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NAC transcription factors in plant immunity

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Abstract

The NAC (NAM, ATAF and CUC) family is one of the largest plant-specific transcription factor (TF) families. Members of this family are implicated in plant growth, development and stress responses. Recent functional studies demonstrate that a number of NAC TFs function as positive or negative regulators of plant immunity to biotrophic, hemibiotrophic or necotrophic pathogens, as modulators of the hypersensitive responses and stomatal immunity or as virulence targets of pathogen effectors. They affect plant immunity through their regulatory impact on signaling of plant hormones, which in turn are key players in plant immune responses. This review summarizes current knowledge and recent progress in our understanding of the biological functions of NAC TFs in plant immunity and discusses perspectives and directions for further study to elucidate the molecular mechanisms of NAC TF functions in immunity and potential application in improvement of crop disease resistance.

Keywords: Transcriptional factor, NAC, Plant immunity

Background

To combat pathogen attack, plants have evolved sophisticated immunity systems. Two distinct types of innate immune responses, known as pathogen/microbe/damage-associated molecular pattern (PAMP/MAMP/DAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI), have been recognized in plants (Jones and Dangl 2006; Boller and He 2009; Spoel and Dong 2012). PTI forms the first layer of immunity toward all potential microbial attack, whereas ETI is a specialized form of immunity triggered by the direct or indirect interactions between plant R proteins and pathogen effector proteins (Zhang and Zhou 2010; Segonzac and Zipfel 2011; Schwessinger and Ronald 2012; Bigeard et al. 2015; Cui et al. 2015). Upon perception of pathogen-derived signals, plants often activate a network of defense signaling pathways (Pieterse et al. 2009; Peng et al. 2018), which ultimately lead to transcriptional reprogramming that coordinately regulates expression of a large set of genes (Tsuda and Somssich 2015; Li et al. 2016). Such large-scale transcriptional reprogramming of gene expression in a specific immune response obviously requires a concerted function of different types of transcription factors (TFs) in both temporal and spatial manners (Buscaill and Rivas 2014; Birkenbihl et al. 2017). Recent genetic studies have demonstrated that a number of TFs from the families of WRKY, AP2/ERF (Apetala2/Ethylene Responsive Factor), NAC (NAM, ATAF and CUC), MYB, bZIP (Basic leucine zipper domain), bHLH (Basic helix-loop-helix), NF-Y (Nuclear Factor Y) and CAMTA (CaM--binding transcription activator) play crucial roles in immune responses against pathogens (Nuruzzaman et al. 2013; Buscaill and Rivas 2014; Huang et al. 2016; Phukan et al. 2016; Noman et al. 2017; Zanetti et al. 2017).

The NAC proteins constitute a large plant-specific TFs family, which contains more than 100 members in Arabidopsis and rice (Ooka et al. 2003; Fang et al. 2008; Nuruzzaman et al. 2010). NAC TFs are characterized by the presence of a highly conserved N-terminal region, known as NAC domain, which functions as a DNA-binding domain and is also responsible for oligomerization into dimer (Olsen et al. 2005; Puranik et al. 2012). The C-terminal region of NAC TFs is more diverse, intrinsically disordered and functions as a transcriptional regulatory domain (Olsen et al. 2005; Jensen et al. 2010). On the structural basis, the NAC TFs can be divided into two classes, typical NAC TFs and atypical NAC TFs. Whereas the typical NAC TFs contain a conserved N-terminal NAC domain and a divergent C-terminal region (Olsen et al. 2005; Puranik et al. 2012), the atypical NAC proteins are characterized, in addition to the NAC domain, by the presence of additional

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conserved domains/motifs in the C-terminal regions or the absence of the C-terminus (Puranik et al. 2012). One type of atypical NAC TFs is the NTL (NAC with transmembrane motif1-like) proteins, which are featured by the presence of a variable transcriptional regulatory domain and a transmembrane (TM) motif in the C-terminal region (Ernst et al. 2004). The TM motif in NTLs is thought to be responsible for anchoring to plasma membrane or endoplasmic reticulum membrane where they could be released by proteolysis to exert their function (Kim et al. 2007a; Liang et al. 2015).

During the last two decades, extensive functional studies have shown that, in addition to their functions in plant growth, development and abiotic stress responses (Zhong et al. 2010; Bollhoner et al. 2012; Nakashima et al. 2012; Puranik et al. 2012; Hussey et al. 2013; Nuruzzaman et al. 2013; Ricachenevsky et al. 2013; Shao et al. 2015; Kim et al. 2016), a large number of the NAC TFs play critical roles in plant immunity. In this review, we summarize the biological functions of the NAC TFs in plant immunity against pathogens and discuss further directions to investigate the molecular mechanisms of NAC TFs in immunity as well as the perspectives to improve crop disease resistance using NAC TFs as targets.

Functions of NAC TFs in plant immunity

Plant pathogens are classified as biotrophs, hemibiotrophs and necrotrophs according to their lifestyles (Wang et al. 2014). The biotrophs generally feed on living hosts while the hemibiotrophs possess a biotrophic phase during the early stage of infection. In contrast, necrotrophs can actively kill hosts to acquire nutrients (Lai and Mengiste 2013). Functional analyses of NAC TFs in plant immunity have been extensively performed using knockout/knockdown mutants and overexpression lines in Arabidopsis, rice and other plant species. Dozens of NAC genes have been demonstrated to play important roles in plant immunity by acting as negative or positive regulators, modulator of hypersensitive response and stomatal immunity or targets of pathogen effectors (Table 1). These reported immunity-related NAC TFs belong to different subfamilies of the NAC family (Fig. 1).

Functions of NAC TFs in plant immunity against biotrophic and hemibiotrophic pathogens

