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In silico Analysis of Transcription Factor Binding Sites and Impact of Defense Responsive Phytohormones in *OsPR1a*

Diksha Kumari [a], Bishun Deo Prasad [b*], Padmanabh Dwivedi [a] and Sangita Sahni [c]

[a] Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, UP, India.
[b] Department of Agricultural Biotechnology & Molecular Biology, College of Basic Sciences & Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar-848125, India.
[c] Department of Plant Pathology, TCA, Dholi, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar-848125, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

A variety of biotic and abiotic stress conditions result in the production and accumulation of pathogenesis related (PR) proteins in plant. Transcriptional regulation of PR genes plays a vital role in defense response in plant. In rice, the role of the *PR1* gene in defense response have been studied, but critical examination of the *OsPR1a* gene after a treatment with defense responsive phytohormones and their regulation via promoter analysis have not been examined indepth. Several signalling mechanisms are involved in the induction and repression of defense genes, which are mediated by salicylic acid (SA) and jasmonic acid (JA). Expression profiling was carried out to determine the effects of phytohormones, salicylic acid (SA), and jasmonic acid (JA) at 12 h, 24 h, and 48 h after treatment. Expression profiling indicates cumulative upregulation of *OsPR1a* gene at 12 h after SA and JA treatments, whereas it downregulates at 24 h and 48 h after JA treatment. We also performed a comprehensive *in silico* analysis of the promoter region of *OsPR1a* gene to predict how the transcription factor binding site (TFBS) regulate its expression.

**Corresponding author: E-mail: bdprasad@rpcau.ac.in;*

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1. INTRODUCTION

Plants are constantly under threat from several phytopathogens and biotic stresses because they are immobile. Plants' ability to endure environmental stress indicates that they are impervious to them [1]. Several studies have demonstrated that plants respond to these environmental stresses through complex defence signalling pathways [1,2]. It has been studied for decades how host-pathogen interactions lead to plant susceptibility or resistance. As stress stimuli are recognized early and defense responses are activated, these interactions take place in a well-organized fashion [1,2]. The defense response is affected by many factors. There are several key factors responsible for pathogenesis, including reactive oxygen species production (ROS), the activation of hypersensitive responses (HR), phytohormone interaction, and the synthesis of pathogenesis-related proteins (PR). In response to biotic and abiotic stresses, PR proteins accumulate in plants. They also accumulate during specific stages of physiological development, such as fruit ripening, pollen development, and leaf senescence [3–6]. Different types of proteins are derived from these proteins: transcription factors, metabolism-promoting enzymes, protease inhibitors, and hydrolases [3–6]. Tobacco mosaic virus isolates PR1-a was the first protein isolated from tobacco leaves. The PR1 group was isolated from several plant species after this isolation [3], but little else is known about the other members of the PR1 group. As the first PR protein to be discovered, PR1 serves as a molecular marker for systemic acquired resistance (SAR), and it plays a role in both biotic and abiotic responses to stress. There are 12 *OsPR1* genes in rice that are well characterized for disease resistance [7, 8], out of 32 predicted *PR1* genes [9]. Identifying ideal promoters depends largely on identifying pathogen-resistant genes. Pathogen-inducible promoters are characterized by rapid activation when multiple phytopathogens are present. In rice, the PR1 gene is known to play a role in defense response, but the *OsPR1a* gene after exposure to defense-responsive phytohormones and its regulation via promoter analysis hasn't been examined thoroughly. The study of gene expression regulation of PR proteins is crucial to understanding plant defense mechanisms. The responses of plants to pests and diseases are controlled by transcription factors that bind to cis-acting regulatory elements (5–20 bp) associated with specific genes [10]. A comprehensive in silico study of *OsPR1a* genes was performed to learn how transcription factor binding sites (TFBSs) in *OsPR1a* promoter sequences regulate gene expression.

2. MATERIALS AND METHODS

2.1 Defense Responsive Phytohormones Treatment

A rice variety, Rajendra Kasturi, was grown to the four-leaf stage and used for SA. We sprayed the four-leaf stage plants with 3 mM sodium salicylate containing 0.05% Triton-X-100, while control plants were treated with distilled water containing 0.05% Triton X-100 and covered with polythene. We collected the treated leaf samples after 12 h, 24 h and 48 h of incubation [8].

For JA treatment, 21 days old seedlings grown in black portrays (9 cm diameter × 9 cm height) containing a small hole (1 cm diameter) at the bottom for water absorption from a tray (20×14× 7 cm) containing 1 liter of water. Seedlings were then placed on another tray (20 × 14 × 7 cm) containing 100 µM JA [11]. Seedlings placed in distilled water were acted as mock. Leaves tissues of JA and mock treated were collected at 12, 24 and 48 h for gene expression studies.

