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Title of Thesis: Studies on the Effect of Abiotic stress on the Expression of Different MicroRNA-Targeted Transcription Factors and MicroRNA Biogenesis Genes in Rice.

Abstract

To achieve the objectives of the thesis entitled, “Studies on the Effect of Abiotic stress on the Expression of Different MicroRNA-Targeted Transcription Factors and MicroRNA Biogenesis Genes in Rice” designing of primers for miRNA targeted TF’s and miRNA biogenesis genes, exposure of rice plants to abiotic stress (drought / salinity) conditions and isolation of total RNA from healthy as well as stress induced plants, cDNA synthesis using Total RNA (from step 2) isolated from plants using OligodT / specific primers, Screening of microRNA targeted transcription factors and micro RNA biogenesis genes and analysis of data to study whether the stress influences the expression of transcription factor that are regulated by miRNA and genes of miRNA biogenesis were performed. In order to achieve the said objective we performed many experiments and the study are as follows:

- Primers were designed and in order to study of expression we exposed rice plants to both abiotic stress conditions i. e. drought and salinity (200mM NaCl) for different time intervals.
- For expression analysis of transcription factors at variable time we used semi quantitative RT-PCR methods. Other than the miRNA its predicted target transcription factors (SBP, MYB, ARF, NAC, SCL, GRF, HD ZIPP, bHLH, AP2) showed gradually reduced expression from zero to 72hrs in both the simulated stress conditions (Drought and Salt). However, corresponding miRNAs showed aberrant expression in both the stress conditions.

- Expression of selected miRNAs (miRNA156, miRNA159, miRNA160, miRNA162, miRNA164, miRNA166, miRNA168, miRNA169, miRNA171, miRNA172, miRNA393 and miRNA396) were checked by stem loop semi quantitative RT PCR method after exposure of rice plants to stress conditions up to 72 hrs. We observed amplification of all miRNA (Twelve conserve miRNA) only up to 18 hrs during our study of 72 hrs exposure to simulated stress conditions.
- When compared with other miRNAs, miRNA169 showed very fine tune of expression and was up regulated during both drought and salt stress conditions.
- Expression of miRNA targeted transcription factors gene (SCL, MYB and bHLH) and miRNAs (miRNA159, miRNA160 and miRNA169) carried out by semi quantitative method were validated by Real time PCR. ABI Step One plus Real time PCR were used in the study and results obtained were more or less similar to semi quantitative expression method.
- A comparative analysis of miRNA and their targeted transcription factor revealed aberrant expression of miRNAs from 0 to 18 hrs whereas expression of transcription factors sharply declined from 0 to 72 hrs. No any definite positive or negative correlation was observed between expression of miRNA and their target transcription factors.
- These introductory data indicate that miRNA might play an important role in abiotic stresses and help to understand the relation of miRNA with abiotic stress, initial point for future studies and sustained efforts are needed to conform the function of miRNAs in stress responses and adaptation.