https://doi.org/10.56093/ijas.v90i1.98553

Indian Journal of Agricultural Sciences **90** (1): 102–6, January 2020/Article

In silico characterization of *WRKY33* TF from *Sinapis alba*

VINOD KUMAR JANGID[1], SHIKHA DIXIT[2], LALIT DEV TIWARI[3], INDRA SINGH[4], ATMAKURI RAMAKRISHNA RAO[5] and ANITA GROVER[6]

ICAR-National Institute for Plant Biotechnology, Pusa, New Delhi 110 012, India

Received: 11 June 2019; Accepted: 05 July 2019

ABSTRACT

The WRKY family of transcription factors modulates the host defense mechanisms in response to various environmental stresses. The role of *WRKY33* in plant defense and its crosstalk with defense hormone was well established in *Arabidopsis* but very few information was noted in *Sinapis alba*. The present study was carried out in 2017, in which computational approaches to characterize the structural and functional features of *SaWRKY33* transcription factor was used. Full length *WRKY33* coding sequence (1509 bp) from *S. alba* has been cloned, sequenced and identified as *AtWRKY33* homolog. The expression of *SaWRKY33* was scored higher in fungal pathogen challenged and jasmonate-treated samples while lower expression was noticed in salicylate-treated samples. Phylogenetic classification, sequence alignment and MEME-based motif scanning demonstrated the remarkable sequential conservation in the WRKY domains and *SaWRKY33* clusters with *Crambe abyssinica* exhibiting the monophyletic origin and paraphyletic evolution from their wild relatives. STRING data showed *SaWRKY33* were interacted with MKS1, MPK3, SIB1, and those are involved in plant defense responses against diverse stress conditions. The homology-based modeling of *SaWRKY33* functional WRKY domains showed acceptable Ramachandran statistics and satisfies all the necessary energy parameters. The Hex Docking server-based analysis of DNA-protein interaction showed that WRKY domain binds to the W-box through WRKYGQK along with few conserved amino acid residues in the flanking sequences and zinc finger motifs.

Key words: Docking, Plant defense, *Sinapis alba*, Transcription factors, *WRKY33*, W-Box

Throughout the course of their entire life, plants encounter multifarious environmental stresses which affect their growth, development and physico-biological processes and ultimately yield. Majority of plants are invulnerable to different pathogens due to presence of highly complex plant defense mechanisms and intricate plant pathogen interactions (Birkenbihl *et al.* 2012). Plants perceive external signals via their membrane anchored receptors and these signals then transmitted to the nucleus in the form of transcription factors, and ultimately initiating expression of defense responsive genes (Lippok *et al.* 2007). Transcription factors bind sequence-specifically to the cis-elements of the target genes and regulate the expression of stress-responsive genes in a highly dynamic manner (Yamasaki *et al.* 2012).

Present address: [1,2]Ph D Student (vinod_shikher@yahoo.co.in, dixitshikha0@gmail.com), [6]Principal Scientist (anitagrover@hotmail.com), ICAR-National Institute for Plant Biotechnology, Pusa, New Delhi, India; [3]Ph D Student (lalit25dev@gmail.com), Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi, India; [4]Research Associate (indrasinghbioinfo@gmail.com), [5]Principal Scientist (arrao@iasri.res.in), ICAR-Indian Agricultural Statistics Research Institute, Pusa, New Delhi, India.

WRKY TFs family is one of the largest plant-specific transcriptional regulator gene family which is integral parts of various signaling pathways and harmonize various plant processes (Zheng *et al.* 2006, Agarwal *et al.* 2011, Mao *et al.* 2011, Lai *et al.* 2011). The WRKY TFs comprise about 60 amino acids conserved region, DNA binding domain possessing an invariant heptapeptide WRKYGQK signature sequence and a zinc-finger motif. The WRKY proteins bind to the W-box sequences-specifically ((T)TTGACY, where Y is C or T) at the promoter regions of the downstream target genes (Rushton *et al.* 1996).

