

# ***Cis*-regulatory module mediated control of sheath blight infection in Rice (*Oryza sativa* L.)**

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## **ABSTRACT**

Plant defense responses are orchestrated at the transcriptional level through *cis*-regulatory elements (CREs) organized as *cis*-regulatory modules (CRMs). This study systematically dissected the CRM architecture of resistance (R) gene promoters in two sheath blight-resistant rice genotypes, Tetep and Pankaj, to unravel the transcriptional logic underlying defense against *Rhizoctonia solani*. RNA-seq analysis identified 3,421 and 1,287 upregulated genes in Tetep and Pankaj, respectively, from which 119 canonical R-genes were annotated. *De novo* motif discovery using the MEME suite revealed six statistically enriched motifs (E-value < 1e-5) within 1 kb promoter sequences of R-genes. TOMTOM annotations mapped these motifs to three major transcription factor families: *DOF* (*DNA-binding with One Finger*), *AP2/ERF* (*APETALA2/Ethylene Response Factor*), and *RAI* (*RAMOSA1*)-like *C2H2* zinc fingers. Motif enrichment analysis via FIMO identified 3,371 highly significant occurrences across the promoter dataset, with *DOF* motifs being most abundant (n=1,690), followed by *C2H2* (n=739) and *AP2/ERF* motifs (n=484). CRM detection, defined as clustering of  $e^{-2}$  distinct TF family motifs within 300 bp windows, revealed combinatorial regulatory architectures in 168 R-gene promoters. Critically, 55 genes exhibited *DOF-ERF* co-occurrence, 65 genes showed *DOF-RAI* co-localization, and 11 genes harbored all three TF families, suggesting synergistic transcriptional control. *In silico* validation against functionally characterized genes confirmed biological relevance; notably, *Os09g0533600* (*BSR1*, a validated broad-spectrum resistance kinase) displayed dense CRM clustering. Several CRM-bearing R-genes co-localized with known quantitative trait loci (QTLs) for sheath blight resistance, including *qSBR-11-1*, *qSB-4*, *qSBR-7*, and *qSB-9*. These findings demonstrate that combinatorial CRM architecture, integrating *DOF*-mediated stress responses, *AP2/ERF* hormone signaling, and *RAI*-like regulatory innovation, orchestrates R-gene transcriptional activation during pathogen challenge. This work provides the comprehensive CRM catalog for rice disease resistance and establishes a framework for promoter engineering and breeding strategies aimed at enhancing quantitative and durable resistance to sheath blight.

**Keywords:** *AP2/ERF*, *Cis-regulatory modules*, *DOF*, *RAMOSA1*, *Resistance genes*, *Sheath blight*, *Transcription factors*