and Related Gene Expression Pattern in Contrasting Rice Genotypes under Drought Stress

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ABSTRACT

In plants, methylation of CpG (5'-C-phosphate-G-3') sites within the promoters of genes is an important epigenetic mechanism that regulates gene expression in response to environmental conditions. In this study, an attempt has been made to identify the DNA methylation marks (CpG Island) in the upstream regions of certain drought responsive genes. Based on the number of CpG Islands, the expression pattern of selected genes in upland rice cultivar was studied by RT-PCR. Furthermore, the effect of a methylation inhibitor was studied on two contrasting rice cultivars AC39020 and BPT5204. These initial findings suggest a possible correlation between gene expression and DNA methylation in rice under stress.

EPIGENETIC modifications play a crucial role in modulating the gene activity in eukaryotes. DNA methylation is one of the important epigenetic modifications that regulate transcription. In plants, DNA methylation occurs at three different sequence contexts, CG, CHH, CHG (H=A, C, or T). Gardiner-Garden and Frommer (1987) defined CpG Island as the genomic region of more than 200bp containing more than 50% GC content and an observed to expected CpG Island ratio of more than 0.6. It has been proposed that during various stresses, cytosine methylation governs the activity of effector genes (Yaish *et al*., 2011).

Drought and salinity are the major abiotic factors that affect rice productivity under upland and semi-irrigated aerobic conditions. Rice germplasm exhibit variable responses to these abiotic stresses; some genotypes tolerate extreme drought and salinity stresses, whereas, many are highly susceptible. This type of phenotypic variability is attributed to genetic and epigenetic modifications. Based on *omics* approaches, attempts were made to study the stress response mechanisms, however, only a few studies have analyzed the epigenetic regulation in rice (Wang *et al*., 2011; Garg *et al*., 2015).

Recently, in contrasting rice cultivars, it was reported that expression of genes associated with the differentially methylated region depend on the methylation status (Garg *et al*., 2015), however, the epigenetic regulation under stress conditions has not been clearly demonstrated. In this study, efforts have been made to identify CpG Islands in drought responsive genes and to understand the correlation between gene expression and methylation pattern under drought in rice.

To identify the candidate genes, drought-specific microarray data generated using contrasting rice varieties such as APO and IR-64 (GSE5978), DK151 and IR-64 (GSE26280), IRAT109 and ZS97 (GSE25176) deposited at the NCBI-GEO (http://www.ncbi.nlm.nih.gov/geo) were obtained and analyzed. The differentially expressed genes between control and drought-stressed conditions were identified by applying FDR correction procedure (Benjamini *et al*., 2000). A total of 5325 up-regulated and 7382 down-regulated unique genes were obtained. Enrichment analysis was performed for the selected genes using web server, DAVID 6.7 (https://david.ncifcrf.gov/). The gene IDs were given as query for carrying out functional and pathway analysis, where the results were filtered using Bonferroni correction method ($P < 0.05$). After filtering out common genes and the ones with putative functions, the analysis yielded 239 and 231 down - and up - regulated genes, respectively. .

CpG Islands were extracted from the 2 kb upstream sequences of all the selected genes

(http://mobyle.pasteur.fr/cgi-bin/portal.py#forms::newcpgreport) obtained from Rice Annotation Project database (RAP-DB) using Os-IDs. The CpG Islands were derived based on the stringent criteria as being the original ones defined by Gardiner-Garden and Frommer (1987). The cut-off for selection of genes was based on the number of CpG Islands (≥ 2) (Oller *et al*., 2009). Among the selected genes, 24 up-regulated ones had 1-2 CpG Islands, whereas, 41 down-regulated genes had more than two Islands (Fig. 1).

