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Function of the NAC transcription factor family genes in the regulation of biotic stresses responses in plants

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Abstract

NAC transcription factor is one of the largest families of transcriptional regulators in plants, and members of the *NAC* gene family have been suggested to play important roles in the regulation of the transcriptional reprogramming associated with plant stress responses. A phylogenetic analysis of *NAC* genes, with a focus on rice and Arabidopsis, was performed. Herein, we present an overview of the regulation of the stress responsive *NAC SNAC*/(*IX*) group of genes that are implicated in the resistance to different stresses. SNAC factors have important roles for the control of biotic stress tolerance and overexpression can improve stress tolerance via biotechnological approaches. We also review the recent progress in elucidating the roles of NAC transcription factor in plant biotic stress. Modification of the expression pattern of transcription factor genes and/or changes in their activity contribute to the elaboration of various signaling pathways and regulatory networks. Though, a single *NAC* gene often responds to several stress factors, and their protein products may participate in the regulation of several seemingly disparate processes as negative or positive regulators. Additionally, the NAC proteins function via auto-regulation or cross-regulation is extensively found among *NAC* genes. These observations assist in the understanding of the complex mechanisms of signaling and transcriptional reprogramming controlled by NAC proteins.

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Introduction

Transcription factors (TFs) function in the promoter region of different stress-related genes, and the overexpression or suppression of these genes may improve the plant's tolerance to biotic stresses. Biotic stresses trigger a wide range of plant responses, from the alteration of gene expression and cellular metabolism to changes in plant growth and development and crop yields. The NAC acronym is derived from three genes that were initially discovered to contain a particular domain (the NAC domain): NAM (for no apical meristem), ATAF1 and -2, and CUC2 (for cup-shaped cotyledon) (Souer *et al.*, 1996; Aida *et al.*, 1997). The *NAC* genes constitute one of the largest families of plant-specific TFs and are present in a wide range of species. Extensive investigation aided by the availability of several complete plant genomic sequences has identified 117 *NAC* genes in Arabidopsis, 151 in rice, 79 in grape, 26 in citrus, 163 in poplar, and 152 each in soybean and tobacco (Rushton *et al.*, 2008; Hu *et al.*, 2010; Le *et al.*, 2011; Nuruzzaman *et al.*, 2010, 2012a).

In the past decade, significant progress has been achieved in determining the molecular mechanisms of innate immune responses in rice, host recognition of pathogens, recognition-triggered early signaling events, and signaling pathways and their involvement in activating defense responses (Liu *et al.*, 2010; Valent and Khang, 2010). A number of regulatory proteins, including several TFs (e.g., OsNAC6), function in regulating defense responses against *Magnaporthe grisea* (Nakashima *et al.*, 2007). Though, a complete understanding of the molecular network regulating the rice immune responses against pathogens remains unclear. Microarray profiling after biotic treatments [rice stripe virus (RSV) and rice tungro spherical virus (RTSV)] in rice seedlings has revealed six *OsNAC* genes induced by both virus infections (Nuruzzaman *et al.*, 2010). Rice plants with a mutation in *rim1-1* are resistant to infection by dwarf virus (Yoshii *et al.*, 2009; Satoh *et al.*, 2011). The *StNAC* (*Solanum tuberosum*) gene is induced in response to *Phytophthora infestans* infection (Collinge and Boller, 2001). The salicylic acid (SA) and ethylene (ET)/jasmonic acid (JA)-mediated signaling pathways are critical in activating innate immune responses in rice and can operate in concert using some common components or biochemical events (Li *et al.*, 2011). Furthermore, numerous *NAC* genes are involved in the response of plants to abiotic stresses, such as drought, salinity, cold, and submergence (Nuruzzaman *et al.*, 2012b).

To date, a few of these genes have been characterized to date. Indeed, most of the NAC family members have yet to be characterized, even though these genes are likely to play important roles in plant physiology, and substantial experimental work will be required to determine the specific biological function of each *NAC* gene. Based on phylogenetic analyses, it is apparent that this large family of TFs consists of groups that are closely related to each other (Tian *et al.*, 2004). The focus of this review is the phylogeny of *NAC* genes with respect to resistance pathways. We also present an overview of the regulation of the *SNAC*/(*IX*) group of genes that are implicated in the resistance to different stresses. Furthermore, we will emphasize on the roles of *NAC* TFs genes in plant biotic stresses.

