Plant Science 227 (2014) 145–156

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

# Tomato WRKY transcriptional factor SlDRW1 is required for disease resistance against *Botrytis cinerea* and tolerance to oxidative stress

Bo Liu[a,b], Yong-Bo Hong[a], Ya-Fen Zhang[a], Xiao-Hui Li[a], Lei Huang[a], Hui-Juan Zhang[a], Da-Yong Li[a], Feng-Ming Song[a,*]

[a] National Key Laboratory for Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, Zhejiang, 310058, China
[b] Weinan Vocational and Technical College, Weinan, Shanxi, China

ARTICLE INFO

*Article history:*
Received 15 May 2014
Received in revised form 1 August 2014
Accepted 3 August 2014
Available online 10 August 2014

*Keywords:*
Tomato (*Solanum lycopersicum*)
WRKY proteins
SlDRW1
*Botrytis cinerea*
oxidative stress
defense response

ABSTRACT

WRKY proteins comprise a large family of transcription factors that play important roles in plant responses to biotic and abiotic stresses; however, only a few of tomato WRKYs have been studied for their biological functions. In the present study, we identified a *Botrytis cinerea*-responsive WRKY gene *SlDRW1* (*Solanum lycopersicum* defense-related WRKY1) from tomato. SlDRW1 is a nucleus localized protein with transactivation activity in yeast. Expression of *SlDRW1* was significantly induced by *B. cinerea*, leading to 10–13 folds of increase than that in the mock-inoculated plants but not by *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000. Silencing of *SlDRW1* resulted in increased severity of disease caused by *B. cinerea*, but did not affect the phenotype of disease caused by *Pst* DC3000. In addition, silencing of *SlDRW1* also resulted in decreased tolerance against oxidative stress but did not affect drought stress tolerance. Furthermore, silencing of *SlDRW1* attenuated defense response such as expression of defense-related genes after infection by *B. cinerea*. Our results demonstrate that SlDRW1 is a positive regulator of defense response in tomato against *B. cinerea* and oxidative stress.



## 1. Introduction

During their lifetime, plants have to confront different kinds of biotic and abiotic stresses around their growth environment. To cope with these stresses to survive, plants have developed a series of defense mechanisms regulated by a complicated signaling network, which is often initiated upon perceiving environmental cues [1–5]. Activation of defense responses against biotic and abiotic stress is always accompanied with significant alterations in expression of a large set of genes, which are regulated by different types of transcription factors (TFs). Thus, TFs are critical regulatory factors that determine the outcome of plant interactions with biotic and abiotic stress through modulating the temporal and spatial expression of the genes involved in defense response. In recent years, many TFs belonging to the NAC, ERF, MYB, WRKY and bZIP families have been identified to play important roles in plant responses to biotic and abiotic stress [6–10].

The WRKY proteins comprise one of the largest families and contain either one or two copies of the conserved WRKY domain, followed by a $C_2H_2$ or $C_2HC$ zinc finger motif [11]. Based on the number of WRKY domains and the type of the zinc finger motifs, the WRKY proteins can be classified into three groups [11,12]. Group I WRKYs contain two WRKY domains whereas Group II and III WRKYs contain only one WRKY domain. Another, WRKYs belonging to Group I and Group II have a $C_2H_2$ zinc finger motif whereas the Group III WRKYs have $C_2HC$ zinc finger motif [11,12].

Although WRKYs have recently been implicated in the regulation of plant growth and development [12,13], the most important functions for WRKYs seem to act as regulators of defense response against biotic and abiotic stresses. Expression of WRKY genes can be induced strongly and rapidly by different biotic and abiotic stresses in numerous plant species [11,14,15]. For example, 49 out of 72 Arabidopsis WRKY genes can respond to bacterial infection or salicylic acid (SA) and most of the Group III WRKY genes can be induced by both SA and pathogens [16,17]. A large body of studies with functional genomic approaches by analyzing phenotypes of the knockout/knockdown or overexpression lines has demonstrated that the WRKY proteins play critical roles in regulating disease resistance responses in plants. In *Arabidopsis thaliana*, WKRY3 and WRKY4 [18], WRKY8 [19], WRKY11 and WRKY17 [20], WRKY18/WRKY40/WRKY60 [21], WRKY22 [22], WRKY27 [23], WRKY25 [24], WRKY33 [25], WRK46 [26], WRKY70 [27,28] and WRKY72 [29] have been shown to

