



Homotypic clustering of OsMYB4 binding site motifs in promoters of the rice genome and cellular-level implications on sheath blight disease resistance



Singh Pooja, Kumari Sweta, A. Mohanapriya, C. Sudandiradoss, Ramamoorthy Siva, Kodiveri M. Gothandam, Subramanian Babu^{*}

School of Bio Sciences and Technology, VIT University, Vellore 632014, India

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ABSTRACT

The promoter regions (1 kb upstream sequences) of 45,836 annotated genes of rice were analyzed for the presence of OsMYB4 binding sites using a Perl program algorithm. Based on the homotypic clustering concept, 113 promoters were found to have more than 4 binding site motifs. Among the downstream genes of these promoters, five genes which are known to have a role in disease resistance were selected and the binding capacity of OsMYB4 protein in the promoter regions was analyzed by docking studies. Expression level of these genes was analyzed by RT-PCR in *Rhizoctonia solani* infected rice seedlings. Upon pathogen challenge, higher expression of aminotransferase, ankyrin and WRKY 12 genes was observed corresponding to higher expression of *Osmyb4*. Over-expression of *Osmyb4* cDNA in rice leaf tissues by agro-infection failed to result in similar over-expression of aminotransferase, ankyrin and WRKY 12 as expected. Although the role of OsMYB4 in sheath blight resistance was found to be definitive based on our initial results, artificial over-expression of this TF was observed to be insufficient in regulating the disease resistance related genes.

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1. Introduction

Transcription factors (TFs) are DNA binding proteins that bind to specific DNA sequences (motifs) in promoter region of genes, thereby controlling the flow of genetic information from DNA to messenger RNA (transcription). They have been reported to regulate genome expression in response to environmental and physiological signals in addition to their involvement in growth and developmental processes.

TF is encoded by a single gene but regulates the expression of several other genes leading to the activation of complex adaptive mechanisms and hence represents major molecular targets to genetically improve the tolerance of crop plants against different stresses (Khong et al., 2008). Plant TFs have been reported to be more diverse when compared to animals and fungi. There are 63 families of TFs reported in rice. MYB is one among the large families of TFs and named after the first observation of DNA binding domain in the protein of myeloblastosis virus. Japonica and indica rice have 138 and 136 families of OsMYB4 TFs,

respectively. The elucidation of the function of these TFs can be used to set up genetic engineering strategies and to rationalize molecular breeding using molecular-assisted selection towards enhancement of rice tolerance to various stresses. Since transcription factors modulate the regulatory network in plants, they are considered to be the best and safest candidate genes for engineering complex traits like stress tolerance (Zhang, 2003; Nakashima et al., 2009; Saibo et al., 2009).

Rice TF *Osmyb4* has been very well explored for the ectopic expression in other plant species. Literature reports are very limited which suggest the role of *Osmyb4* in rice. For example, ectopic expression of *Osmyb4* gene (GenBank accession no. Y11414) in *Arabidopsis thaliana* induced a high level of tolerance to cold and freezing and resulted in multiple biochemical changes commonly observed in plants during cold acclimation (Vannini et al., 2004; Mattana et al., 2005).

Previous studies using microarray analysis of transgenic plants versus the WT showed that the *myb4* activates genes involved in tolerance to different abiotic stresses (such as drought and salt), as well as in pathogen resistance, indicating that this transcription factor is able to integrate the activation of multiple components of the stress response (Vannini et al., 2006). Vannini et al. (2007) developed transgenic tomato plants over-expressing rice *Osmyb4* and observed the plants acquiring higher tolerance to drought stress and viral disease. Similarly, ectopic expression of *Osmyb4* gene improved the physiological and biochemical adaptation to cold and drought in apple (Pasquali et al., 2008)

Abbreviations: AMV, avian myeloblastosis virus; CaMV, cauliflower mosaic virus; GMC, glucose-methanol-choline; RT-PCR, reverse transcriptase PCR; TF, transcription factor; TFBm, transcription factor binding motif; YEP, yeast extract peptone

^{*} Corresponding author.

E-mail address: babu.s@vit.ac.in (S. Babu).

and cold in *Osteospermum ecklonis* (Laura et al., 2010). Recently, enhanced tolerance to frost and improved germination of transgenic barley plants expressing rice *Osmyb4* have been observed (Soltesz et al., 2012).

The results of the effect of ectopic expression of the rice *Osmyb4* in other plant species prompted us to study the effectiveness in rice, the native plant of this gene. In the homologous transgenic system, the supra-optimal expression of *Osmyb4* was found to up-regulate 4193 genes and down-regulate 5362 genes (Park et al., 2010). However, the role of this gene in rice disease resistance remains largely unexplored other than our preliminary report on sheath blight and bacterial blight (Singh et al., 2013). When expressed in other plants, rice *Osmyb4* is reported to confer systemic acquired resistance against fungi, bacteria and viruses by inducing expression of pathogenesis related proteins and their activators (Vannini et al., 2006). Hence an attempt was made to systematically study the role of OsMYB4 in rice through a genome-

wide analysis of binding locations and further validation in relevance to rice sheath blight plant–pathogen system.