Several NAC TFs have been demonstrated to play roles in different stages of plant immunity against biotrophic and hemibitrophic pathogens. In the non-host *Blumeria graminis* f. sp. *hordei* (*Bgh*)-Arabidopsis interaction, ATAF1, belonging to the ATAF subfamily (Fig. 1), promotes penetration resistance as the *ataf1–1* mutant plants showed decreased penetration resistance to *Bgh* (Jensen et al. 2007). Similarly, silencing of *HvNAC6*, the ATAF1 homologue in barley (Fig. 1), compromised penetration resistance in epidermal cells towards virulent *Bgh* while transient overexpression of *HvNAC6* increased the occurrence of penetration resistant cells towards *Bgh* attack (Jensen et al. 2007). Thus, it was proposed that ATAF1 and its homologues play a conserved role in penetration resistance in dicot and monocot plants. Overexpression of *ATAF2*, closely related to ATAF1 in the ATAF subfamily (Fig. 1), led to a significant reduction in virus accumulation, accompanied with upregulated expression of defense genes *PR1*, *PR2* and *PDF1.2*, indicating that ATAF2 functions in the regulation of host basal defense (Wang et al. 2009b). Overexpression of *ATAF1* resulted in increased susceptibility while suppression of *ATAF1* led to enhanced resistance in transgenic Arabidopsis plants to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (Wang et al. 2009a; Wu et al. 2009). The ANAC019, ANAC055 and ANAC072 are three members of the NAM subfamily (Fig. 1) and the *anac019 anac055 anac072* triple mutant plants exhibited enhanced resistance to *P. syringae* pv. *maculicola* ES4326 (Bu et al. 2008; Zheng et al. 2012). Overexpression of *ANAC042/JUB1*, a reactive oxygen species-responsive NAC gene belonging to NAM subfamily (Fig. 1), attenuated Arabidopsis resistance to *Pst* DC3000, while the *anac042/jub1* knockdown mutant exhibited reduced disease symptoms and growth of the bacterial pathogen *Pst* DC3000, indicating that ANAC042/JUB1 suppresses Arabidopsis immunity against *Pst* DC3000 (Wu et al. 2012; Shahnejat-Bushehri et al. 2016a, b).

The Arabidopsis NCBAC/NTL9, a member of the NTL subfamily (Fig. 1), plays distinct roles in different stages of plant immunity. The *cbnac/ntl9* mutant plants displayed enhanced resistance to a virulent strain of *Pst* DC3000 while resistance was reduced in *CBNAC/NTL9*-overexpressing plants, and the changes in resistance were correlated with changes in defense gene expression (Kim et al. 2012; Guo et al. 2017). By contrast, the *ncbac1/ntl9* mutant plants displayed reduced resistance to *P. syringae hopD1* mutant and ETI-inducing *P. syringae* strains and failed to induce the expression of SA biosynthesis genes in response to flg22, whereas transgenic plants expressing a constitutively active CBNAC/NTL9 derivative showed increased resistance to these *P. syringae* strains (Zheng et al. 2012; Block et al. 2014). These contradictory results may indicate that NCBAC1/NTL9 plays distinct roles at different stages or aspects of plant immunity. NTL6 (ANAC062), another member of the NTL subfamily (Fig. 1), is cold-induced and its transcriptionally active form, 6$^{\Delta}$C, which was modified by deleting its C-terminal region, entered the nucleus and induced a subset of cold-responsive defense genes *PR1*, *PR2* and *PR5*. Transgenic plants overexpressing the active NTL6 form, 6$^{\Delta}$C, exhibited enhanced disease resistance, whereas *NTL6*-RNAi plants with

Table 1 Functional and biochemical features of the reported immunity-related NAC transcription factors

Plants	Gene	Trans-activity	Functions	Possible mechanisms	Target genes
Arabidopsis	ANAC019 ANAC055 ANAC072	Activators/ Repressors	*anac019 anac055* mutant increased resistance to *Botrytis cinerea*; Stomatal immunity to *Pseudomonas syringae*	SA or JA signaling, stomatal immunity	*VSP1*, *ICS1*, *SAGT1* and *BSMT1*
	ANAC042/ JUB1	Activator	Overexpression decreased resistance while *anac042* mutant increased resistance to *P. syringae* pv. *tomato* (*Pst*) DC3000	Phytoalexin biosynthesis	
	ATAF1	Activator	Overexpression decreased resistance while loss-of function mutants increased resistance to *Pst* DC3000, *B. cinerea*, and *Alternaria brassicicola*. *ataf1* mutant decreased penetration resistance to *Blumeria graminis* f. sp. *hordei* (*Bgh*)	ABA signaling	
	ATAF2	Unknown	Overexpression decreased resistance to *Fusarium oxysporum*, but induced response to TMV	Interaction with TMV replicase protein	
	ANAC062/ NTL6	Activator	Overexpression of an active form increased resistance to *Pst* DC3000	PR gene expression	*PR1*, *PR2*, *PR5*
	CBNAC/ NTL9	Repressor	*cbnac* mutant increased resistance to *Pst* DC3000 Stomatal immunity to *Pst* DC3000	ETI and SA signaling, stomatal immunity	*PR1*, *ICS1*, *EDS1*, *PAD4*, *PBS3*
	NAC4	Unknown	Overexpression increased *Pst* DC3000-induced cell death	Targets of *miR164*, programmed cell death	*LURP1*, *WRKY40*, *WRKY54*
Rice	OsNAC6	Activator	Overexpression increased resistance to *Magnaporthe oryzae*	Defense gene expression	*AK104277* (peroxidase), *AK110725* (DUF26)
	RIM1	Activator	*rim1* mutant increased resistance to *Rice dwarf virus*	JA signaling	
	ONAC122, ONAC131	Activator	Silencing decreased resistance to *M. oryzae*	Defense gene expression	
	OsNAC111	Activator	Overexpression increased resistance to *M. oryzae*	PR gene expression	*PR2* and *PR8*
	OsNAC066	Activator	Overexpression increased resistance to *M. oryzae* and *Xanthomonas oryzae* pv. *oryzae*	ABA signaling	*LIP9* and *NCED4*
	OsNAC60	Unknown	Mutation increased susceptibility to *M. oryzae*	SA signaling	
	OsNAC58	Unknown	Overexpression increased resistance to *Xanthomonas oryzae* pv. *oryzae*		
Tomato	SlSRN1	Activator	Silencing attenuated resistance to *B. cinerea* and *Pst* DC3000	PR gene expression	
	SlNAC35	Activator	Overexpression improved resistance to *Ralstonia solanacearum*	PR gene expression	
	JA2	Activator	JA2-suppressed plants increased susceptibility to *Pst* DC3118	ABA-mediated stomatal closure	*NCED1*
	JA2L	Activator	JA2L-suppressed plants increased resistance to *Pst* DC3118	SA accumulation and stomatal reopening	*SAMT1* and *SAMT2*
Barley	HvNAC6	Activator	RNAi plants reduced resistance to *Bgh*	ABA signaling	
Wheat	TaNAC21/ TaNAC22	Activator	Silencing increased resistance to *Puccinia striiformis* f. sp. *tritici*	Target of *tae-miR164*	
	TaNAC1	No activity	Overexpression decreased	SA and JA signaling	

Table 1 Functional and biochemical features of the reported immunity-related NAC transcription factors *(Continued)*