The *WRKY33* is one of the members performing vital roles in modulating and fine-tuning of plant processes and signaling cross-talks which are involved in plant defense mechanism against diverse biotic and abiotic stresses (Zheng *et al.* 2006, Birkenbihl *et al.* 2012). Birkenbihl *et al.* (2012) experimentally confirmed that AtWRKY33 plays as a positive regulator in plant defense against necrotrophic pathogens such as *Botrytis cinerea* and *Alternaria brassicicola* along with induced expression of the JA-responsive plant defensin *PDF1.2* gene. In this study, our emphasis is on detailed characterization of cloned *Sinapis alba WRKY33* gene by deduced amino acid sequence analysis, expression study, *in silico* analysis including domain prediction, protein interactive network analysis and homology-based docking

of *WRKY33* protein to the DNA W-box.

MATERIALS AND METHODS

Plant materials and treatments: In the present study, white mustard (*Sinapis alba* L.) plants were grown in the growth chamber under suitable growth conditions (22°C, 16/8 h light/dark photoperiod) in 2017. *Alternaria brassicae* fungal pathogen was collected from the field and cultured on oat meal agar medium plates and kept for incubation in BOD for 10 days at 26°C temperature. 2 mM salicylic acid and 100 µM Methyl Jasmonate working solution was sprayed on four-week-old plants separately. For the disease assay, fungal spore suspension (5×10^5 spores/ml of distilled water) was inoculated directly onto leaf surface and sterile distilled water as control treatment. Leaf samples were harvested from treated and control plants at different time intervals and stored in -80°C till further use.

Cloning and expression profiling of SaWRKY33 *gene*: Total RNA was extracted from 100 mg of leaf tissues using TRIzol™ reagent as per manufacturer's protocol. 1 µg of total RNA was reverse transcribed into cDNA using oligo(dT) primers with the help of SuperScript III First-Strand synthesis kit. Full-length *SaWRKY33* coding sequence was PCR amplified using gene specific primer sets (W33_F - CTTTATGTATGGCTGCTTCTTCC; W33_R - CTTTATCTTCACGACAAGAACG) and the purified insert was cloned into pGEM®-T Easy vector. Colony PCR was conducted for screening positive colonies and the presence of insert was confirmed by *Eco*RI digestion of the plasmid DNA. For transcript profiling, semi-quantitative PCR was set with optimized parameters for individual target genes. For sqPCR, 20 µL reaction volume containing 1X Taq Buffer, 0.2 Units DNA Taq polymerase, 0.2 mM dNTP, 10 picomole of each primer set (qW33_F- TCAGATGCTGCACAACAACA; qW33_R- AACGAATCGAAAAACGAGGA) and PCR reactions were following as 94°C for 5 min, 30 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 35 sec and final extension at 72°C for 5 min. The amplification products were checked on 1% agarose gel and α-tubulin was used as internal control (Tub_F- AATGTGCTTTCGTTCACTGGT; Tub_R- TTCGTCATCCTCATCGTCAC).

In silico *sequence analysis of WRKY33*: *SaWRKY33* amino acid sequential homologs were identified from different dicots and monocots and retrieved from NCBI-BLAST tool (https://www.ncbi.nlm.nih.gov) and Brassica database (http://brassicadb.org/brad/). ExPASy translate tool (https://web.expasy.org/translate/) was used to deduce amino acid sequences from nucleotide sequences. The physico-chemical properties such as molecular weight, isoelectric point (pI), hydropathy index and half-life of *SaWRKY33* protein were determined using ExPASy Protparam Server (http://web.expasy.org/cgi-bin/protparam/protparam). The CELLO v.2.5 server (http://cello.life.nctu.edu.tw/cgi/main.cgi) was used for predicting subcellular localization of SaWRKY33 proteins. Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used for multiple sequence alignment and unrooted phylogenetic tree was constructed using MEGA7 software (http://megasoftware.net/) following Neighbour-Joining method and 1500 bootstrap replicates. The functional WRKY domains were identified within the *WRKY33* protein using ExPASy-Prosite scan (http://prosite.expasy.org/scanprosite/). MEME Suite 4.12.0 (http://meme-suite.org/tools/meme) was used to discover the conserved motifs present within the WRKY33 amino acid sequences with optimum motif width between 6–40 residues and maximum number of motifs up to 8. The STRING version 11.0 servers (http://string-db.org/) was employed for predicting the *SaWRKY33* protein interaction network with selecting maximum 10 interactors for first shell of interactions and 5 for second shell of interactions. The prediction was based on *Arabidopsis* datasets due to unavailability of *Sinapis* datasets and interactive scores were evaluated at high confidence level (0.700).