2.2 Analysis of cis-regulatory Elements

The 1000 bp upstream FASTA files of the promoter sequence of *OsPR1a* retrieved from Rice Annotation Project Database (RAP-DB) (http://rapdb.dna.affrc.go.jp/tools/dump) and retrieved sequences were analysed for the presence of TFBSs using the PlantPan3.0 (http://plantpan3.itps.ncku.edu.tw/) tool. The promoter sequences were further used for PlantCARE databases of plant cis- regulatory DNA elements analysis. Promoter analysis tools provides an informative resource for detecting transcription factor binding sites (TFBSs). PlantPan3.0 contains maximum number of TFs and matrices of TFBSs among 76 plant species covering major families of plants.

2.3 RNA Isolation and Quantitative Real-time RT-PCR (qRT-PCR)

The relative expression of *OsPR1a* genes in treated rice seedlings after SA, JA, and EBR,

treatments is measureed by qRT-PCR. The total RNA was extracted from frozen plant tissue using the SV Total RNA Isolation System (Promega, Madison, WI). Total RNA (1µg) was reverse transcribed for cDNA synthesis using random hexamer primers by following the manufacture's protocol (Promega, Madison, WI). qRT-PCR was carried out using SYBR Green dye on Light Cycler system (Applied Biosystem). Each qRT-PCR quantification was carried out using three biological replication using gene specific primers. The PCR program initial denaturation at 95°C for 2 min, followed by amplification of 40 cycles of denaturation (95°C for 20 s), annealing (53°C for 30 s), and extension (72°C for 30 s). The specificity of amplification was confirmed by melting curve analysis after 40 cycles. The expression value of *ACTIN* was used to normalize the expression data of genes. The expression levels of genes investigated in this study were calculated with the formula given by [12].

3. RESULT

3.1 *OsPR1* a Gene Expression in Response to Hormonel Treatment

Plant hormones SA and JA are the known modulators of plant defense system. The expression studies of *OsPR1a* after defense hormone treated rice seedlings were performed at 12 h, 24 h and 48 h dpi. using qRT-PCR. The upregulation of *OsPR1a* in all the time points analyzed indicates the onset of plant defense mechanism after SA and JA treatments. The differential expression of all the *OsPR1a* was shown in Fig. 1. *OsPR1a* gene was upregulated after SA and JA treatment at 12 h dpi, whereas at 24 h and 48 h dpi shows antagonistic relation. *OsPR1a* gene was downregulates after JA treatment at 24 h and 48 h dpi.

Leaf samples were collected at the 12 h, 24 h, 48 h time points. Transcript levels were analyzed by qRT-PCR analysis and expressed relative to the mock treatment at each time point. Results are representative of three independent experiments. Error bars represent standard error (SE) of mean for three replicates.

3.2 Retrieval of Promoter Regions and Analysis of Plant cis-acting Regulatory Elements (PCAREs) and Transcription Factor Binding Sites (TFBSs) in Rice

Promoter sequences up to 1 kb upstream from the translation start site of each PR gene of *O. sativa* were scanned using PlantCARE and PlantPAN 3 programmes for the identification of PCAREs and TFBSs. Several important defense responsive TFBSs (bHLH, bZIP, C2H2, EIN3, GATA, LEA, MYB, Myb/SANT, NAC, NAM, and WRKY) were identified in the promoter sequences of *OsPR*1a genes (Fig. 2). The maximum number of developmental related TFBSs SBP (62), TBP (41), TCP (34) and TBP (34) are present in *OsPR1a* gene. This gene does not contain WRKY, MADF, and MADS TFBSs.