Plants	Gene	Trans-activity	Functions	Possible mechanisms	Target genes
			resistance to *Pst* DC3000		
	TaNAC30	Activator	Silencing of *TaNAC30* increased resistance to *P. striiformis* f. sp. *tritici*		
	TaNAC6s	Activator	Overexpression of *TaNAC6-A* enhanced resistance to *Blumeria graminis* f. sp. *tritici*	JA signaling	
Cotton	GbNAC1	Unknown	Silencing decreased resistance to *Verticillium dahliae*		
	GhATAF1	Activator	Overexpression decreased resistance to *B. cinere* and *V. dahliae*	SA and JA signaling	
Potato	StNTP1, StNTP2	Unknown	Silencing decreased resistance to *Phytophthora infestans*	Targets of the RxLR effector Pi03192	
Grapevine	VvNAC1	Activator	Overexpression increased resistance to *B. cinerea* and *Hyaloperonospora arabidopsidis*	Defense gene expression	
Chinese wild grape	VpNAC1	Activator	Overexpression increased resistance to *Erysiphe cichoracearum* and *Phytophthora parasitica* var. *nicotianae*	PR gene expression	
Artemisia annua	AaNAC1	Activator	Overexpression increased resistance to *B. cinerea*	Increase artemisinin content	

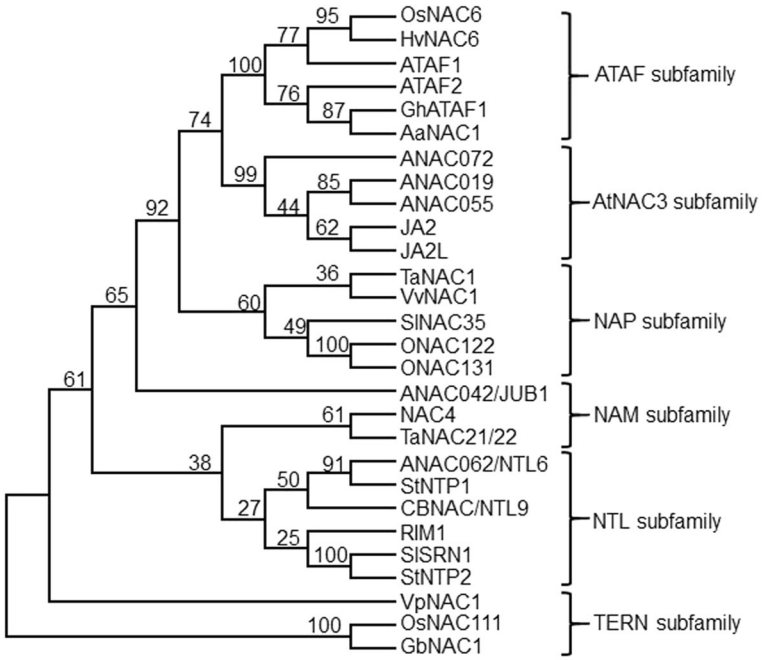

Fig. 1 Phylogenetic tree of the reported immunity-related NAC TFs. The tree was constructed using the Neighbour-Joining algorithm method and bootstrap values from 1000 replicates are indicated at each node. The GenBank accession numbers are as follows: OsNAC6, BAA89800; HvNAC6, AM500854; ATAF1, NP_171677.1; ATAF2, NP_680161; GhATAF1, DT549350.1; AaNAC1, AQU15092.1; ANAC072/RD26, NP_001078452.1; ANAC019, NP_175697; ANAC055, NP_188169.1; JA2, NP_001233972.1; JA2L, NP_001306107.1; TaNAC1, AEI00649.1; VvNAC1, XP_002282566; SlNAC35, XM_004236996; ONAC122, BAF27472.1; ONAC131, BAT15671.1; ANAC042/JUB1, NP_181828; NAC4, NP_568182.2; TaNAC21/22, AGV08300.1; ANAC062/NTL6, NP_190522.1; StNTP1, AGY49284.1; CBNAC/NTL9, NP_001119122.1; RIM1, BAS82017.1; SlSRN1, NP_001304297.1; StNTP2, AGY49285.1; VpNAC1, GU393316; OsNAC111, XP_015615861.1; GbNAC1, AOW72925.1

reduced NTL6 were more susceptible to infection by a virulent strain of *Pst* DC3000 at low temperatures, indicating that NTL6 integrates cold signals into plant defense responses (Seo et al. 2010).

In rice, transgenic plants overexpressing *OsNAC6*, the rice *ATAF1* homologue (Fig. 1), displayed increased resistance to *Magnaporthe grisea* (Nakashima et al. 2007), the causal agent of rice blast disease. Silencing of *ONAC122* or *ONAC131*, two closely related rice NAC TFs that belong to the NAP subfamily (Fig. 1), caused increased susceptibility to *M. oryzae* and downregulated expression of several defense- and signaling-related genes (i.e. *OsLOX*, *OsPR1a*, *OsWRKY45* and *OsNH1*), suggesting that both ONAC122 and ONAC131 positively regulate rice blast resistance (Sun et al. 2013). *OsNAC111*-overexpressing plants showed increased resistance to *M. oryzae* and constitutively expressed several defense genes, suggesting that OsNAC111, a member of TERN subfamily (Fig. 1), positively regulates the expression of a specific set of defense genes and contributes to disease resistance (Yokotani et al. 2014). Overexpression of *OsNAC066*, which was induced by the blast fungus, increased resistance to *M. oryzae* and *Xanthomonas oryzae* pv. *oryzae* (Liu et al. 2018). Mutation in *OsNAC60* increased susceptibility of the *osnac60* plants to *M. oryzae* (Wang et al. 2018a), while overexpression of *OsNAC58* increased resistance to *X. oryzae* pv. *oryza*e (Park et al. 2017). Disruption of *RIM1*, coding for a rice NAC TF belonging to the NTL subfamily (Fig. 1), resulted in reduced rice susceptibility to *Rice dwarf virus* (RDV) but remains susceptible to other viruses. The accumulation of RDV capsid proteins was drastically reduced in *rim1* mutant plants, indicating that the multiplication of RDV is specifically impaired in the *rim1* mutant. It was proposed that RIM1 negatively regulates rice immunity to RDV by acting as a host factor that is required for multiplication of RDV in host plants (Yoshii et al. 2009).