Structural modeling of WRKY domains and their interaction with DNA W-box: Based on homology approach, WRKY domains of *SaWRKY33* protein was modeled using Discovery Studio 4.1. Using BLASTp tool against Protein Data Bank (PDB; http://www.rcsb.org/pdb/), the searched template 2AYD (CTD of AtWRKY1) was found to be suitable for protein modeling and 2LEX (complex of CTD of AtWRKY4 and W-box DNA) for docking purpose with >65% sequence homology. Using PROCHECK module of PDBSum server (http://www.ebi.ac.uk/pdbsum/, models were validated and evaluated for stereo-chemically quality by Ramachandran plot. The stabilities of models were further improved by energy minimization employing implementation of CHARMM force field. HEX 6.1 standalone tool was employed for study of molecular interaction between WRKY domains of *SaWRKY33* with DNA W-box using 2LEX as a template and Shape + Electro parameters. Further, DS Studio 4.1 was used for visualization and interaction analysis of the docked complexes.

RESULTS AND DISCUSSION

Molecular cloning and insight into SaWRKY33 *sequence*: The response of *WRKY33* gene in plant defense against diverse stresses has been unravelled in model plants such as *Arabidopsis* (Lippok *et al.* 2007), but scanty information has been found in *S. alba*. Considering this, full length *SaWRKY33* coding sequence was cloned and sequence characterized. The cloned *SaWRKY33* sequence was confirmed through colony PCR and *Eco*RI restriction digestion by releasing of 1509 bp insert (Fig S1A-B) and sequence was submitted to GenBank database with accession number MG001179.1 with 75.09% sequence similarity with *AtWRKY33*. Our ProtParam results revealed that *SaWRKY33* encodes a protein of 502 amino acid residues with molecular mass of 55.56 KDa, $C_{2368}H_{3651}N_{721}O_{808}S_{13}$ atomic composition and predicted pI of 8.10 (Table 1). The SaWRKY33 protein was shown to have hydrophilic and soluble nature based on low Grand Average Hydropathicity (GRAVY) score -1.030 and unstable nature with a half-life of less than 5 h and exhibiting instability index of 56.37

Table 1 Physico-chemical properties of SaWRKY33 protein

Organism/ Sequence	No. of AAs	Molecular weight	pI	+ve AAs	-ve AAs	EC	Ii	Ai	GRAVY*	Most abundant amino acid (%)	Least abundant amino acid (%)
SaWRKY33	502	55565.16	8.10	54	52	51340	56.37	43.90	-1.030	Serine (15.5 %)	Trp (1.0 %), Cys (1.2 %) Met (1.4 %)

pI, Isoelectric point; EC, Extinction Coefficient (/M/cm); Ii, Instability index; Ai, Aliphatic index; GRAVY, Grand average hydropathy.

(Table 1). Agarwal *et al*. (2011) correlated the protein thermostability with positive and high aliphatic index (Ai) which may be showing responses to diverse biotic and abiotic stresses. In our result, the Ai of *SaWRKY33* protein was found to be 43.90 (Table 1). Mao *et al.* (2011) demonstrated the potential MAPK phosphorylation site was serine at N-terminus of *WRKY33* protein in response to pathogen invasion. From the results of *SaWRKY33* amino acid composition, tryptophan, cysteine and methionine were the least abundant amino acids while serine was the most abundant (15.5%) (Table 1). CELLO v.2.5 server predicted *SaWRKY33* protein localized in nucleus with a highest reliability index of 4.287.

Expression analysis of SaWRKY33 *genes under biotic stresses*: The *AtWRKY33* gene showed differential expression patterns to diverse stresses and variety of hormonal treatments which were analyzed based on microarray and mutant study (Sham *et al.* 2017). Our sqPCR results revealed that *SaWRKY33* gene displaying higher expression in fungal pathogen challenged and JA treated leaf samples while comparatively lower expression in SA treated samples (Fig S1C). Birkenbihl *et al.* (2012) reported higher transcript levels in fungal pathogen challenged *Arabidopsis* leaf samples about 6 folds at 8 hpi and that was further increased up to 48 hpi during infection.