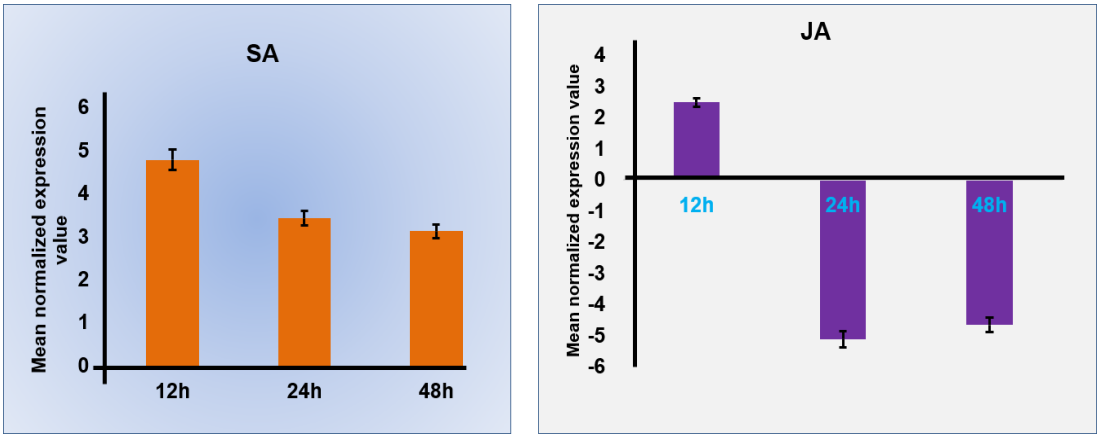

Fig. 1. Response of *OsPR1a* in salicylic acid (SA) and jasmonic acid (JA) treated rice seedlings Leaf samples were collected at the 12 h, 24 h, and 48 h time points. Transcript levels were analyzed by qRT-PCR analysis and expressed relative to the mock treatment at each time point. Results are representative of three independent experiments. Error bars represent the standard error (SE) of the mean for three replicates

Table 1. List of Cis-Regulatory elements in OsPR1a

Site	Position Strand (+)	Position Strand (-)	sequence	Functions
ABRE	139, 137, 140	136, 303, 705	GACACGTACGT	Abscisic acid responsiveness
ABRE3a	139	705, 303	TACGTG	
ABRE4	139	705, 303	CACGTA	
ACE		137	GACACGTATG	light responsiveness
ATCT-motif	831		AATCTAATCC	part of a conserved DNA module involved in light responsiveness
AT~ABRE	139		TACGTGTC	
AT~TATA-box	43, 220, 218, 327	757, 563, 810, 759, 751, 755, 761, 680, 753	TATATA	
Box 4		620, 644, 636, 804, 624, 640	ATTAAT	part of a conserved DNA module involved in light responsiveness
CAAT-box	7, 359, 247, 829, 241, 830, 261, 246, 778, 285, 510, 214, 746	774, 264, 13, 685, 815, 883, 9,	CCAAT	common cis-acting element in promoter and enhancer regions
CGTCA-motif	176, 573		CGTCA	MeJA-responsiveness
G-box	139, 300,	705, 144, 303	TACGTG, TAACACGTAG, CACGAC	light responsiveness
GTGGC-motif	16		GATTCTGTGGC	part of a light responsive element
MYB	916	542	CAACCA	
MYC	7	713	CAATTG	
Myb-binding site	916		CAACAG	
TATA-box	27, 33, 43, 45, 216, 218, 219, 220, 221, 222, 232, 233, 316, 318, 327, 329, 379, 465, 749,	29, 30, 31, 32, 217, 317, 562, 563, 565, 680, 681, 682, 750-763, 809-812, 833, 965, 982, 984	TATACA, TATATAA	core promoter element around -30 of transcription start
TC-rich repeats	418		GTTTTCTTAC	defense and stress responsiveness
TCA	452		TCATCTTCAT	
TGACG-motif		176, 573	TGACG	MeJA-responsiveness
Unnamed__4	49, 869, 956, 450, 783, 402, 597	661, 886,	CTCC	
as-1		176, 573	TGACG	
dOCT	586		CACGGATC	