Structural features of the nac proteins

The N-terminus of NAC proteins is a highly homologous region containing the DNA-binding NAC domain. NAC proteins commonly possess a conserved NAC domain at the N-terminus that consists of approximately 150 to 160 amino acids and is divided into five sub-domains (A to E) (Ooka *et al.*, 2003). The function of the NAC domain has been associated with nuclear localization, DNA binding, and the formation of homodimers or heterodimers with other NAC domain-containing proteins (Olsen *et al.*, 2005). The structure of the DNA-binding NAC domain of Arabidopsis *ANAC019* has been solved by X-ray crystallography (Ernst *et al.*, 2004), and the functional dimer formed by the NAC domain was identified in the structural analysis. The NAC domain structure of a rice stress-responsive NAC protein

(SNAC1; STRESS-RESPONSIVE NAC 1) was also reported (Chen *et al.*, 2011) and shares structural similarity with the NAC domain from Arabidopsis *ANAC019*. In contrast, the C-terminal regions of NAC proteins are highly divergent (Ooka *et al.*, 2003) and are responsible for the observed regulatory differences between the transcriptional activation activity of NAC proteins (Jensen *et al.*, 2010). The divergent C-terminal region of these proteins generally operates as a functional domain, acting as a transcriptional activator or repressor (Kim *et al.*, 2007b). The C-terminal region is large and possesses protein-binding activity.

Structural conservation of snac group

The evolutionary analysis of developmental processes of *NAC* genes through the correlation of function and phylogeny is a well-known approach in plant research (Figure 1; Nuruzzaman *et al.*, 2010, 2012a). The NAC TF family has experienced extensive expansion through gene duplication events. Although NAC structural diversity has been constrained within the 60-amino acid conserved domain, which comprises a unique DNA-interacting β-sheet structure, structural conservation outside this conserved domain is extremely limited. Additional highly conserved motifs can be identified only within specific groups (e.g., SNAC, TIP, and SND), and most members in the same group share one or more motifs outside the NAC domain (Nuruzzaman *et al.*, 2012a). A phylogeny of the SNAC group, which includes the *ANAC019* and *OsNAC6* genes, indicates the existence of multiple co-orthologs in dicots and monocots (Figure 1).

Roles played by nac transcription factors

Since the early research into NAC TFs, it was evident that these factors play roles in regulating several different plant processes. For convenience, some of these processes are discussed individually below. The recent data presented here provided new insight, namely, that it is common for a single NAC NF to regulate transcriptional reprogramming that is associated with multiple plant programs: the dynamic web of signaling in which NAC factors operate has multiple inputs and outputs.

NAC function in biotic stresses

The majority of reports concerning NAC TFs have indicated that numerous members of the multigene family play roles in the transcriptional reprogramming associated with plant immune responses. This is an active research area that has been extensively reviewed and therefore will only be briefly considered here. To date, it is clear that NAC NFs are central components of many aspects of the plant innate immune system, basal defense, and systemic acquired resistance.

There are many examples in which the overexpression or knockdown of *NAC* gene expression has effects on plant defense, observations that have allowed the resolution of some components of the web of signaling pathways (Table 1 and Figures 2-4) (Jensen *et al.*, 2007, 2008, 2010).

Table 1. Function of NAC transcription factors in biotic infections.

Genes/ target genes	Functions	Method	Species	References
HvNAC6	*HvNAC6* positively regulates penetration resistant towards *Bl. gramini f.sp. hordei* (*Bgh*) attack	Knockdown/ overexpression	*H. vulgare*	Jensen *et al.*, 2007
ataf1-1	Loss-of-function mutants have attenuated penetration resistance towards *Bgh* attack	Knockout	*A. thaliana* (*At*)	Jensen *et al.*, 2008
ATAF1, PR1	ATAF1 negatively regulates resistance to *B. cinerea*	Overexpression/ *ataf1-1* and *ataf1-2*, knockout	*A. thaliana*	Wu *et al.*, 2009
ATAF1, PR-1, PR-5, NPR1, PDF1.2	ATAF1 negatively regulates resistance to *P. syringae*, *B. cinerea*, *A. brassicicola*	Overexpression/ *ataf1-2*, knockout	*A. thaliana*	Wang *et al.*, 2009a
ATAF2 , PR1, PR2, PR4, PR5, PDF1.1, PDF1.2	ATAF2 negatively regulates resistance to *F. oxysporum*,	Overexpression/ knockout	*A. thaliana*	Delessert *et al.*, 2005