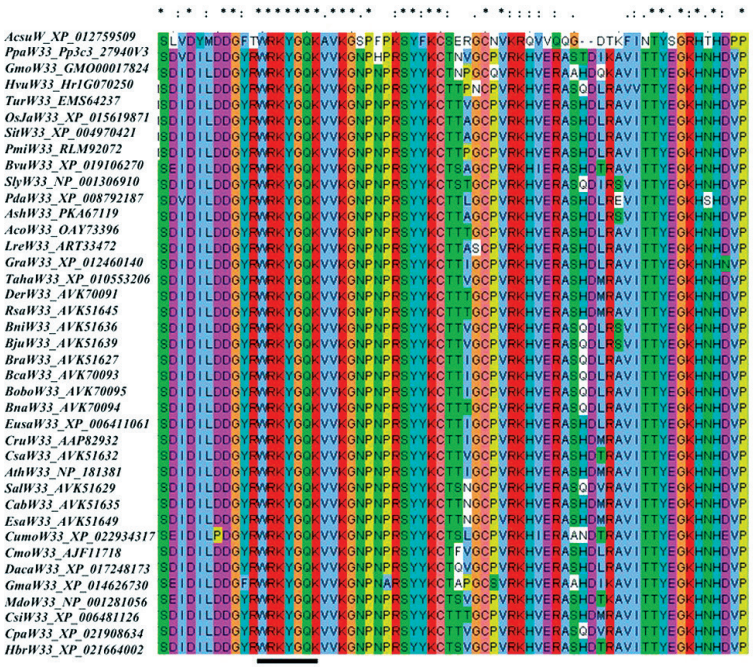

Fig 1 The amino acid sequence alignment of CTD of WRKY33 protein.

Multiple sequence alignment and phylogenetic analysis: The maximum conservation of amino acid residues was found in both C-terminal (CTD, Fig 1) and N-terminal (NTD, Fig S2) WRKY domains of the *SaWRKY33* protein from various plant species with few amino acid substitutions of very similar or different chemical properties. Within the functional WRKY domains, least disturbed and most conserved core residues elucidate their evolutionary significant role in phylogenetic origin and their vital role in defense and other plant physiological activities which mediated through their interaction with specific ligand molecules for triggering responses to various environmental stresses (Brand *et al.* 2013). Phylogenetic analysis results showed that *SaWRKY33* (AVK51629.1) exhibiting the close relationship with *Crambe abyssinica* (AVK51635.1) with monophyletic origin while paraphyletic origin with other mustard family members like AtWRKY33 (NP_181381.2) and BjWRKY33 (AVK51639.1) (Fig 2A). The list of plant species with their accession number and family name is given in Table S1.

C-terminal WRKY domain displays the strong conservation; Above the sequences, asterisk (*), colon (:) and period (.) marks represent the identical or fully conserved amino acids, residues with strongly similar properties and residues with weakly similar properties respectively; Below the sequences, a horizontal bar represents the invariant heptapeptide WRKYGQK signature sequence.

Prediction and analysis of functional domains and motifs: At least one WRKY DBD region which encompassing the highly conserved WRKYGQK signature sequence along with zinc finger motif possessed by the WRKY TF superfamily members which in turn participate in sequence specific binding to the cis-elements (Yamasaki *et al.* 2012). We identified two highly conserved and functional sites within the WRKY DBDs based on ExPASy-Prosite scan of the *SaWRKY33* amino acid sequences. The NTD of *SaWRKY33* stretches from Arg^{172} to Pro^{236} residues with the core signature sequences existed from Glu^{178} to Pro^{236} whereas the CTD stretches from Ile^{326} to Pro^{397} having signature sequences from Ser^{332} to Pro^{397} (Fig S3). Hence, *SaWRKY33* protein can be classified into group I of *WRKY* gene superfamily based on two WRKY DBDs and C-X_4-C-X_{22-23}-H-X-H-type zinc finger motifs.