Functions of NAC TFs from other plant species have also been explored through virus-induced gene silencing or antisense-mediated suppression in host plants and ectopic expression in Arabidopsis or tobacco. Tomato JA2 and JA2L, two closely related NAC TFs belonging to the AtNAC3 subfamily (Fig. 1), play different roles in regulating immunity against *Pst* DC3118 by modulating pathogen-induced stomatal closure and reopening (Du et al. 2014). Silencing of *SlSRN1* in tomato plants resulted in decreased resistance against *Pst* DC3000 (Liu et al. 2014), whereas ectopic overexpression of *SlNAC35* in tobacco improved resistance to *Ralstonia solanacearum*, accompanied with increased expression of SA-responsive defense genes *PR1a*, *NPR1*, *PR2* and *PR5* (Wang et al. 2016a). Overexpression of grapevine *VvNAC1* and *VpNAC1* or *Artemisia annua AaNAC1* in Arabidopsis or tobacco plants led to enhanced resistance to *Hyaloperonospora arabidopsidis* and *Erysiphe cichoracearum* (Zhu et al. 2012; Le Hénanff et al. 2013; Lv et al. 2016). Knockdown of *TaNAC1*, *TaNAC21/22* or *TaNAC30* in wheat enhanced resistance against *Puccinia striiformis* f. sp. *tritici*, indicating that TaNAC1, belonging to NAP subfamily (Fig. 1), TaNAC21/22, belonging to NAM subfamily (Fig. 1), and TaNAC30 negatively regulate the stripe rust resistance in wheat (Feng et al. 2014; Wang et al. 2015a; Wang et al. 2018b). By contrast, overexpression of *TaNAC6-A* enhanced resistance against *Blumeria graminis* f. sp. *tritici* (*Bgt*), while silencing of each *TaNAC6s* compromised the resistance, suggesting that TaNAC6s play positive roles in broad-spectrum resistance against *Bgt* (Zhou et al. 2018).

Functions of NAC TFs in plant immunity against necrotrophic pathogens

In Arabidopsis, at least seven NAC proteins ATAF1, ATAF2, ANAC019, ANAC055, ANAC072, NTL9/CBNAC (Calmodulin-binding NAC protein) and ANAC042/JUB1 (JUNGBRUNNEN1) are implicated in the regulation of plant immunity to necrotrophic fungal pathogens including *Botrytis cinerea*, *Alternaria brassicicola*, and *Fusarium oxysporum*. We and others found that transgenic plants overexpressing *ATAF1* (ATAF1-OE) displayed increased susceptibility while transgenic plants expressing an *ATAF1* chimeric repressor (ATAF1-SRDX) construct enhanced resistance to *B. cinerea* and *A. brassicicola* (Wang et al. 2009a; Wu et al. 2009). Expressions of defense genes were upregulated in the ATAF1-SRDX plants but attenuated or unchanged in the ATAF1-OE plants, after inoculation with *B. cinerea* or *Pst* DC3000 (Wang et al. 2009a). Similarly, overexpression of *GhATAF1*, a homologue of *ATAF1* in cotton (Fig. 1), decreased resistance to *B. cinerea* and *Verticilium dahlia*, coupled with the suppression of JA-mediated signaling and the activation of SA-mediated signaling (He et al. 2016). The *anac019 anac055* double mutant plants showed increased resistance to *B. cinerea* (Bu et al. 2008; Zheng et al. 2012). The JA-induced expression of *VSP1* and *LOX2* in *anac019 anac055* double mutant plants was attenuated while their expression in transgenic plants overexpressing either *ANAC019* or *ANAC055* was upregulated (Bu et al. 2008). It was found that overexpression of *ATAF2* increased susceptibility to *Fusarium oxysporum* and reduced expression of defense genes in transgenic Arabidopsis plants (Delessert et al. 2005). Furthermore, mutation in *ANAC042/JUB1* led to enhanced susceptibility to *A. brassiscicola* and reduced accumulation of camalexin, indicating that ANAC042/JUB1 regulates immunity against *A. brassicicola* through modulating the camalexin biosysnthesis in Arabidopsis plants (Saga et al. 2012).

Additionally, silencing of *SlSRN1* in tomato plants resulted in increased susceptibility to *B. cinerea* (Liu et al.

2014), whereas silencing of potato *StNTP1* and *StNTP2*, two membrane-localized NAC TFs belonging to the NTL subfamily (Fig. 1), in *Nicotiana benthamiana* increased susceptibility to *Phytophthora infestans* infection (McLellan et al. 2013). *GbNAC1*-silenced cotton plants attenuated resistance to *Verticillium dahlia* while *GbNAC1*-overexpressing Arabidopsis plants enhanced resistance to *V. dahliae* (Wang et al. 2016b). Overexpression of grapevine *VvNAC1* or *Artemisia annua AaNAC1* in Arabidopsis or tobacco plants enhanced resistance to *B. cinerea* or *P. parasitica* var. *nicotianae* (Zhu et al. 2012; Le Hénanff et al. 2013).

NACs as modulators of hypersensitive response

Hypersensitive response (HR) is a form of programmed cell death (PCD) and is an active and efficient primary immune response that prevents pathogen invasion in plants (Coll et al. 2011). The function of rice OsNAC4 in HR cell death has been well studied (Kaneda et al. 2009). *OsNAC4* was found to be upregulated during non-host defense response and regulate the occurrence of HR cell death. In response to an avirulent strain of *Acidovorax avenae*, the *OsNAC4* knockdown plants decreased while the *OsNAC4*-overexpressing plants enhanced HR cell death, accompanied by loss of plasma membrane integrity, nuclear DNA fragmentation and typical morphological changes (Kaneda et al. 2009), which are characteristics of PCD (Coll et al. 2011). On the other hand, Arabidopsis NAC4, a member of NAM subfamily (Fig. 1), plays an essential role in the regulation of HR cell death. HR cell death was noticeably enhanced in *NAC4*-overexpressing plants in response to an avirulent strain of *Pst* DC3000 (*AvrRpm1*), demonstrating that NAC4 is a positive regulator of HR cell death (Lee et al. 2017).