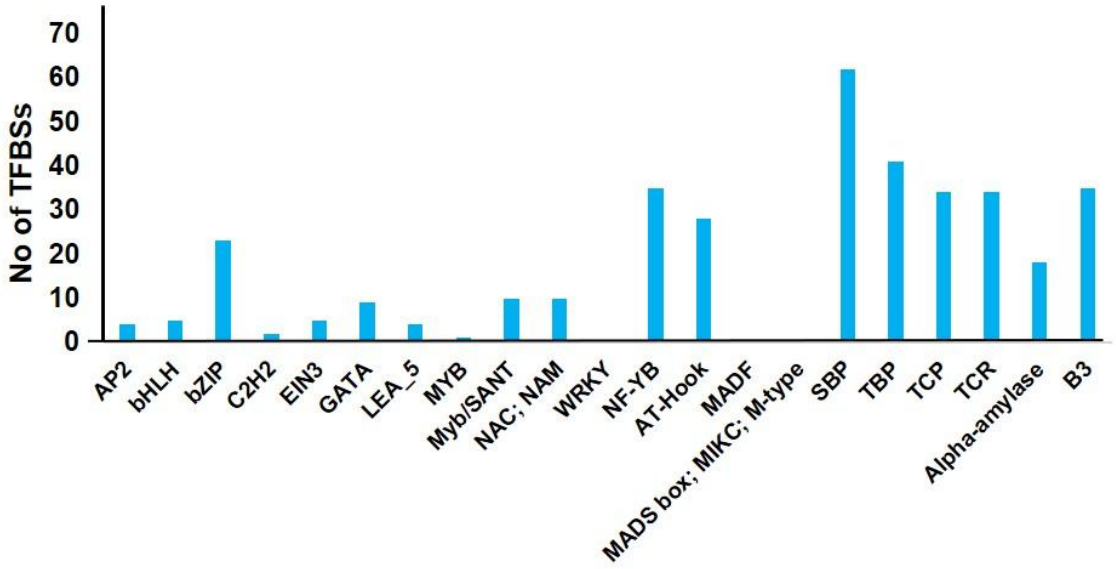

Fig. 2. Putative cis-acting defense related TFs identified in the promoter of *OsPR1a* genes

In addition, this study revealed a total of 21 PCAREs found in *OsPR1a* and their length varied from 4–10 bp. The frequency of occurrence of different cis-elements at different positions in the 1 kb of both reverse and forward strands is almost the same. The majority of the defense responsive cis-elements were located between 16–493 bp on the forward strand (+) and 546–842 bp on the reverse strand (-) (Table 1). The numbers of cis-elements TATA, CAAT box, and Box 4, were greater in *OsPR1a* genes. The phytohormones MeJA-responsiveness consensus sequence CGTCA-motif, TGACG-motif present in the positive and negative strands of 176, 573. This gene also contains defense responsive TC-rich repeats (GTTTTCTTAC) present at 418 positions. *OsPR1a* gene was enriched with abscisic acid responsiveness and maximally found at proximal region (<500) of promoter (Table 1).

4. DISCUSSION

It is undeniable that plant pathogens possess a greater range of ecological adaptions and devastation of plant growth than any other organism. Plants have yet to develop natural resistance to disease, but several strategies have been carried out for plant resistance such as signalling pathway modification, gene pyramiding, overexpression disease responsive genes including PR-genes [13]. The regulation of the defense network that translates the pathogen-induced early signaling events into activation of effective defense responses depends profoundly on the action of plant phytohormones [14]. The importance of SA and JA as primary signals in the regulation of the plant's immune response is well established [14–16]. The SA pathway is primarily induced by and effective in mediating resistance against biotrophic pathogens, whereas the JA pathway is primarily induced by and effective in mediating resistance against herbivores and necrotrophic pathogens [17]. In SA treatment, the induced expression of *OsPR1a* was recorded whereas, down regulation of *OsPR1a* was observed in JA treated samples. The relationship between SA/JA treatments on *PR1* gene expression is extensively studied in plants [18]. A plethora of reports indicate that treatment with salicylic acid up-regulates the *PR*-1 gene expression in plants [18] including rice [7]. In rice, it has been reported that *OsPR1a* genes are induced through the coordinated action of SA, JA and ACC signaling pathways and underlie the Systemic Acquired Resistance [7]. Transcription factors (TFs) regulate gene expression through binding to cis-regulatory specific sequences in the promoters of their target genes [19]. During the last few years, the advance in the determination of TF-binding sites using bioinformatics tools has helped research to decipher expression of genes in different conditions including abiotic and biotic stresses [20, 21]. The expression of a large number of defense-related plant genes is regulated at the transcriptional level in response to pathogen infection [22]. Timely transcriptional regulation of defense-related genes is crucial for effective

responses to pathogens [23]. We analyze the promoter sequences of the *OsPR1a* using PlantPAN2 and PlantCare software. The basic region/leucine zipper motif (bZIP) regulates various stress and developmental responses by binding to various TFBSs. Plant bZIP proteins can recognize the ACGT core in DNA sequences, preferentially the A-box, C-box and G-box [24], CCAAT-box, TGA-element, NON, ABRE [24–26] AS-1 [27], TATCCAT/C-motif [28]. In a previous report was found that the AS-1cis element is an oxidative stress-responsive element and activated by SA by binding TGA cis element [29]. bZIP proteins have imparted a major role in activating several defense genes [30]. AP2/ERF TFs were known to induce the synthesis of ET, SA and JA which enhanced the expression of *PR* genes during pathogen infestation [31, 32]. It is worthy to mention that, presence of AP2/ERF TFBSs in defence responsive genes enhanced resistance against specific abiotic and biotic stresses [33]. In our study we found that Calmodulin signaling responsive gene is mostly associated with ABRE (CGTG) cis-elements [34, 35].

5. CONCLUSION

This study insight light on complex view of PR1 proteins in rice. Even though PR1 proteins are an important family for linking factor of biotic and abiotic stress but some of their characteristics have not been illuminated until now.

DISCLAIMER

The products used for this research are commonly and predominantly used in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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