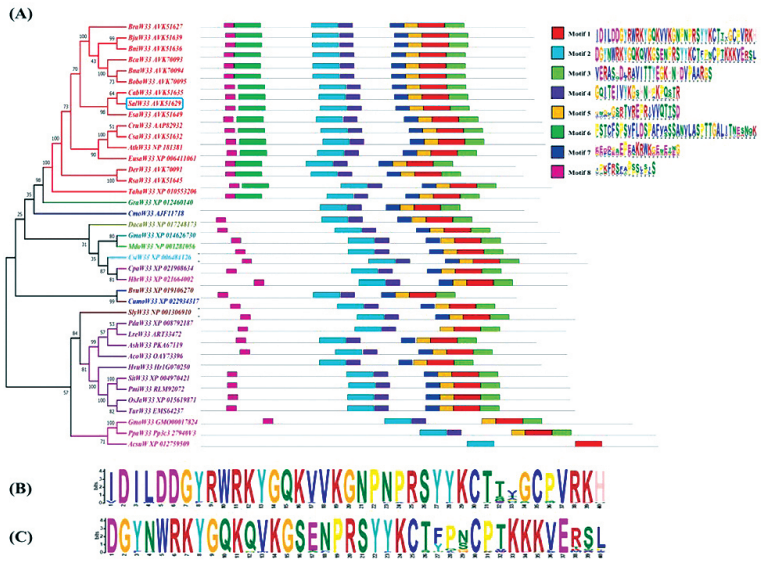

Fig 2 Unrooted phylogenetic tree of *WRKY33* proteins and distribution of their predicted motifs.

The MEME motif search results revealed the more uniformly distribution of conserved motifs across the diverse plant members which participate in forming functional WRKY domains. Our MEME results clearly demonstrated that two fundamental motifs are present in all the plant members which encompasses in the functional WRKY signature sequences and they revealing functional and structural conservation (Fig 2A). Due to substitution of one or more amino acid residues, plant members showed variation in motif numbers which reflect the sequence divergence and forming separate clusters. The closely related plant members showing more similar patterns of motif distribution. The C-terminal of *SaWRKY33* represented by motif 1 (*p*-value 1.9e–51; Fig. 2B) and N-terminal by Motif 2 (P-value 4.9e–53; Fig 2C) in which motif 1 shows least P-values, therefore C-terminal exhibited more conserved residues.

Protein interactive network study: Since, *SaWRKY33* have been shown great extent of conservation with AtWRKY33, therefore, they participating most of common protein interacting partners in their functional associative protein networks. Mao *et al.* (2011) reported two pathogen-responsive MAPKs (MPK3 and MPK6), which are involved in induction of the antimicrobial compound camalexin in biosynthesis via WRKY33 phosphorylation and regulation of *PAD3* gene transcription. Our STRING-based results predicted various protein partners of *SaWRKY33* which are involved in diverse biological functions and forming protein interactive networks (Fig 3). The interaction of *SaWRKY33* protein and MKS1 (MAP Kinase Substrate 1) was predicted with maximum interactive score of 0.997 (Fig 3; Table S2). MKS1 mainly participates in plant defense regulation as it functions like MPK4 adaptor protein which in turn affects WRKY protein activities (Andreasson *et al.* 2005). Other predicted protein partners which include MPK3, MPK4, SIB1 protein (Sigma factor binding protein 1), SIB2, ACS6 (ACC synthase 6), STZ (Cys2/His2-type zinc-finger proteins), SZF1 (Zinc finger CCCH domain-containing protein 47), MYB51 and ERF6 (Ethylene responsive element binding factor 6) in the first shell of interaction and MKK2, MKK6, MEK1, MKP2 and VIP1 proteins in the second shell of interaction. Majority of the interacting partners for *SaWRKY33* protein belong to the plant defense-related proteins which are involved in plant defense activities against biotic and abiotic stresses. It was demonstrated that the DNA-binding activity of *WRKY33* is enhanced through specific recognition and modulation of the WRKY CTD mainly by three VQ proteins, viz. VQ23 (SIB1), VQ16 (SIB2) and VQ21 (MKS1) (Lai *et al.* 2011, Cheng *et al.* 2012). Li *et al.* (2012) revealed the role of MPK3 and MPK6 in the regulation of ACS2 and ACS6 gene expression and their protein stability post-translationally via direct phosphorylation of *WRKY33* and its binding to the promoter regions. Under osmotic stress conditions, MPK3 and MPK6 phosphorylate ERF6 which in turn activates the expression of the stress-related transcription factor genes such as *STZ*, *MYB51* and *WRKY33* (Dubois *et al.* 2013).