NACs as modulators of stomatal immunity

Stomata are not just a passive port for pathogen entry but also play an active role in the plant innate immune response (Arnaud and Hwang 2015). The control of stomatal closure is one of the first lines of defense against pathogen invasion and thus stomatal opening or closing, governed by guard cells, is the main battleground during the early stage of plant-pathogen interaction. Recent discoveries have revealed that some specific NAC TFs such as the Arabidopsis ANAC019/ANAC055/ANAC072 and CBNAC/NTL9 and tomato JA2 and JA2L participate in signaling pathways that regulate stomatal immunity. In the Arabidopsis *anac019 anac055 anac72* triple mutant plants, *Pst* DC3000-induced stomatal closure occurred at 1 hours post inoculation (hpi) but *Pst* DC3000-induced stomatal reopening did not occur and the stomatal apertures remained small at 4 hpi, suggesting that ANAC019/ANAC055/ANAC072 are required for *Pst* DC3000-triggered stomatal reopening and thus play critical role in stomatal-mediated immunity. Both *JA2* and *JA2L*, encoding two NAC TFs that are closely related to ANAC019/ANAC055/ANAC072, were preferentially expressed in guard cells of tomato leaves (Du et al. 2014). The *Pst* DC3000-induced stomatal closure at 1 hpi was impaired and the pathogen-triggered stomatal reopening at 4 hpi remained normal in JA2-SRDX plants, indicating that JA2 is required for *Pst* DC3000-induced stomatal closure but not stomatal reopening (Du et al. 2014). By contrast, the *Pst* DC3000-induced stomatal closure at 1 hpi was largely normal but the pathogen-triggered stomatal reopening at 4 hpi was substantially impaired in JA2L-AS plants, indicating that JA2L is required for pathogen-regulated stomatal reopening (Du et al. 2014). It was speculated that JA2 acts in ABA-mediated stomatal closure while JA2L acts in JA-mediated stomatal reopening, showing distinct functions of JA2 and JA2L in stomatal immunity against *Pst* DC3000. Furthermore, the Arabidopsis CBNAC/NTL9 is preferentially expressed in guard cells (Yoon et al. 2008; Zheng et al. 2015) and the *cbnac*/*ntl9* mutant plants impaired flg22-induced SA biosynthesis in guard cells and *Psm* ES4326-induced change of stomatal aperture, demonstrating that CBNAC/NTL9 is specifically required for SA synthesis in guard cells and plays an essential role in the flg22-triggered stomatal immunity (Zheng et al. 2015).

NACs as targets of pathogen effectors

During infection process, pathogens often secrete and inject numerous effectors into host plant cells to suppress immune response. Recent studies have indicated that some NAC proteins are targets of pathogen effectors to facilitate infection by inhibiting plant immune responses. In Arabidopsis, CBNAC/NTL9 was found to physically interact with one effector from *P. syringae* and four effectors from *Hyaloperonospora arabidopsidis* (Mukhtar et al. 2011). A recent study revealed that the *P. syringae* effector HopD1, a strong repressor of ETI, interacted with CBNAC/NTL9 in endoplasmic reticulum (ER) and inhibited CBNAC/NTL9-mediated induction of certain defense genes during ETI (Block et al. 2014). The potato StNTP1 and StNTP2 were found to be targets of RxLR effector Pi03192 secreted by *P. infestans* (McLellan et al. 2013). StNTP1 and StNTP2 were released from the ER membrane, where they are normally localized, in response to treatment with *P. infestans* culture filtrate (CF) and then translocated and accumulated in the nucleus, where they contribute to potato immunity to inhibit disease progression. By contrast, Pi03192 prevented CF-triggered re-localization of StNTP1 and StNTP2 from the ER into the nucleus. It is likely that, during infection process, *P. infestans*-secreted Pi03192 interacts with StNTP1 and StNTP2 and prevents the

ER-nucleus translocation of these two host NAC TFs to promote disease progression (McLellan et al. 2013).

It has been found that, during viral infection, NAC TFs can be hijacked by interacting with viral proteins to facilitate viral replication or suppress host immunity involved in plant-virus interactions. An earlier study characterized two wheat NAC TFs, GRAB1 (Geminivirus Rep A-binding) and GRAB2, which interacted with *Wheat dwarf geminivirus* (WDV) RepA protein (Xie et al. 1999). Overexpression of *GRAB1* and *GRAB2* in cultured wheat cells severely inhibited WDV DNA replication, indicating the involvement of GRAB1 and GRAB2 in viral DNA replication (Xie et al. 1999). Tomato SlNAC1 was found to interact with geminivirus replication enhancer REn from *Tomato leaf curl virus* (TLCV), and overexpression of *SlNAC1* enhanced the accumulation of TLCV DNA (Selth et al. 2005). Geminiviral replication initiator protein Rep from *Mungbean yellow mosaic India virus* (MYMIV) was found to interact with Arabidopsis AtNAC083, which plays a possible role in MYMIV DNA replication (Suyal et al. 2014).

Arabidopsis ATAF2 interacted with the helicase domain of the *Tobacco mosaic virus* (TMV) 126−/183 kDa replicase protein. Overexpression of *ATAF2* significantly reduced virus accumulation, indicating that the TMV replicase-ATAF2 interaction suppresses basal host defenses to promote systemic virus accumulation (Wang et al. 2009b). The capsid protein (CP) of *Turnip crinkle virus* (TCV) interacts with an Arabidopsis NAC protein TIP (TCV-interacting protein) and the TCV CP-TIP interaction prevented nuclear localization of TIP (Ren et al. 2005, 2012). Although mutation in TIP resulted in increased replication of TCV and *Cucumber mosaic virus* (CMV), TIP is required for basal resistance to CMV but not for resistance to TCV (Jeong et al. 2008). Lack of TIP-CP interaction in TCV mutant with a mutated CP caused more severe symptoms, implying that TCV regulates antiviral basal immunity in host plants through TIP-CP interaction (Donze et al. 2014).

NACs in plant immune signaling

Upon pathogen attack, plants timely perceive the pathogen-derived signals and often activate efficient and complicated but fine-tuned network of defense hormone-mediated signaling pathways (Zhang et al. 2017; Peng et al. 2018). Emerging evidence has indicated that some NAC proteins participate in modulating these immune signaling pathways to execute their critical functions in plant immunity.

NACs in SA signaling

Salicylic acid (SA) is a critical signaling molecule and mediates SA signaling pathway that regulate immunity against biotrophs and hemibiotrophs (Glazebrook 2005; Shigenaga and Argueso 2016). Pathogen-induced SA production is largely synthesized via the isochorismate pathway, in which the isochorismate synthase 1 (ICS1) is a critical enzyme responsible for approximately 90% of the SA production (Vlot et al. 2009). Meanwhile, most of the SA produced in plant is converted into SA O-β-glucoside (SAG) by a pathogen-inducible SA glucosyltransferase (SAGT) (Vlot et al. 2009). It was recently found that, in the *anac019 anac055 anac072* triple mutant Arabidopsis plants, the basal transcript level of *ICS1* was higher, whereas the basal transcript level of *SAGT1* was lower than that in the wild-type plants, suggesting that ANAC019/ANAC055/ANAC072 may decrease SA synthesis, but increase SA metabolism through transcriptional repression of *ICS1* and transcriptional activation of *SAGT1*, respectively (Zheng et al. 2012). Further ChIP experiments revealed that ANAC019-GFP was enriched in some NAC core-binding sites in promoters of the *ICS1*, *SAGT1* and *SA methyl transferase 1* (*BSMT1*, coding an enzyme that converts SA to the inactive methyl SA) (Zheng et al. 2012). Thus, it is likely that ANAC019/ANAC055/ANAC072 act as negative regulators of SA accumulation through the induction of *SAGT1* and *BSMT1* and repression of *ICS1* via direct binding to their promoters. The N-terminal fragment CBNAC/NTL9$_{1-330}$ of the Arabidopsis CBNAC/NTL9 not only strongly activated the expression of the reporter *LUC* gene under the control of *ICS1* promoter but also interacted with the promoters of *EDS1*, *PAD4* and *PBS3* genes (Zheng et al. 2015), revealing that CBNAC/NTL9 may coordinately regulate these four SA synthesis-related genes and thus modulate SA accumulation in plants. In tomato, the *Pst* DC3000-induced expression of *SAMT1* and *SAMT2*, coding for SA methyl transferases that convert SA to the inactive methyl salicylate was markedly weakened in the JA2L-AS plants and the GFP-JA2L proteins were enriched in fragments containing NAC core-binding sites in promoters of the *SAMT1* and *SAMT2* genes in ChIP experiments, indicating that JA2L represses SA accumulation by activating the transcription of two tomato SAMT genes, *SAMT1* and *SAMT2* (Du et al. 2014).