	represses pathogenesis-related proteins			
ATAF2, PR1, PR2, PDF1.2	OX = Reduced tobacco mosaic virus accumulation, increased pathogenesis-related genes	Overexpression/ knockout	*A. thaliana*	Wang *et al.*, 2009a
ATAF2, NIT2	Defense hormones, pathogen infection	Overexpression/ knockout	*A. thaliana*	Huh *et al.*, 2012
ANAC019, ANAC055	Defense disease, JA pathway	Overexpression	*A. thaliana*	Bu *et al.*, 2008
NTL6, PR1, PR2, PR5	Positive regulator of pathogen resistance against *P. syringae*	Gene silencing/ overexpression	*A. thaliana*	Seo *et al.*, 2010
ANAC042, P450	Regulation of camalexin biosynthesis, pathogen infection	β- Glucuronidase (GUS)-reporter assays	*A. thaliana*	Saga,12
SlNAC1	Increased tomato leaf curl virus (TLCV) DNA accumulation	Transient overexpression	*N. benthamiana*	Selth *et al.*, 2005
OsNAC4	Inducer of HR cell death upon *Acidovorax avenae* infection, loss of plasma membrane integrity, nuclear DNA fragmentation	Overexpression/ knockdown	*Oryza* (O) *sativa*	Kaneda *et al.*, 2009
OsNAC6 , PR protein 1, Probenazoleinducible proteins (PBZ1s), DUF26- like Ser/Thr protein kinase,Thioredoxin, Peroxidase,Lipoxygenase,	Slightly increased tolerance to rice blast disease	Overexpression	*O. sativa*	Nakashima *et al.*, 2007
rim1-1	Resistance to rice dwarf virus (RDV), susceptible to *rice* transitory yellowing virus (RTYV) and RSV	Knockout	*O. sativa*	Yoshii *et al.*, 2009
Os02g34970, Os02g38130, Os11g03310, Os11g03370, Os11g05614, Os12g03050	RSV, RTSV infections	Microarray	*O. sativa*	Nuruzzaman *et al.*, 2010
OsNAC19	Disease resistance	Infection	*O. sativa*	Lin *et al.*, 2007
GRAB1, GRAB2	Inhibited wheat dwarf virus replication	Transient Overexpression	*T. monococcum*	Xie *et al.*, 1999
ATAF2	Tobacco mosaic virus	Transgenic	Tobaco	Wang *et al.*, 2009b
ONAC122 and *ONAC131* brome mosaic virus (BMV)	Defense responses against *Magnaporthe grisea*	-----	*O. sativa*	Sun, 2013
SlNAC1	Upregulated during pseudomonas infection	Pathogen infection	*S.lycopersicum*	Huang *et al.*, 2012
CaNAC1	Defense responses against pathogen	Infection	*C. arietinum*	Oh *et al.*, 2005
GmNAC6	Responses to biotic signals, osmotic stress-induced	Transctiption	*G. max*	Faria, 11
TLCV, SlNAC1	Enhances viral replication	Overexpression	*L. esculentum*	Selth *et al.*, 2005
BnNAC14, BnNAC485, ATAF1 or *ATAF2*	Response to biotic and abiotic stresses including wounding	cDNA libraries	-----	Hegedus *et al.*, 2003
Stprx2, StNAC	Wounding and pathogen response	Transcriptome	*S. tuberosum*	Collinge and Boller, 2001
NT L4	ROS under abscisic acid, leaf senescence	Transgenic	*A. thaliana*	Lee *et al.*, 2012
NTL9	Osmotic stress responses, leaf senescence	Overexpression /knocout	*A. thaliana*	Yoon *et al.*, 2008
MtNAC969	Symbiotic nodule senescence	Overexpresion	*M. truncatula*	de Zélicourt *et al.*, 2012
VNI2 , OR/RD	Leaf senescence	Transcription	*A. thaliana*	Seo *et al.*, 2011
Os07g37920, Wheat GPC	Senescence	Overexpression/ RNAi	*O. sativa, T. aestivum*	Distelfeld *et al.*, 2012
AtNAP	Leaf senescence	Overexpression/ RNAi	*A. thaliana*	Guo *et al.*, 2006

