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TaNAC2 is a negative regulator in the wheat-stripe rust fungus interaction at the early stage

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ABSTRACT

NAC transcription factors play important roles in plant response to various stresses. In this study, the roles of *TaNAC2* in wheat against biotic and abiotic stresses were investigated. The expression of *TaNAC2* was highly induced in wheat-*Puccinia striiformis* (*Pst*) interactions and ABA treatment. TaNAC2 was localized in the nuclei of Arabidopsis protoplasts. The C-terminus of TaNAC2 was necessary for transcriptional activation by the yeast one-hybrid assay. Furthermore, silencing of *TaNAC2* enhanced tolerance to *Pst* via a significant increase of H_2O_2 generation and suppressing hyphal growth at the early interaction stage.

1. Introduction

Plants possess a dedicated immune system to fend off infection by pathogens such as viruses, bacteria, fungi, oomycetes, and nematodes [1]. To combat invading pathogens, plants have evolved innate immune systems that are capable of recognizing potential invading pathogens and initiating effective defense responses [2]. The multiple defense mechanisms include basal resistance, such as PAMP (pathogen-associated molecula pathogen-associated molecular patterns) -triggered immunity (PTI), a basal defense mechanism in plants, and effector-triggered immunity (ETI) [2,3], which requires plant resistance (R) proteins for specific recognition of effectors and causes a rapid localized cell death termed the hypersensitive response (HR) [4]. However, plants attract pathogens, enable their host entry and accommodation, and facilitate nutrient provision. The plant immune system is rendered ineffectively by adapted pathogens that have evolved ways to interfere with host defense. Susceptibility to infectious diseases caused by pathogens affects most plants in their natural habitat and leads to yield losses in agriculture [1].

To protect plants against biotic and abiotic stresses, many stress-inducible genes are regulated by transcription factors (TFs) in down-stream signaling pathways. Among these TFs, the NAC TFs, one of the largest TF families in plants, comprise a complex plant-specific superfamily present in a wide range of species. The NAC acronym is derived from the three earliest characterized proteins with a NAC domain: petunia NAM (no apical meristem) and

Abbreviations: Pst, *Puccinia striiformis* f. sp. *tritici*; PAMP, pathogen-associated molecula pathogen-associated molecular patterns; PTI, PAMP-triggered immunity; ETI, effector-triggered immunity; HR, hypersensitive response; TF, transcription factors; ABA, abscisic acid; MeJA, methyl jasmonate; DAB, 3,3-diaminobenzidine; dpi, days post-inoculation; hpt, hours post treatment; hpi, hours post inoculation; qRT-PCR, quantitative reverse transcriptase PCR or quantitative real-time PCR; BSMV, barley stripe mosaic virus; VIGS, virus induced gene silencing; PDS, phytoene desaturase; SA, salicylic acid; cDNA, complementary DNA; ORF, longest open reading frame.

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Arabidopsis ATAF1/2 and CUC2 (cup-shaped cotyledon) [5,6]. Members of the NAC family have a highly conserved N-terminal NAC domain and a variable C-terminal transcriptional regulation domain [7,8]. Owing to the availability of an ever-increasing number of complete plant genomes and EST sequences, large numbers of putative *NAC* genes have been identified in many sequenced species at genome-wide scale, including 168 in durum wheat, 117 in *Arabidopsis*, 151 in rice, 79 in grape, 204 in Chinese cabbage, and 152 in maize [9]. Numerous reports have demonstrated that TFs are necessary for plant development and are crucial in the conversion of perceived stress signals to stress-responsive gene expression.

The NAC proteins are involved in various plant biological regulation processes, including developmental programs, plant senescence control, secondary cell wall formation, and various biotic and abiotic stress responses [8–10]. In plant responses to biotic stresses, some NAC proteins have dual roles, acting as positive or negative regulators in response to different pathogens [11]. For example, *ATAF1* and its barley homolog *HvNAC6* enhance resistance to the biotrophic fungus *Blumeria graminis* f. sp. *graminis* (*Bgh*) [12], but attenuate resistance to pathogens such as *Pseudomonas syringae*, *Botrytis cinerea* and *Alternaria brassicicola* [13,14]. GhATAF1, a NAC transcription factor, confers abiotic and biotic stress responses by regulating phytohormonal signaling networks in cotton. Overexpressing *GhATAF1* increases cotton plant susceptibility to the fungal pathogens *V. dahliae* and *Botrytis cinerea* coupled with suppression of JA-mediated signaling and the activation of SA-mediated signaling [15]. However, knowledge of the functions of most NAC members in disease response is limited. Orthologous NAC transcription factors show divergent functions in wheat and rice. For example, wheat Grain Protein Content genes (GPC) show higher transcript levels in leaves than in flowers and delay senescence by mutation but exhibit normal anther dehiscence and fertility [16]. The rice gene Os07g37920, the closest rice homolog to both wheat GPC genes, shows the opposite transcription profile [16].