2. Materials and methods

2.1. Genome-wide computation analysis

Genome-wide computational analysis was performed on promoter sequences of rice genes to determine the genes that are controlled by OsMYB4. Promoter analysis was carried out using sequence analyzing Perl programs incorporating various bioinformatics resources (Fig. 1).

2.2. Sequence retrieval and data preprocessing

Initially, Rice TOGO Browser database (Nagamura et al., 2011) was used to retrieve 1 kb upstream sequence of the promoter region of

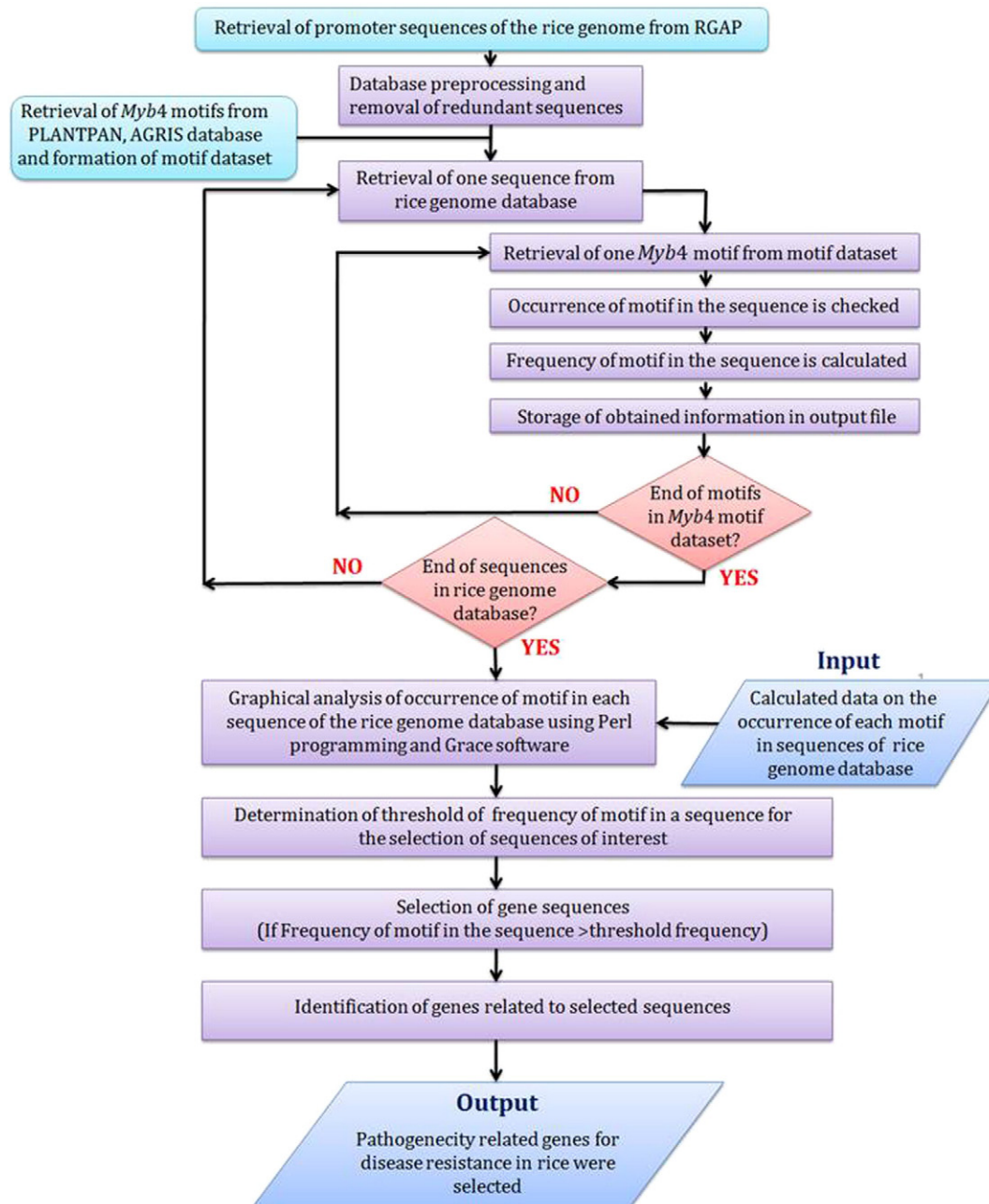


Fig. 1. Flow chart representing the data flow and analysis of promoter sequences in the rice genome.

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