Regulation of NAC TFs by pathogen infection

Sun and co-workers applied Virus-induced gene silencing (VIGS) system to investigate the function of NAC TFs (ONAC122 and ONAC131) in disease resistance against *M. grisea* (Sun *et al.*, 2013). VIGS is a useful tool for the rapid analysis of gene function in plants (Scofield and Nelson, 2009). Some VIGS vectors have been developed for dicotyledonous plants among which the tobacco rattle virus (TRV)-based VIGS vector is the most successful example for members of Solanaceae, such as *Nicotiana benthamiana* and *Lycopersicon esculentum* (Liu *et al.*, 2002; Chakravarthy *et al.*, 2010). The barley stripe mosaic virus (BSMV)-based VIGS vector was used to characterize multiple genes for their roles in disease resistance in wheat and barley (Zhou *et al*., 2007; Sindhu *et al*., 2008). Several scientists have developed a brome mosaic virus (BMV)-based VIGS vector, and this vector was demonstrated to be a versatile tool for rapid gene function analysis in barley, rice, and maize (van der Linde *et al*., 2011; Biruma *et al*., 2012). In rice seedlings, 19 and 13 *NAC* genes were up-regulated after RSV and RTSV infection, respectively, at different days after inoculation (Nuruzzaman *et al*., 2010). Several NAC proteins can either enhance or inhibit virus multiplication by directly interacting with virus-encoded proteins (Figure 2; Jeong *et al*., 2008; Yoshii *et al*., 2009), and increases in the expression level of *NAC* genes have been monitored in response to attack by viruses, several fungal elicitors, and bacteria (Figures 3 and 4; Collinge and Boller, 2001; Jensen *et al*., 2007; Jeong *et al*., 2008; Wang *et al*., 2009a, 2009b; Xia *et al*., 2010a, 2010b). Such dual modulation in plant defense implies the association of NAC proteins with distinct regulatory complexes.

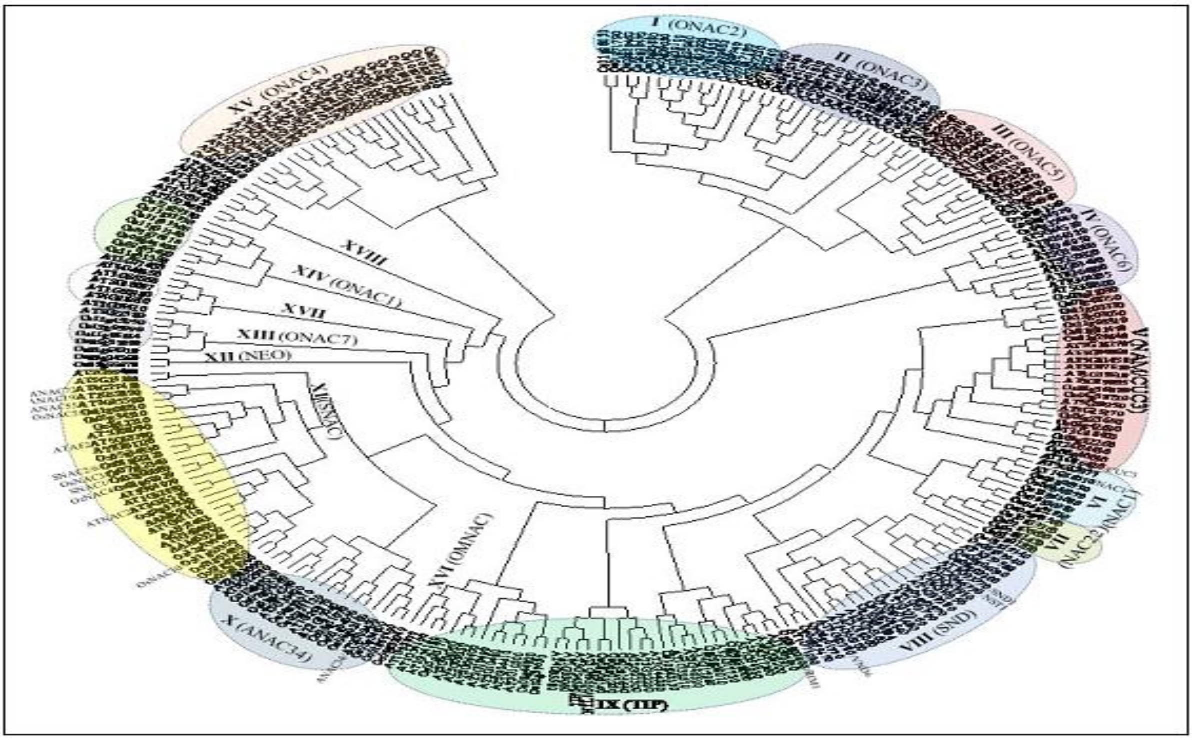

Fig. 1. An unrooted phylogenetic tree of the NAC transcription factors of rice and Arabidopsis. The amino acid sequences of the NAC domain of 135 rice NAC family proteins and 117 Arabidopsis NAC proteins were aligned by ClustalW, and the phylogenetic tree was constructed using MEGA 4.0 and the NJ method. Bootstrap values from 1000 replicates were used to assess the robustness of the trees. The classification by Nuruzzaman *et al.* (2010) is indicated in parentheses.