NACs in JA signaling

Jasmonates (JA) and ethylene (ET) are critical defense signaling molecules, mediating the JA/ET signaling that regulates immunity against necrotrophs and herbivorous insects (Glazebrook 2005; Pieterse et al. 2012; Shigenaga and Argueso 2016; Zhang et al. 2017). In Arabidopsis, JA- and *Psm* ES4326-induced expression of *ANAC019*, *ANAC055* and *ANAC072* was dependent on MYC2, a master regulator of the JA signaling (Kazan and Manners 2013), and ChIP experiments confirmed the interaction of MYC2 with G box elements in promoters of the *ANAC019*, *ANAC055* and *ANAC072* genes,

indicating that expression of the *ANAC019*, *ANAC055* and *ANAC072* genes was activated by MYC2 through direct interaction (Bu et al. 2008; Zheng et al. 2012). In *anac019 anac055* double mutant plants, JA-induced expression of *VEGETATIVE STORAGE PROTEIN1* (*VSP1*) and *LIPOXYGENASE2* (*LOX2*) was attenuated, whereas JA-induced *VSP1* and *LOX2* expression was enhanced in transgenic plants overexpressing the *ANAC019* or *ANAC055* gene, which implies that ANAC019 and ANAC055 act downstream of MYC2 to regulate JA-signaled defense responses (Bu et al. 2008). In rice, the expression of genes encoding JA biosynthetic enzymes lipoxygenase (LOX), allene oxide synthase 2 (AOS2) and OPDA reductase 7 (OPR7) was up-regulated in the *rim1* mutant and a rapid and massive accumulation of endogenous JA was detected in the *rim1* mutant after wounding, suggesting that RIM1 may represent a new molecular link in JA signaling (Yoshii et al. 2010). *TaNAC1*-overexpression in Arabidopsis suppressed the expression levels of resistance-related genes *PR1* and *PR2* involved in SA signaling and *AtWRKY70*, which functions as a connection node between the JA- and SA-signaling pathways (Wang et al. 2015a).

NACs in ABA signaling

Abscisic acid (ABA) is a well-known stress hormone, and ABA signaling plays an important role in plant immunity (Lievens et al. 2017). Nine-*cis* epoxycarotenoid dioxygenases (NCEDs) are rate-limiting enzymes of ABA biosynthesis while CYP707As negatively regulate ABA accumulation by catalyzing ABA metabolism (Lievens et al. 2017). The JA2-SRDX-1 tomato plants contain reduced ABA levels and exhibit reduced expression of *NCED1*, indicating that JA2 regulates *NCED1* expression for ABA biosynthesis in tomato (Du et al. 2014). Further biochemical experiments revealed that JA2 specifically interacted with the NAC core binding sites of the *NCED1* promoter and selectively activated the expression of *NCED1*, confirming regulation of ABA biosynthesis by JA2 through direct binding to the promoter of *NCED1* (Du et al. 2014). In rice, overexpression of *ONAC066* remarkably suppresses the expression of ABA-related genes and decreases endogenous ABA level, but does not affect SA- and JA-related genes as well as the endogenous SA and JA levels (Liu et al. 2018). Y1H assays demonstrate that ONAC066 directly binds to the promoters of *LIP9* and *NCED4* to modulate their expression (Liu et al. 2018). It was also found that *HvNAC6*-RNAi plants display an altered light/dark rhythm of ABA level, compromised expression of the two ABA biosynthetic genes *HvNCED1* and *HvNCED2*, elevated expression of an ABA conjugating enzyme gene *HvBG7*, and impaired basal resistance against *Bgh* by exogenous application of ABA. These observations, suggest that HvNAC6 regulates ABA signaling by modulating circadian control of ABA level (Chen et al. 2013). Comparative transcriptome analyses revealed that an ABA biosynthesis gene *ABSCISIC ALDEHYDE OXIDASE3* and a number of ABA-responsive genes were significantly induced by *Bgh* in *ataf1* plants and that *Bgh* induced reduction in endogenous ABA content was dependent on ATAF1, indicating that ATAF1 attenuates ABA signaling for efficient penetration resistance in Arabidopsis against *Bgh* (Jensen et al. 2008). However, it was observed that ANAC019/ANAC055/ANAC072-mediated stomatal immunity against *Psm* ES4326 did not require the ABA signaling, although their *Psm* ES4326-induced expression depends on ABA signaling pathway (Zheng et al. 2012).

Regulation of NAC TF activity

miRNA-mediated regulation of NAC transcript abundance

miRNAs are small, single-stranded, noncoding RNAs that can fine-tune the transcript abundance of their target genes and thus play roles in plant immunity (Seo et al. 2013). Bioinformatics and experimental analyses revealed some *NAC* genes are targets of several conserved miRNAs and miRNA-mediated regulation of NAC gene transcript abundance is one of the mechanisms that govern the activity of NAC TFs in plant cells. Arabidopsis *NAC4* (*ANAC079/080*), one of the six targets of *miR164*, was found to promote pathogen-induced cell death under negative regulation by *miR164* (Lee et al. 2017). The *NAC4*-overexpressing plants and the *mir164a*, *mir164b* and *mir164c* mutant plants showed noticeably enhanced cell death after inoculation with an avirulent strain of *Pst* DC3000 (*AvrRpm1*). The expression kinetics of *NAC4* and *miR164* after infection with *Pst* DC3000 (*AvrRpm1*), and *NAC4-miR164* cleavage assays in protoplasts, demonstrated that *NAC4* expression is fine-tuned by the negative action of *miR164* through specific cleavage of the *NAC4* mRNA during NAC4 promotion of HR cell death (Lee et al. 2017). In rice, miR164a suppresses the expression of *OsNAC60*, a blast fungus-inducible NAC TF, and overexpression of miR164a leads to enhanced susceptibility to *M. oryzae* (Wang et al. 2018a). It was also observed that *TaNAC21/22* and tae-miR164 showed contrasting divergent expression patterns in wheat in response to *Puccinia striiformis* f. sp. *tritici* and that tae-miR164 cleaved the mRNA of *TaNAC21/22* genes when both *TaNAC21/22* and tae-miR164 genes were transiently co-expressed in tobacco leaves (Feng et al. 2014).

