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Research article

Six NAC transcription factors involved in response to TYLCV infection in resistant and susceptible tomato cultivars

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ABSTRACT

NAC transcription factors (TFs) belong to plant-specific TFs, which have been identified in many plant species. The NAC TFs act as the nodes of a regulatory network in plant's response to abiotic and biotic stresses. Till now, response of tomato NAC TFs involved in Tomato yellow leaf curl virus (TYLCV) infection is unknown. In the present study, six NAC TFs were identified to respond to TYLCV infection in tomato. We observed that transcripts of four NAC genes (*SlNAC20*, *SlNAC24*, *SlNAC47*, and *SlNAC61*) were induced after TYLCV infection in resistant tomato cultivar. Virus-induced gene silencing analysis (VIGS) indicated that *SlNAC61* played positive roles in response to TYLCV infection. Tomato NAC TFs were not only involved in defense regulation but in development and stress progress. These NAC TFs interacted with other proteins, including protein phosphatase and mitogen-activated protein kinase. Some defense response TFs, such as WRKY, TGA, MYB, NAC, could interact with NAC proteins by binding *cis*-elements in promoter regions of NAC TFs. These identified tomato NAC TFs cooperated with other TFs and proteins, indicating the complex response mechanism of described NAC TFs involved in TYLCV infection. The results will offer new evidence to further understand the NAC TFs involved in response to TYLCV infection in tomato.

1. Introduction

Owing to their sessile lifestyle, plants are frequently threatened by a broad range of abiotic (low/high temperature, chilling, drought, and salinity) and biotic stresses (fungi, viruses, bacteria, and nematodes) (Fujita et al., 2006). To survive under these stresses, plant cells can activate regulated defense reactions which accompany expression alteration of corresponding genes. Different types of transcription factors (TFs) mediate transcriptional regulation of gene expression (Liu et al., 2014a, b).

Over the past decades, plant signal regulations by TFs have been demonstrated as one of the most effective defense responses (Nakashima et al., 2009). TFs, as critical regulatory factors, can determine interaction outcomes between plants and adverse stress by modulating related genes expression (Nakashima et al., 2009; Du et al., 2014). Numerous studies have demonstrated the important roles of TFs played in plant tolerance against abiotic and biotic stresses (Nakashima et al., 2009; Du et al., 2014). NAC TFs, which were identified in many plant species including *Arabidopsis*, *Oryza sativa*, *Populus trichocarpa*, *Brassica rapa*, *Triticum aestivum*, *Camellia sinensis*, were known to pose diverse roles in plant growth and development processes (Ooka et al., 2003; Uauy et al., 2006; Hu et al., 2010; Ma et al., 2014; Wang et al., 2016b). NAC TFs have been shown to play important roles in transcriptional regulation for increasing tolerance to abiotic and biotic stresses (Ohnishi et al., 2005; Jensen et al., 2007; Jin et al., 2017). Overexpression of *Pbe-NAC1* in tobacco, isolated from *Pyrus betulifolia*, enhanced tolerance to cold and drought (Jin et al., 2017). *OsNAC6* was found to be induced by cold, drought, abscisic acid (ABA), methyl jasmonate (MeJA), and high sality (Ohnishi et al., 2005).

NAC TFs also take part in the plant–pathogen interaction. Two NAC TFs of *Arabidopsis* (ATAF1 and CBNAC) were identified as transcriptional regulators in pathogen defense (Delessert et al., 2005; Mauch-Mani and Flors, 2009). A potato NAC TF, StNAC was identified to be involved in response to wounding and pathogen infection (Collinge and Boller, 2001). Two maize NAC TFs were induced in response to fungal attack (Voitsik et al., 2013). Two wheat NAC TFs (TaNAC4 and TaNAC8) acted as transcriptional activator in defense response against strip rust pathogen infection (Xia et al., 2010a, b).

As a destructive disease pathogen, Tomato yellow leaf curl virus

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Abbreviations

ABA	abscisic acid
CDPK	calcium-dependent protein kinase
dpi	day post infection
ET	ethylene
GFP	green fluorescent protein
JA	jasmonic acid
MAPK	mitogen-activated protein kinase
MeJA	methyl jasmonate
MEME	multiple em for motif elicitation
PCR	polymerase chain reaction
PDS	phytoene desaturase
PP	protein phosphatase
PR	pathogenesis-related protein
PSTVd	potato spindle tuber viroid
REn	replication enhancer protein
RT-qPCR	quantitative real-time polymerase chain reaction
SA	salicylic acid
TF	transcription factor
TLCV	Tomato leaf curl virus
TRV	tobacco rattle virus
TSWV	tomato spotted wilt virus
TYLCV	Tomato yellow leaf curl virus
VIGS	virus-induced gene silencing.

(TYLCV) seriously affects many plant species, such as tomato, tobacco, and cotton (Moriones and Navas-Castillo, 2000). TYLCV belongs to the genus *Begomovirus* of the family Geminiviridae and contains single-stranded DNA genomes (Ghanim et al., 1998). Transferred by the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), TYLCV adversely affects quality and productivity of plants by causing serious symptoms, including yellowing and curling leaves, stunted growth, and flower abscission (Morilla et al., 2005; Huang et al., 2016a). Control of TYLCV infection was difficult due to outbreak and uncontrollable spread of whiteflies.