Homology modeling of WRKY domains and docking analysis of SaWRKY33: To unravel interaction patterns of WRKY domains with other proteins and DNA W-box, WRKY domains were modeled using homology modeling-based approach. Best 3D models were selected based on associated electrostatic energies, percent coverage and their stereo quality which are very crucial for determining protein structure stability and model reliability. Our validated results showed that 99.1% amino acid residues were found in most favored regions, while 0.9% in allowed regions in Ramachandran plot which shows quality and reliability of

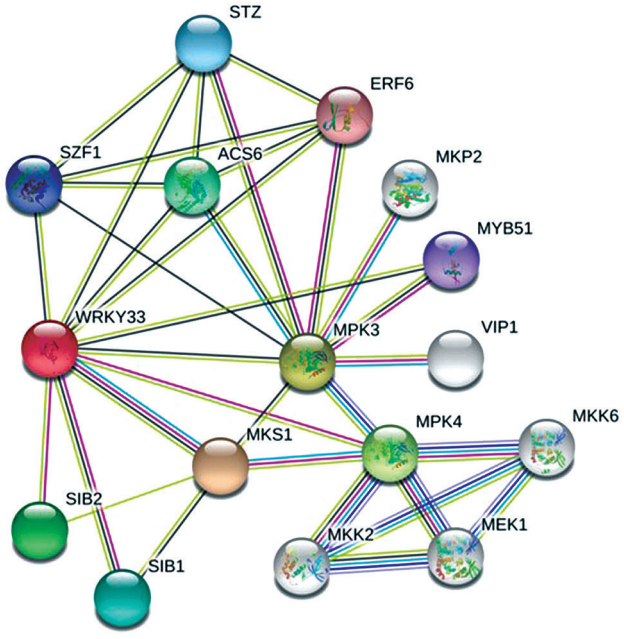

Fig 3 Protein interaction network for *SaWRKY33* proteins.

protein domains (Fig S4A-B).

WRKY TFs have stereotypic preferential binding with DNA cis-regulatory element, W-box of the targeted genes and regulate the gene expression spatio-temporally at transcriptional level (Brand *et al.* 2013, Ciolkowski *et al.* 2008). The involvement of WRKYGQK sequence in the DNA binding was investigated using mutational experiments and identified very crucial amino acid residues for DNA binding. Those are particularly Trp, Tyr, and two Lys residues which are playing significant role in maintaining and stabilizing of correct structure of DNA-protein complex (Maeo *et al.* 2001, Yamasaki *et al.* 2005, Yamasaki *et al.* 2012). Our docking results of *SaWRKY33* protein over DNA W-box substantiated that Arg^{175}, Lys^{176}, Glu^{178}, Arg^{216}, Thr^{223}, Glu^{224}, His^{231} and Lys^{235} residues of NTD are mainly participate in the interaction with T^5, G^8, A^9, G^{22}, T^{24} and C^{25} DNA bases (Fig S4D). Whereas, Arg^{325}, Val^{327}, Gln^{329}, His^{373}, Glu^{375}, Ser^{378} and Gln^{379} residues of C-terminal are involved in interaction with T^6, G^8, A^{26}, A^{28}, G^{29} and G^{30} DNA bases (Fig S4E). The lysine residue in the conserved WRKYGQK motif favors contact with negatively charged DNA phosphate backbone, whereas glutamine residue favors the binding with nucleotide bases due to its partial negative charge (Brand *et al.* 2013). The two WRKY domains from group I exhibited different roles in DNA-binding process as the CTD play a crucial role in binding to DNA W-box, whereas increasing the binding affinity and specificity to target gene as determined by NTD (Ciolkowski *et al.* 2008).

Using computational approaches for the structural and functional interpretation of WRKY TFs provides clear understanding about sequence specific attributes which show functional conservation within members that regulates transcriptional reprogramming of stress responsive genes under environmental stress conditions. This study would provide information regarding their evolutionary divergence, ancestral origins and phylogenetic relationships within family members and even distant members, thus the possible biological roles of gene family could be elucidated. The computational approaches along with mutant and gene complementation techniques would be helpful in functional characterization of individual gene. Against various environmental stresses including biotic and abiotic stresses, these studies may provide some positive perspective for Brassica breeding program.