Kaneda *et al.* (2009) reported that *OsNAC4* is a key positive regulator of hypersensitive cell death in plants, and hypersensitive cell death is markedly decreased in response to avirulent bacterial strains in *OsNAC4*-knock-down lines.

After induction by an avirulent pathogen recognition signal, *OsNAC4* is translocated into the nucleus in a phosphorylation-dependent manner. Conversely, the overexpression of *OsNAC6* does not lead to hypersensitive cell death (Kaneda *et al*., 2009), whereas transgenic rice plants overexpressing *OsNAC6* exhibited tolerance to blast disease (Nakashima *et al*., 2007). *ATAF2* overexpression resulted in increased susceptibility toward the necrotrophic fungus *Fusarium oxysporum* under sterile conditions due to the repression of pathogenesis-related (*PR*) genes (Delessert *et al.*, 2005) but induced *PR* genes, reducing tobacco mosaic virus accumulation in a non-sterile environment (Wang *et al.*, 2009b). RNA interference and overexpression studies have also revealed the function of NAC TFs in various plant–pathogen interactions (Figure 3). A number of NAC proteins may positively regulate plant defense responses by activating *PR* genes, inducing a hypersensitive response (HR), and cell death at the infection site (Figure 3; Jensen *et al.*, 2007, 2008; Kaneda *et al.*, 2009; Seo *et al.*, 2010). *ATAF1* and its barley homolog *HvNAC6* positively regulate penetration resistance to the biotrophic fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) (Jensen *et al.*, 2007, 2008) but attenuate the resistance to other pathogens, such as *Pseudomonas syringae*, *Botrytis cinerea*, and *Alternaria brassicicola* (Wang *et al.*, 2009a; Wu *et al.*, 2009). Unlike *ATAF2*, *ATAF1* and *HvNAC6* are transcriptional activators and may indirectly regulate the repression of *PR* genes via a hypothetical negative regulator (Figure 3). Hence, the ATAF subfamily clearly appears to have a conserved but non-redundant function in regulating the responses to different pathogens. The immune response in plants elicited upon pathogen infection is characterized by activation of multiple defense responses including expression of a large set of defense-related genes (Van Loon *et al.*, 2006), which are regulated by different types of TFs. Many TFs belonging to the NAC, ERF, and WRKY families have been identified (Eulgem *et al.*, 2007; Gutterson *et al.*, 2004) and revealed to play important roles in regulating expression of defense-related genes.