* Corresponding author. Tel.: +86 571 88982481.
*E-mail address:* fmsong@zju.edu.cn (F.-M. Song).

http://dx.doi.org/10.1016/j.plantsci.2014.08.001
0168-9452/

be either positive or negative regulators of defense response against different types of pathogens. Similarly, at least 10 rice WRKYs, i.e. OsWRKY03 [30], OsWRKY71 [31], OsWRKY13 [32], OsWRKY45 [33,34], OsWRK89 [35], OsWRKY31 [36], OsWRKY22 [37], OsWRKY30 [38], OsWRKY28 [39], OsWRKY76 [40] and OsWRKY62 [41], have been implicated in immune responses against fungal and bacterial pathogens. Alternatively, some of the WRKY proteins have also been shown to play important roles in regulating abiotic stress tolerance (for review see [42]), such as Arabidopsis WRKY6 and WRKY75 in low phosphorus stress [43,44], WRKY25 and WRKY39 in heat stress [45,46], WRKY63 (ABO3) and WRKY57 in drought stress [47,48], WRKY34 in cold stress [49]), WRKY18, WRKY40, WRKY70 and WRKY54 in osmotic stress [50,51], WRKY30 in oxidative stress [52] and WRKY46 in aluminum toxicity [53], and rice OsWRKY30 in drought stress [54]. These observations suggest that, compared with other TF families, a relatively larger proportion of the WRKY family members in a given plant species play roles in regulating defense response against diverse biotic and abiotic stresses, demonstrating the importance of the WRKY proteins in plant stress responses.

Recent genome-wide bioinformatics analysis identified a total of 81 WRKY genes in tomato genome [55]. However, only a few of tomato WRKYs have been characterized at molecular level for their biological functions. It was recently reported that SlWRKY70 and SlWRKY72 are required for *R* gene *Mi-1*-mediated resistance to aphids and nematodes [29,56] and SlWRKY72 also contributes to basal immunity against *Pseudomonas syringae* [29]. Overexpression of a tomato WRKY gene in transgenic tobacco plants resulted in increased expression of defense genes and improved abiotic stress tolerance [57]. Furthermore, some tomato WRKY genes were found to show differential expression patterns after infection with viral, bacterial and fungal pathogens [55,58–60] or treatment with pathogen-derived elicitors [61]. However, the biological functions for the majority of the tomato WRKYs are not clear yet. In this study, we performed VIGS-based assays to identify putative WRKYs that are involved in defense response in tomato against *B. cinerea*, the causal agent of grey mold disease on a number of economically important crops. We found that silencing of one tomato WRKY gene led to increased severity of disease caused by *B. cinerea* and designated this putative WRKY gene as *SlDRW1* (*Solanum lycopersicum* defense-related WRKY1). Results from further experiments demonstrate that SlDRW1 plays important roles in defense response against *B. cinerea* and oxidative stress tolerance in tomato.

## 2. Materials and methods

### 2.1. Plant growth and treatments

Tomato (*S. lycopersicum*) cv. Suhong 2003 was used in this study. Tomato and *Nicotiana benthamiana* plants were grown in a mixture of perlite:vermiculite:plant ash (1:6:2) in a growth room at 22 °C under a 16 h light (350 μmol $s^{-1}$ $m^{-2}$ photons $m^{-2}$ $s^{-1}$) and 8 h dark regime. For analysis of gene expression in response to defense signaling hormones, 4-week-old tomato plants were treated by foliar spraying with 100 μM methyl jasmonate (MeJA), 100 μM 1-amino cyclopropane-1-carboxylic acid (ACC), 1 mM salicylic acid (SA) or water as a control. For analysis of gene expression in response to pathogen infection, 4-week-old plants were inoculated with spore suspension of *B. cinerea*, bacterial suspension of *Pst* DC3000 or with same volume of buffer as a mock-inoculation control (see below "Disease assays" for details). Leaf samples were collected at indicated time points after treatment or inoculation and stored at −80 °C until use.