ACKNOWLEDGMENTS

The authors are grateful to Department of Biotechnology, Ministry of Science and Technology, Govt. of India, for providing the financial assistance in form of DBT-JRF (DBT/2014/IARI/234).

REFERENCES

Agarwal P, Reddy M P and Chikara J. 2011. WRKY: its structure, evolutionary relationship, DNA-binding selectivity, role in stress tolerance and development of plants. *Molecular Biology Reports* **38**(6): 3883–96.

Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen N H T and Zhu S. 2005. The MAP kinase substrate MKS1 is a regulator of plant defense responses. *The EMBO Journal* **24**: 2579–89.

Birkenbihl R P, Diezel C and Somssich I E. 2012. *Arabidopsis* WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. *Plant Physiology* **159**: 266–85.

Brand L H, Fischer N M, Harter K, Kohlbacher O and Wanke D. 2013. Elucidating the evolutionary conserved DNA-binding specificities of WRKY transcription factors by molecular dynamics and *in vitro* binding assays. *Nucleic Acids Research* **41**(21): 9764–78.

Cheng Y, Zhou Y, Yang Y, Chi Y-J, Zhou J and Chen J-Y. 2012. Structural and functional analysis of VQ motif-containing proteins in *Arabidopsis* as interacting proteins of WRKY transcription factors. *Plant Physiology* **159**: 810–25.

Ciolkowski I, Wanke D, Birkenbihl R P and Somssich I E. 2008. Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function. *Plant Molecular Biology* **68**: 81–92.

Dubois M, Skirycz A, Claeys H, Maleux K, Dhondt S and Bodt S D. 2013. ETHYLENE RESPONSE FACTOR6 acts as a central regulator of leaf growth under water-limiting conditions in *Arabidopsis*. *Plant Physiology* **162**: 319–32.

Lai Z, Li Y, Wang F, Cheng Y, Fan B and Yu J Q. 2011. *Arabidopsis* sigma factor binding proteins are activators of the WRKY33 transcription factor in plant defense. *The Plant Cell* **23**: 3824–41.

Li G, Meng X, Wang R, Mao G, Han L and Liu Y. 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*. *PLoS Genetics* **8**(6): e1002767.

Lippok B, Birkenbihl R P, Rivory G, Brümmer J, Schmelzer E and Logemann E. 2007. Expression of *AtWRKY33* encoding a pathogen- or PAMP-responsive WRKY transcription factor is regulated by a composite DNA motif containing W box elements. *Molecular Plant-Microbe Interactions* **20**(4): 420–29.

Maeo K, Hayashi S, Kojima-Suzuki H, Morikami A and Nakamura K. 2001. Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family proteins. *Bioscience, Biotechnology, and Biochemistry* **65**(11): 2428–36.

Mao G, Meng X, Liu Y, Zheng Z, Chen Z and Zhang S. 2011. Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. *Plant Cell* **23**: 1639–53.

Rushton P J, Torres J T, Parniske M, Wernert P, Hahlbrock K and Somssich I E. 1996. Interaction of elicitor-induced DNA binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *EMBO Journal* **15**: 5690–5700.

Sham A, Moustafa K, Al-Shamisi S, Alyan S, Iratni R and AbuQamar S. 2017. Microarray analysis of Arabidopsis *WRKY33* mutants in response to the necrotrophic fungus *Botrytis cinerea*. *PLoS ONE* **12**(2): e0172343.

Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T and Yabuki T. 2005. Solution structure of an *Arabidopsis* WRKY DNA binding domain. *Plant Cell* **17**: 944–56.

Yamasaki K, Kigawa T, Watanabe S, Inoue M, Yamasaki T and Seki M. 2012. Structural basis for sequence-specific DNA recognition by an *Arabidopsis* WRKY transcription factor. *Journal of Biological Chemistry* **287**(10): 7683–91.

Zheng Z, Qamar S A, Chen Z and Mengiste T. 2006. Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. *Plant Journal* **48**: 592–605.