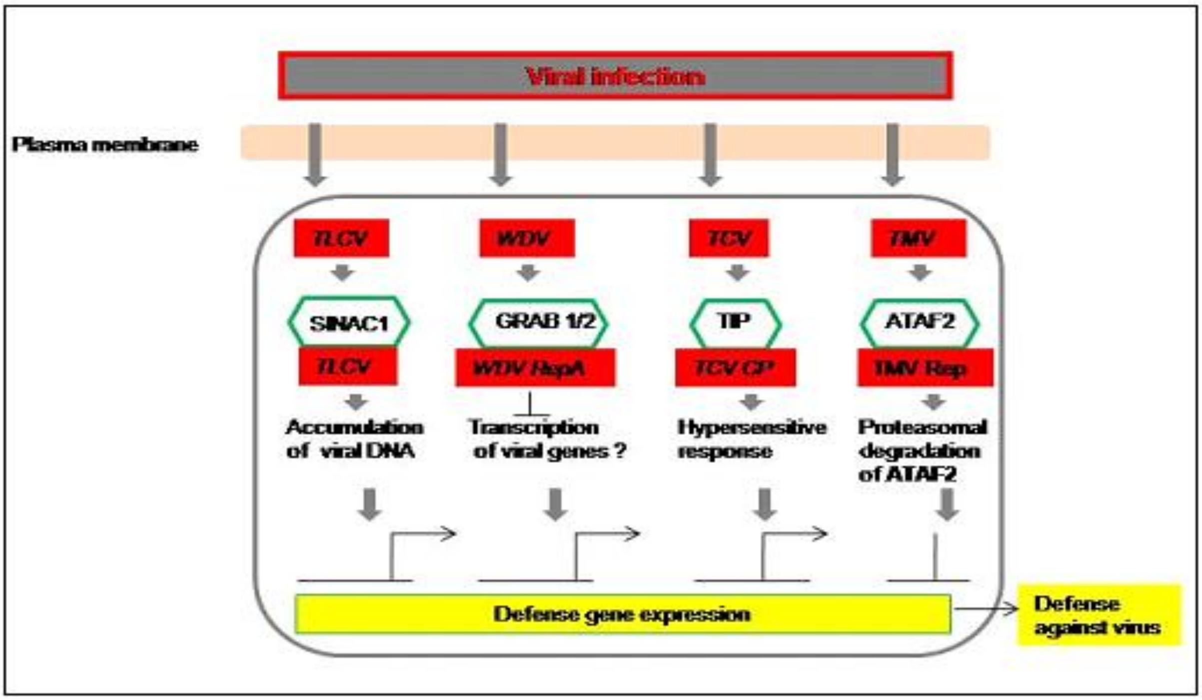

Fig. 2. NAC transcription factor as key components in the transcriptional regulation of gene expression during virus infection. Abbreviations: TCV, turnip crinkle virus; TIP, TCV-interacting protein; TLCV, tomato leaf curl virus; TMV, tobacco mosaic virus; WDV, wheat dwarf geminivirus.

Arabidopsis stress-responsive *NAC* genes, such as *RD26*, respond to JA, a well-described phytohormone that is functionally involved in regulating wounding and biotic stress responses (Fujita *et al.*, 2006).

Hence, it is reasonable to consider that JA-responsive SNAC factors might function in both biotic and abiotic stress responses. In rice, most of the genes in the SNAC group respond to JA. Among them, *SNAC1*, *OsNAC3*, *OsNAC4*, *OsNAC5*, *OsNAC6*, and *OsNAC10* are present in the same phylogenetic SNAC/(IX) group (Figure 1). In particular, the SNAC group (Figure 1) comprises several genes that regulate disease resistance pathways, as inferred from the increased resistance to pathogens upon overexpression under the control of a constitutive promoter. Data indicate that NAC TFs also have an important role in the regulation of plant defense responses to different pathogens in addition to wounding and insect feeding (Figure 4).

The application of exogenous phytohormones, such as JA, SA, and ET, has also been shown to induce *NAC* genes in several species (Nakashima *et al.*, 2007; Hu *et al.*, 2006, 2008; Yoshii *et al.*, 2010; Xia *et al.*, 2010a, 2010b; Nuruzzaman *et al.*, 2012b). Hence, NAC TFs can possibly modulate the phytohormonal regulation of the biotic stress cellular network for convergent and divergent adaptive pathways.

