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Effect of DNA methylation change on seed development and functional analysis of three rice PIL5-like bHLH transcription factors during seed germination

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Viable seed production is one of the most important events in plant life cycle and agriculture. Successful seed germination also offers plant to survive and spread its genetic constituents in the environment. Knowledge of environmental signals which affect seed germination and molecular mechanism affects seed development can help us to make useful crops in a future. Arabidopsis PIL5, a phytochrome-interacting bHLH transcription factor, was identified as a key negative regulator of light-promoted seed germination. It is curious to know if PIL5-like bHLH transcription factors also play important roles in other modelplant species such as rice. Using RT-PCR samples from various rice tissues, six of nine bHLH transcription factor genes were able to be amplified. Three of them had correct reading frame and are being used for functional analysis in rice. Central cell-specific and pollen-specific promoters are cloned to understand how the different methylation in female and male gametophyte, respectively, affects functional seed production. To induce hypomethylation in the central cell, DME promoter is cloned and ligated to antisense-MET1 methyltransferase. Furthermore, LPAT3 promoter, which shows pollen-specific expression, is ligated with DME cDNA to understand the effect of ectopically expressing previously silenced genes in pollen and further seed development. To identify significant cis-elements in DME expression, DME-deleted promoter constructs fused to GUS and GFP are transformed. Moreover, rice OsDME gene promoter, pDME1, as well as two deletion promoters with reporter gene are constructed. From our further works, we hope to get the answers for the important biological questions we addressed above.

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