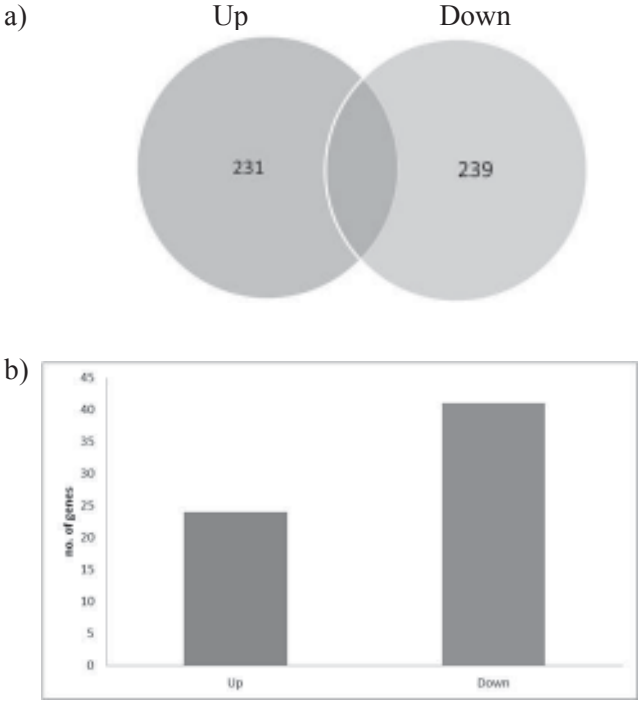

Fig. 1. a) Number of genes up-regulated and down-regulated b) Number of genes having two or more CpG Islands in 2kb upstream region of transcription start site (Up: up-regulated, Down: down-regulated genes)

To demonstrate that these methylation marks could vary in contrasting genotypes, targeted gene expression analyses were carried out using select genes. A basal transcription factor (BTF3) whose upstream region lacked CpG Island, and superoxide dismutase (Cu-Zn SOD) which showed the presence of CpG Island, were selected for this analysis.

The RAP Os-IDs of the selected genes were used for the electronic Northern analysis (eNorthern) (http://bbc.botany.utoronto.ca/; Toufighi *et al*., 2005). The expression levels of drought responsive genes in shoot under different abiotic stresses were analyzed. BTF3 was down-regulated under drought and salinity stress but up-regulated under cold stress, whereas, SOD was up-regulated under all abiotic stresses.

Further, to study the pattern of expression of the select genes under drought stress in contrasting rice genotypes, semi-quantitative reverse transcription PCR (RT-PCR) was performed. Healthy plants of two rice genotypes (AC39020 and BPT5204) were raised in small pots and drought stress was imposed to 30 days old plants by controlled irrigation. Soil field capacity of 60 per cent was maintained by gravimetric approach (Karaba *et al*., 2007). The extent of stress induced damage was assessed by quantifying chlorophyll content (Hiscox and Israelstam, 1979). Significant difference in chlorophyll content was observed between the treatments in both the genotypes (Fig. 2a). BPT5204 showed higher reduction in chlorophyll content (54.34 %) than AC39020 (47.27 %) under drought compared to well irrigated conditions.