### 2.2. Extraction of total RNA

Total RNA was extracted from leaf tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and treated with PrimeScript RT reagent Kit With gDNA Eraser (Takara, Dalian, China) to eliminate DNA according to the manufacturer's protocols. The total RNA samples were stored at −80 °C until use.

### 2.3. Cloning of SlDRW3 and bioinformatics analysis

First-strand cDNA was synthesized using the AMV reverse transcriptase (Takara, Dalian, China) with oligo d(T) primer according to the manufacturer's instructions. Full-length cDNA of *SlDRW1* was amplified using a pair of *SlDRW1*-specific primers SlDRW1-orf-1F (5′-ATG GCT GCT TCA AGT TTC TCT TTT CC-3′) and SlDRW1-orf-1R (5′-TTC ATA AAC TTC AAT TCG TGC TCC T-3′). The PCR product was purified and cloned into pMD-19T vector (Takara, Dalian, China), followed by sequencing for confirmation. Similarity analyses of nucleotide and amino acid sequences were carried out using BLAST program at the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Plant WRKY protein sequences were retrieved from NCBI GenBank. Sequence alignment was performed using ClustalX (version 2.0.8) and phylogenetic tree was generated by neighbor-joining algorithm with *p*-distance method using MEGA version 6.05. A bootstrap statistical analysis was performed with 1000 replicates to test the phylogeny.

### 2.4. Construction of VIGS vector and agroinfiltration

A 437 bp fragments of *SlDRW1* was amplified with a pair of *SlDRW1*-specific primers SlDRW1-vigs-1F (5′-GCG TCT AGA TGA CGA CTT CTT TCA CCG ACC TT-3′, a *Xba*I site underlined) and SlDRW1-vigs-1R (5′-ATA GGA TCC TGT GGG CTC TTG ACA ATT CCA T-3′, a *Bam*HI site underlined) using plasmid pMD19T-SlDRW1 as templates. The resulting products were digested and cloned into pYL156, yielding TRV-SlDRW1 construct. For construction of TRV-GUS, a 396 bp fragments of the GUS gene was amplified with primers GUS-vigs-1F (5′-CGG TCT AGAACC TGG GTG GAC GAT ATC AC-3′, a *Xba*I site underlined) and GUS-vigs-1R (5′-CGG GGA TCC GTG CAC CATC AGC ACG TTA T -3′, a *Bam*HI site underlined), and cloned into pYL156, yielding TRV-GUS construct. The recombinant plasmids TRV-SlDRW1 and TRV-GUS were transformed into *Agrobacterium tumefaciens* strain GV3101 by electroporation. Agrobacteria carrying TRV-SlDRW1 or TRV-GUS construct were cultivated in YEP liquid medium with 50 μg/mL kanamycin, 50 μg/mL rifampicin and 25 μg/mL gentamicin to $OD_{600}$ = 0.8∼1.0. Cells were centrifuged and resuspended in infiltration buffer containing 10 mM $MgCl_2$, MES (pH 5.7) and 150 μM acetosyringone. The agrobacteria carrying TRV-GUS or TRV-SlDRW1 were mixed with agrobacteria carrying pTRV1 in a ratio of 1:1 and maintained at $OD_{600}$ = 1.5 for 3 h at room temperature. The mixed agrobacteria suspension was infiltrated into the abaxial surface of the 2-week-old seedlings using a 1 mL needleless syringe. Efficiency of the VIGS protocol was evaluated using phytoene desaturase (PDS) gene as a marker of silencing in tomato plants according to Liu et al. [62]. The VIGS-infiltrated plants were allowed to grow for 3 weeks under the same conditions as mentioned above and then used for all experiments.

### 2.5. Transient expression in N. benthamiana

The coding sequence of *SlDRW1* was amplified using a pair of primers SlDRW1-oe-1F (5′-ATA GGA TCC ATG GAA TTT ACC AGT TTG GT-3′, a *Bam*HI site underlined) and SlDRW1-oe-1R