Five major loci (Ty-1 to Ty-5) with polymorphic DNA markers from wild tomato species were identified to be associated with resistance to TYLCV (Ji et al., 2007; Anbinder et al., 2009). In tomato, some genes have been identified to respond to TYLCV infection. *SlVRSLip*, a lipocalin-like gene from tomato, showed resistance to TYLCV inoculation (Sade et al., 2012). Eugenol induced transcription increase in *SlPer1* contributed to high anti-TYLCV efficiency (Sun et al., 2016). Overexpression of *SlMAPK3* in tomato enhanced resistance to TYLCV infection by increasing the expression levels of salicylic acid/jasmonic acid (SA/JA)-mediated defense-related genes (Li et al., 2017). Several reports showed that some genes of *B. tabaci* were also involved in response to TYLCV infection in tomato. Overexpression of *GroEl* gene of *B. tabaci* in tomato showed mild or no disease symptom of TYLCV infection during three consecutive generations (Akad et al., 2007). Silencing of *Knot-1* gene of *B. tabaci* increased accumulation of TYLCV (Hariton-Shalev et al., 2016).

Tomato (*Solanum lycopersicum*) is one of the most consumed and economically important vegetables worldwide. Most of tomato cultivars were susceptible to TYLCV infection. In China, TYLCV was first identified in 2006 in Shanghai, and then spread to Zhejiang, Shandong, and Jiangsu, affecting growth, quality, and yield of tomato (Wu et al., 2006; Mugiira et al., 2008). Understanding response mechanism to TYLCV infection in tomato was important for improving its tolerance. Currently, several family TFs (AP2/ERF, bHLH, and WRKY) in tomato have been identified to respond to TYLCV infection (Wang et al., 2015; Huang et al., 2016a, b). A few reports documented the roles of tomato NAC TFs involved in pathogen infection (Selth et al., 2005; Du et al., 2014; Liu et al., 2014b). In tomato, 74 NAC TFs were identified and divided into 12 subgroups (Kou et al., 2014). However, functions of NAC TFs in response to TYLCV infection remain unclear.

Here, we identified and analyzed six NAC TFs (*SlNAC20*, *SlNAC24*, *SlNAC39*, *SlNAC47*, *SlNAC61*, and *SlNAC69*), which participated in tomato response to TYLCV infection based on the transcriptome database. Expression patterns of the six NAC genes differed in resistant and susceptible tomato cultivars. A tobacco rattle virus (TRV) mediated virus-induced gene silencing analysis (VIGS) system was used to silence *SlNAC61*. Compared with control 'Zhefen-702' plants, TYLCV DNA accumulation in *SlNAC61*-silencing plants increased, as observed by quantitative real-time polymerase chain reaction (RT-qPCR) analysis. The present study aimed to provide a basis for studying responses of NAC TFs involved in TYLCV infection. Isolation and identification of these six NAC TFs in tomato uncovered the molecular mechanism and improved tolerance to TYLCV infection.

2. Materials and methods

2.1. Cultivation of tomato and TYLCV infection

In this study, the used tomato cultivar 'Zhefen-702' (resistant to TYLCV infection) was a hybrid of female parent T7969F_2-19-1-1-3 (progenies of Israel tomato NEMO-TAMMI F_1 and T9179) and male parent T4078F_2-3-3-3 (progenies of Holland Qirouya F_1 and T9178) produced by conventional breeding. Susceptible tomato cultivar 'Jinpeng-1' was derived from Holland tomato cultivar 99-13A and American tomato cultivar 9708B. 'Zhefen-702' and 'Jinpeng-1' were obtained from Zhejiang Academy of Agricultural Sciences and Xi'an Jinpeng Seed Co., Ltd, respectively. Seedlings of two tomato cultivars were grown in a controlled environment chamber with 320 $\mu mol \cdot m^{-2}\ s^{-1}$ light intensity in a 12/12 h light/dark cycle. The process of TYLCV infection was referred to our previous study (Huang et al., 2016a). After reaching the two-leaf stage, tomato seedlings were transferred to an insect-proof greenhouse for TYLCV infection at 2, 4, 6, 7, and 14 days. Uninfected tomato plants served as control plants in the study. Leaves of TYLCV-infected and uninfected tomato plants (control plants) were collected and frozen in liquid nitrogen and subjected to RNA extraction.

2.2. Sequence alignment and phylogenetic analyses of NAC TFs

Tomato sequences were downloaded from Sol Genomics Network (https://solgenomics.net/organism/Solanum_lycopersicum/genome) (Fernandez-Pozo et al., 2015). The NAC TFs family members of tomato related to TYLCV infection were obtained from transcriptome data of resistant and susceptible tomato cultivars (Chen et al., 2013). Clustal X performed the multiple alignments of protein sequences (Chenna et al., 2003). MEGA 5.0 was used to construct the phylogenetic tree by the neighbor-joining method with 1000 bootstrap replicates (Tamura et al., 2011). Common motifs of protein sequences were screened by multiple em for motif elicitation (MEME, http://meme-suite.org/) (Bailey et al., 2009). The upstream 1500 bp sequences of NAC genes were analyzed to identify the *cis*-regulatory elements by using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/