Post-translational modification of NAC proteins

Post-translational modifications such as phosphorylation and ubiquitination can affect the activity, distribution and abundance of proteins in plant cells and have been implicated in plant immunity (Meng and Zhang 2013;

Zhou and Zeng 2017). In rice, nuclear localization of the PCD-related OsNAC4 was found to be critical for PCD. OsNAC4 was translocated into nuclei after infection by an avirulent strain of *Acidovorax avenae*; however, the amount of OsNAC4 accumulation in nuclei was decreased with the absence of staurosporine, a potent inhibitor of serine/threonine protein kinases, indicating that the translocation of OsNAC4 into the nucleus might be regulated by phosphorylation (Kaneda et al. 2009). The tomato SlNAC1, which interacts with the geminivirus replication enhance (REn) protein of *Tomato leaf curl virus*, was required for tomato immunity against bacterial pathogens (Selth et al. 2005; Huang et al. 2013), and was found to be stable in the presence of proteasome-specific inhibitor MG132 or MG115, indicating ubiquitination of SlNAC1 in plant cells (Huang et al. 2013). Recently, it was found that NAC1 specifically interacts with SINA3, a member of SEVEN IN ABSENTIA family, in the nucleus of plant cells and that SINA3 is a functional ubiquitin E3 ligase capable of promoting NAC1 degradation *in planta* via polyubiquitination (Miao et al. 2016). It is likely that the function of SlNAC1 in tomato immunity is fine-tuned by SINA5. In addition, the rice PCD-related RIM1 was destabilized by JA treatment and degraded by a 26S proteasome complex (Yoshii et al. 2010).

Interactions with other proteins

Many TFs exert their biochemical functions through interactions with other proteins including other types of TFs to coordinately regulate the expression of their target genes or enhance their binding capacity to the *cis*-elements in promoters of their target genes (Puranik et al. 2012). The Arabidopsis CBNAC/NTL9, a transcriptional repressor (Kim et al. 2007b), physically interacted with SNI1, a negative regulator of *PR1* expression. SNI1 enhanced the DNA-binding activity of the CBNAC/NTL9 to the *cis*-elements in promoter of *PR1*, indicating that CBNAC/NTL9 acts synergistically with SNI1 as a transcriptional repressor of *PR1* and thus functions as a negative regulator of Arabidopsis immunity (Kim et al. 2012). Another, CBNAC/NTL9 interacts with CRWN1, a member of CROWDED NUCLEI family that are considered as lamin-like candidates. The interaction between CRWN1 and CBNAC/NTL9 enhanced the binding of CBNAC/NTL9 to the promoter of the *PR1* gene, increased the repressive function of CBNAC/NTL9 on *PR1* expression (Guo et al. 2017). Furthermore, the ANAC019/ANAC055/ANAC072 suppressed whereas TCP8 and TCP9, two members of the TEOSINTE BRANCHED1, CYCLOIDEA and PCF (TCP) family TFs, enhanced *ICS1* expression during pathogen infection (Zheng et al. 2012; Wang et al. 2015b). ANAC019 interacted with TCP8 and this interaction may be involved in the orchestrated regulation of *ICS1* expression to regulate SA accumulation (Wang et al. 2015b).

Target genes regulated by immunity-related NACs

Transcriptional activity

Most of the NAC TFs functionally characterized have been shown to possess transcriptional activator function. Arabidopsis ATAF1, NTL6, rice OsNAC6, ONAC122, ONAC131, OsNAC111 and RIM1, barley HvNAC6, tomato SlSRN1, and cotton GhATAF1 are transcriptional activators in yeast or in plant protoplasts (Jensen et al. 2007; Lu et al. 2007; Nakashima et al. 2007; Yoshii et al. 2009; Seo et al. 2010; Sun et al. 2013; Yokotani et al. 2014; He et al. 2016). Interestingly, wheat TaNAC1 does not display transcriptional activity although it has a transcription activation domain in its C-terminal (Wang et al. 2015a). On the other hand, a few of the NAC proteins with known biological functions are transcriptional repressors. The Arabidopsis CBNAC/NTL9 and NAC4 function as transcriptional repressors (Kim et al. 2007b; Lee et al. 2017). Notably, previous studies showed that ANAC019, ANAC055 and ANAC072 had transactivation activity (Tran et al. 2004; Bu et al. 2008); however, a recent study indicated that these three NAC TFs may also function as transcriptional suppressors by binding to the *ICS1* promoter and repressing its expression (Zheng et al. 2012). This dual transcriptional capability of NAC TFs, functioning either as activators or repressors, may be achieved by recruiting or interacting with different transcriptional partners.

DNA binding sites

As TFs, NAC proteins exert their biological functions through binding to their target sites in promoters of downstream target genes and thus drive the transcription of the target genes. A NAC recognition sequence (NACRS), containing CATGT and harboring CACG, was characterized as a conserved core binding site for some stress-responsive NACs across plant species (Tran et al. 2004) and single NACRS could be enough to drive the binding of NAC TFs to promoters of target genes (Lindemose et al. 2014). The immunity-related ATAF1, ANAC019, ANAC055 and ANAC072 have been shown to be capable of binding to this NACRS core site in stress-responsive genes (Tran et al. 2004; Lindemose et al. 2014). In vitro binding assays revealed that ANAC019 bound directly to the CATGT and CACG motifs in the promoter of the defense gene *VSP1* (Bu et al. 2008). The flanking bases next to the core CGT[G/A] of NACRS in promoters may determine the binding specificities and fine-tune affinity for different NAC TFs in vivo (Lindemose et al. 2014). The Arabidopsis CBNAC/NTL9 is

capable of binding to a calmodulin-binding NAC binding sequence (CBNACBS) that consists of a GCTT core sequence (Kim et al. 2007b), whereas Arabidopsis NAC4 contains a 9 bp consensus binding sequence ACAA GCAAC (Lee et al. 2017). Genomic pull-down assays identified a 25 bp specific A/T-rich consensus binding sequence with repeating [GC]AAA motifs for ATAF2 and transcriptional analyses confirmed this binding sequence is enough to promote ATAF2-mediated transcription of genes whose promoters contain this consensus sequence (Wang and Culver 2012). According to the current knowledge, it is likely that immunity-related NAC TFs have diverse DNA binding sites to regulate the transcription of the downstream target genes and therefore exert their biological functions in plant immunity.