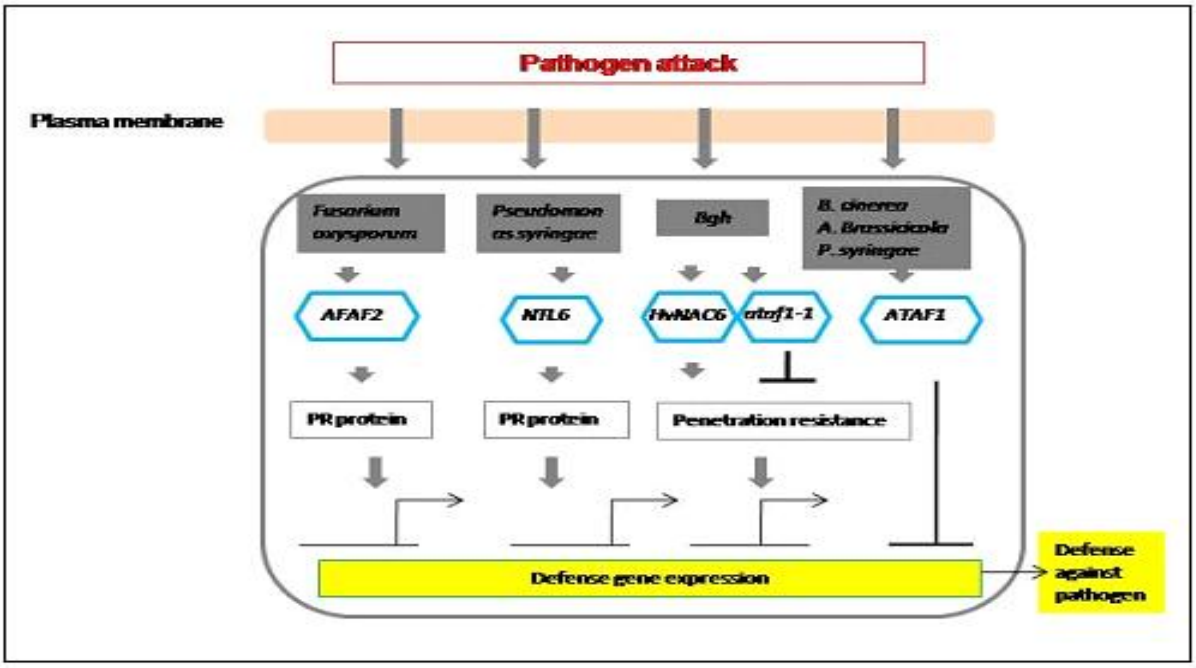

Fig. 3. NAC transcription factor as key components in transcriptional regulation of gene expression during pathogen attack, integrating both positive (arrows) and negative (bars) regulatory mechanisms.

NAC TFs in ROS and senescence signaling pathways

Reactive oxygen species (ROS) is an active molecule in most biotic plant stress. Such ROS as H_2O_2 act as important signal transduction molecules, mediating the acquisition of tolerance to various stresses (Bhattacharjee, 2005). In rice, *OsNAC6* gene is involved in both response and tolerance to biotic stress (Nakashima *et al.*, 2007).

In Arabidopsis, ATAF subfamily (*ATAF1*, *ATAF2*, and *RD26*) is also involved in biotic stress. The expression of *RD26* is induced by JA and H_2O_2, and pathogen infections (Zimmermann *et al.*, 2004). Large-scale transcriptiome analysis with both types of mutants revealed that *RD26*-regulated genes are involved in the detoxification of ROS, defense, and senescence (Fujita *et al.*, 2006). The role of stress-responsive NAC proteins in senescence is poorly understood. Recently, the *NTL4*, (Lee *et al.*, 2012), *MtNAC969* (de Zélicourt *et al.*, 2012), *Os07g37920*, wheat *GPC* (Distelfeld *et al.*, 2012) genes were found to be induced senescence in different plants.

Leaf senescence is a unique developmental process that is characterized by massive programmed cell death and nutrient recycling. Leaf senescence is induced by pathogen infection (Buchanan-Wollaston 2003, Gepstein 2003). *AtNAP* gene, which belongs to the closest NAC subfamily of the ATAF subfamily, has been shown to be involved in senescence (Guo and Gan, 2006). In addition all ATAF subfamily *NAC* genes, including *ATAF1*, *ATAF2*, and *RD26*, are upregulated during senescence in Arabidopsis leaves (Guo *et al.*, 2004). These findings suggest that *RD26* may function at the node of convergence between the pathogen defense and senescence signaling pathways. Taken together, these results support the notion that ROS and senescence may be closely related to NAC-mediated stress responses.