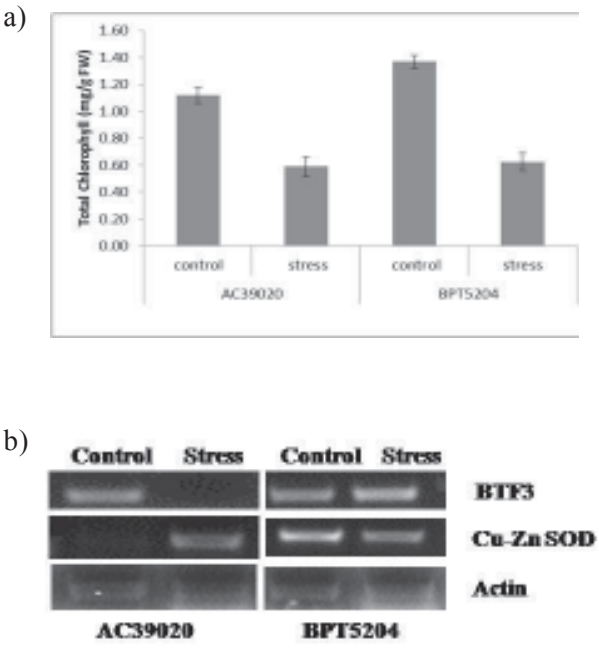

Fig. 2. a) Total chlorophyll content in control and drought stressed tissues of AC39020 and BPT5204 (Error bars represent SE calculated from three replicates); b) Semi quantitative RT- PCR analysis to examine the pattern of expression of genes with or without CpG Islands in rice genotypes under drought stress.

The expression analysis suggests a differential expression pattern for BTF3, a gene having no methylation marks. However, SOD with a CpG Island was found to be down-regulated under stress in the susceptible genotype, whereas, it exhibited stress responsive nature in the tolerant genotype (Fig. 2b). CpG Islands govern hypo- or hyper-methylation of upstream regions of stress responsive genes thereby modulating their expression to drive differential responses under stress conditions.

To reiterate the fact that methylation could play a role in differential stress response pattern across contrasting genotypes, the effect of 5'-Azacytidine, a demethylating agent, on the growth of the seedlings under different abiotic stresses was studied. Rice seedlings were allowed to grow in 10 µM 5'-Azacytidine for 3 days and later subjected to salinity or oxidative stress for another three days (Zhong *et al*., 2010). The tolerant genotype showed significant reduction in root growth compared to the susceptible genotype (Fig. 3). Alteration of DNA methylation could have induced a change in expression of certain

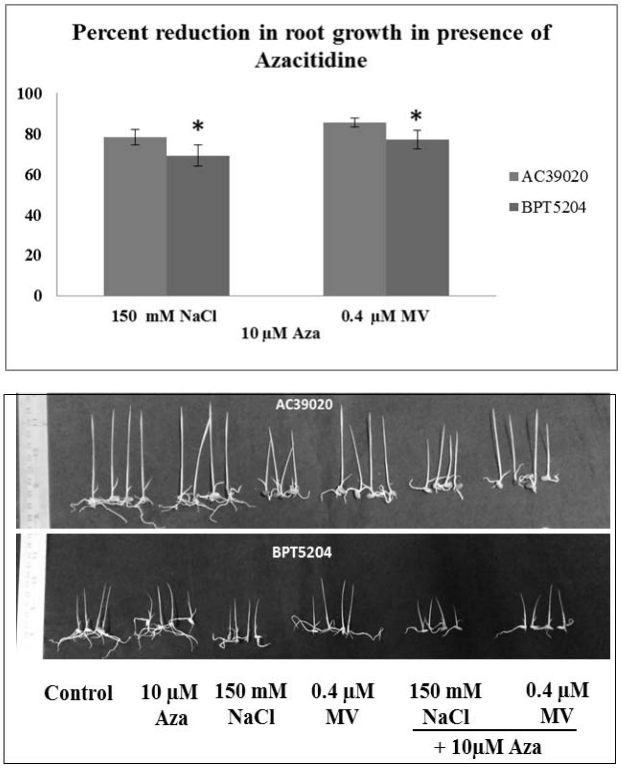

Fig. 3 : Response to stress in the presence of Azacytidine (10 µM Aza) under salinity (150mM NaCl) and oxidative (0.4 µM Methyl Viologen, MV) stresses

developmental and stress related genes, resulting in the observed phenotype, which needs to be examined.

The study indicated high frequency of CpG Islands in down-regulated genes in rice, and genotypic variation in the gene expression under drought (Figure 2). The differential expression of the select genes studied cannot be fully attributed to DNA methylation in the upstream region. However, based on the azacytidine experimental results and its comparison with the RT-PCR data, it can be suggested that there exists a correlation between methylation and gene expression. Global methylation analysis needs to be carried out to have a clear picture about the correlation between gene expression and methylation under abiotic stresses.

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