Target genes

Experimental identification of DNA binding sequences, bioinformatics analyses of the presence of DNA binding sites in gene promoters and comparative analyses of co-expression patterns, along with ChIP verification of in vivo DNA binding events, enable the characterization of putative target genes whose transcription is regulated by specific immunity-related NAC TFs (Table 1). In Arabidopsis, SA biosynthesis genes *ICS1*, *PAD4*, *EDS1* and *PBS3* are the targets for ANAC019/ANAC055/ANAC072 (Zheng et al. 2012) while *ICS1* and defense gene *PR1* are targets of CBNAC/NTL9 (Kim et al. 2012; Zheng et al. 2015). Microarray, ChIP and qRT-PCR analyses identified at least three NAC4-targeted genes *LURP1*, *WRKY40* and *WRKY54*, which act as negative regulators of cell death (Lee et al. 2017). The active form of Arabidopsis NTL6 bound to a region of − 105 to − 76 bp in *PR1* promoter in vitro and induced the expression of *PR1*, *PR2* and *PR5* by directly binding to a conserved sequence in their promoters (Seo et al. 2010) while the rice OsNAC111 was shown to be capable of enhancing the promoter activity of defense genes *PR2* and *PR8* (Yokotani et al. 2014). Microarray analysis revealed that many abiotic and biotic stress inducible genes were upregulated in *OsNAC6*-overexpressing rice plants. Among these upregulated stress inducible genes, *LOC_Os01g73200* (AK104277, coding for a cationic peroxidase) and *LOC_Os04g25060* (AK110725, coding a protein containing a conserved DUF26 domain) were identified as putative target genes of OsNAC6 through transient transactivation assays (Nakashima et al. 2007). In tomato, the JA2L targets the SA metabolism genes *SAMT1* and *SAMT2* by binding to the NAC core-binding sites in their promoters; whereas JA2 specifically interacted with the NAC core binding sites of the *NCED1* promoter and selectively activated the expression of *NCED1* (Du et al. 2014). However, the target genes of other reported immunity-related NAC TFs remain to be characterized.

Conclusions and further directions

During the last two decades, functional studies have established our current knowledge on the functions of dozens of NAC TFs as critical regulators of various aspects of plant immunity through impacts on different immune signaling and as virulence targets of pathogen effectors to suppress host plant immunity or benefit pathogen replication. Functions of these immunity-related NAC TFs and their mechanisms of action provide new insights into our understanding of the regulation of plant immunity against pathogens. Further studies should focus on the following three aspects. First, the discovery of novel immunity-related NAC TFs. As a relatively large family of TFs, family-wide functional studies using knockout/knockdown and overexpression approaches will identify novel immunity-related NAC TFs, with emphasis on the NAC TFs in economically important crop plants. For this aspect, CRES-T, tag line, RNAi, and antisense technologies are useful to analyze the biological functions of NAC TFs. For example, the chimeric repressor silencing technology CRES-T system, which is achieved by fusing the NAC proteins with the SRDX repression domain (for SUPERMAN repression domain X), successfully identified some immunity-related NAC TFs such as ATAF1, OsNAC111 and JA2. Second, studies are needed for in-depth understanding of the regulatory network of immunity-related NAC TFs. Further biochemical, genetic and molecular studies will be helpful to elucidate the detailed mechanisms for the immunity-related NAC TFs in transcriptional regulation of their target genes and how these immunity-related NAC TFs integrate into the well-established immune signaling network. Another, global mapping of the genome-wide DNA-binding sites and specificities by high-throughput approaches will discover and characterize the regulatory networks for the immunity-related NAC TFs. Third, emphasis should be given to the potential application of immunity-related NAC TFs. The NAC TFs possess a great potential in improvement of disease resistance in economically important crops. This can be achieved by directly manipulating the expression of the immunity-related NAC TFs via overexpression or knockout/knockdown approaches and by modifying the binding sites in promoters of the immunity-related NAC TFs-regulated target genes through CRISPR/Cas9 genome editing. Notably, some immunity-related NAC TFs such as ATAF1, ATAF2 and CBNAC/NTL9 show distinct and even opposite functions in immunity against necrotrophic and biotrophic pathogens in dicot and monocot plants. Thus, risks from pleiotropic effect of NAC TFs on plant immunity against different pathogens as well as on abiotic stress tolerance and agronomic traits must be

taken into consideration when immunity-related NAC TFs are used for genetic improvement of disease resistance in crop plants.

Abbreviations

AP2/ERF: Apetala2/ethylene responsive factor; *Bgh*: *Blumeria graminis* f. sp. *hordei*; *Bgt*: *Blumeria graminis* f. sp. *tritici*; bHLH: Basic helix-loop-helix; bZIP: Basic leucine zipper domain; CAMTA: CaM-binding transcription activator; CBNAC: Calmodulin-binding NAC protein; CBNACBS: Calmodulin-binding NAC binding sequence; CF: Culture filtrate; CMV: *Cucumber mosaic virus*; ER: Endoplasmic reticulum; ETI: Effector-triggered immunity; GRAB: Geminivirus Rep A-binding; hpi: Hours post inoculation; HR: Hypersensitive response; JUB1: JUNGBRUNNEN1; MYMIV: *Mungbean yellow mosaic India virus*; NAC: NAM, ATAF and CUC; NACRS: NAC recognition sequence; NF-Y: Nuclear Factor Y; NTL: NAC with transmembrane motif1-like; OE: Overexpression; PCD: Programmed cell death; PTI: PAMP/MAMP/DAMP-triggered immunity; RDV: *Rice dwarf virus*; RNAi: RNA interference; TCV: *Turnip crinkle virus*; TF: Transcription factor; TIP: TCV-interacting protein; TLCV: *Tomato leaf curl virus*; TM: Transmembrane; TMV: *Tobacco mosaic virus*; VIGS: Virus-induced gene silencing; WDV: *Wheat dwarf geminivirus*

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Authors' contributions

XY and FS wrote the manuscript; HW, JC and DL discussed on some parts of the manuscript. All authors read and approved the final manuscript.

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