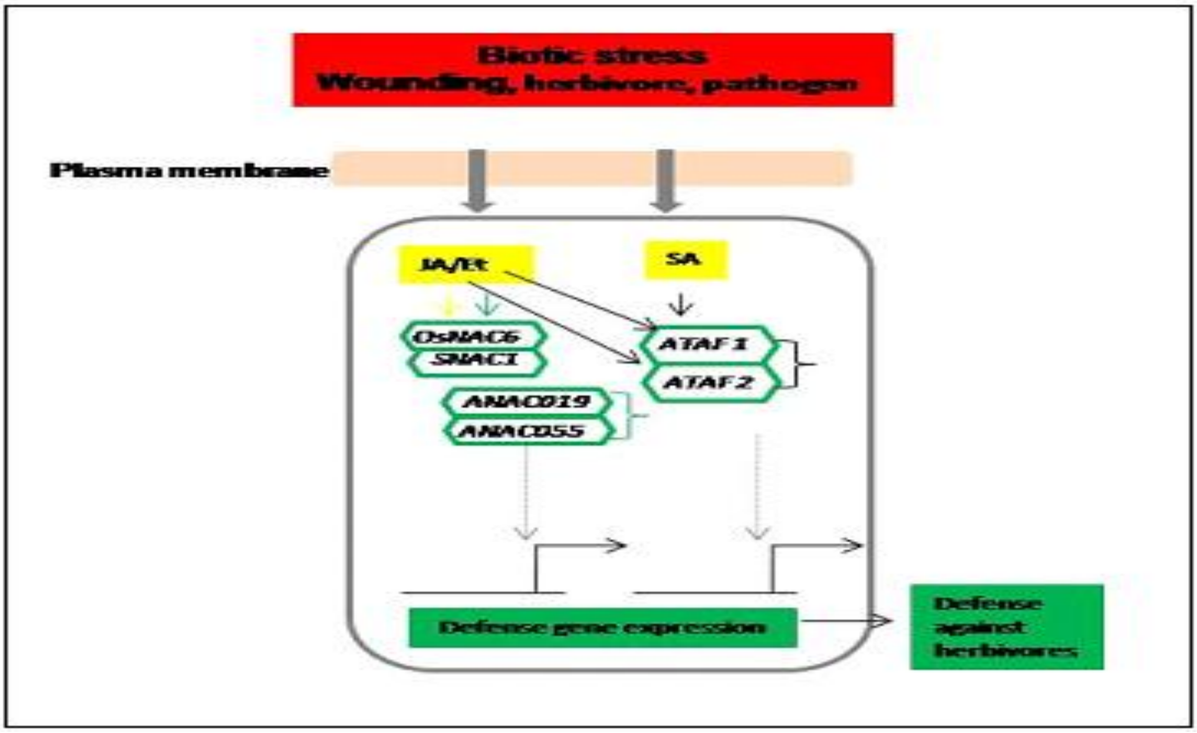

Fig. 4. The role of NAC transcription factor in the herbivore/biotic response signaling pathway.

One nac for multiple processes

Numerous studies have demonstrated that a single transcription factor may function in several seemingly disparate signaling pathways, as can be deduced from their induced expression profiles by various stress factors. *OsNAC6* was induced by JA, a plant hormone that activates defense responses against herbivores and pathogens (Figure 4, Ohnishi *et al.*, 2005). Studies on an *NAC* gene (*Os04g0477300*) showed that it functions in at least three different processes, including pathogen defense, senescence, and responses to phosphate and boron deficiency (Nilsson *et al.*, 2010; Ochiai *et al.*, 2011). A number of *NAC* genes (e.g., *AtNAC2*) in plants are affected by auxin, ethylene (Xie *et al.*, 2000; He *et al.*, 2005), and ABA (e.g., *OsNAC5*; Sperotto *et al.*, 2009). *Os05g34830* (SNAC group, Figure 1) was specifically induced in the roots of a tolerant line under severe and mild drought conditions and was activated by ABA treatment (Nuruzzaman *et al.*, 2012b). *OsNAC5*/*ONAC009*/*ONAC071* and *OsNAC6* are homologs that are induced by pathogen infection and such abiotic stresses as drought and high salinity and ABA (Takasaki *et al.*, 2010). *AtNAC1* and *AtNAC2* are induced by auxin and ABA, respectively, and *AtNAC1* mediates auxin signaling to promote lateral root development in Arabidopsis (Xie *et al.*, 2000; He *et al.*, 2005). The *TaNAC4* gene functions as a transcriptional activator involved in wheat responses to abiotic and biotic stresses (Xia *et al.*, 2010a). *SiNAC* transcripts mostly accumulate in young spikes and were strongly induced by dehydration, salinity, ethephon, and methyl jasmonate (Distelfeld *et al.*, 2012). These data demonstrate that a single *NAC* gene can function as regulator of several different processes and may also mediate the cross-talk between different signaling pathways.

Conclusion

The responses to the environment are specialized through the diversification of the structure of stress-response regulators, which are involved in stress-response pathways via binding motifs (CATGTG) in their target genes. Thus, the components and regulatory structure of specific pathways must be delimited for an understanding of the evolutionary genetics of environmental stress responses. This review summarizes the current knowledge of the genes and NAC TFs that comprise a portion of this network. Interestingly, all of the SNAC sequences known to play a role in disease resistance responses are in one group of the NAC family. Much progress in NAC TF functional research has been attained over the past decade. However, most of these advances are related to the involvement of biotic stresses.

The identification of NAC functions in biotic stresses will remain a substantial challenge in the coming years. To achieve a better understanding of their role under biotic stresses, it is very important to identify the interacting partner of NAC proteins that cooperates in regulating the transcription of downstream target genes under a specific condition. It is also crucial to identify the key components of the signal transduction pathways with which these factors physically interact. Applying data obtained from microarrays could help to directly determine the specific NAC DNA-binding sites on a global scale under conditions of biotic stresses. Accordingly, we may then appreciate the complex mechanisms of signaling and transcriptional reprogramming controlled by NAC proteins and the plant processes in which they participate. Certainly, further molecular studies of NAC NFs under different biotic stresses will clarify the fine-tuning mechanisms that are controlled by NAC proteins in plants, with economical benefits to agricultural production.

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