In recent years, the functional elucidation of more wheat NAC TFs, particularly in response to abiotic and biotic stresses, has been carried out [9]. For example, the novel NAC TF genes *TaNAC8* and *TaNAC4* in wheat have been reported to respond to stripe rust pathogen infection and abiotic stresses [17]. *TaNAC69* from the NAC superfamily of TFs is up-regulated by abiotic stresses in wheat and recognizes two consensus DNA-binding sequences [18]. Overexpression of *TaNAC69* in transgenic bread wheat enhances transcript levels of stress up-regulated genes and dehydration tolerance [19]. The target gene of tae-miR164, the novel NAC transcription factor TaNAC21/22 from the NAM subfamily, negatively regulates resistance of wheat to stripe rust [20]. TaNAC1 acts as a negative regulator of stripe rust resistance in wheat, enhances susceptibility to *Pseudomonas syringae*, and promotes lateral root development in transgenic *Arabidopsis thaliana* [21]. The Arabidopsis NAC transcription factor NAC4 promotes pathogen-induced cell death under negative regulation by microRNA164 [22]. Therefore, these TFs have the potential to be used in transgenic breeding to improve abiotic stress tolerance in crops.

As one of the top 10 plant-pathogenic fungi, *Pst* damages wheat crops, and influences yield and quanlity of wheat around the world [23]. NAC genes should be of great importance and be involved in wheat responses against *Pst*. In the previous research, we have isolated an EST sequence encoding a *TaNAC22* gene, which is differentially expressed during stripe rust infection. TaNAC2 has been confirmed to be located in the nucleus, and it may function as a transcriptional activator. In this study, expression patterns of *TaNAC2* in response to biotic and abiotic stresses were examined by qRT-PCR in wheat. By gene silencing of individual cDNA clones, we found that *TaNAC2* negatively regulates resistance to stripe rust. Together, our results indicated that *TaNAC2* plays an important role in the regulation of wheat defense response against fungal attack.

2. Materials and methods

2.1. Plant materials, biotic and abiotic stress

In this study, wheat (*Triticum aestivum* L.) genotype Suwon11 and two *Pst* pathotypes, CY23 and CY31, were used as the biological materials. Suwon 11 has been reported to contain the stripe rust resistance gene *YrSu* [24], which is highly resistant to CY23 and is incompatible with infection type 0 according to Stakman et al. [25]. CY31 is highly virulent on Suwon 11, forming a reaction compatible with infection type 4. Plants were grown and maintained according to the procedure described by Kang and Li [26]. Freshly collected urediniospores were applied with a paintbrush to the surfaces of primary leaves of 7-day-old wheat seedlings. Parallel mock inoculation was also carried out with sterile water. After inoculation, all plants, were incubated for 24 h in dark in a 100% humidity chamber and were subsequently transferred to a growth chamber with a 16-h photoperiod. Inoculated and mock inoculated leaf tissues were each harvested at 0, 12, 18, 24, 48, 72, 120 h post inoculation (hpi), respectively.

For chemical treatments, leaves of 4-week-old plants were sprayed with a solution of 2 mM salicylic acid (SA), 100 mM methyl jasmonate (MeJA), 100 mM ethephon (ETH) and 100 mM abscisic acid (ABA) that were dissolved in 0.1% (v/v) ethanol. In addition, the mock control plants were similarly treated with 0.1% (v/v) ethanol. The leaves of wheat seedlings with chemical treatments along with control plants were sampled at 0, 2, 6, 12, 24 and 48-h post treatment (hpt). In these experiments, all samples were collected quickly and frozen in liquid nitrogen and stored at −80 °C prior to the extraction of RNA. For each experiment, three replications were analyzed [20].

2.2. Gene expression analyses

Total RNA from wheat leaves infected with *Pst*, treated with exogenous hormones, and challenged with abiotic stresses was prepared by using an RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. To obtain the entire TaNAC2 sequence, a pair of primers called TaNAC2-F (5'-: GACCAGCTTTCTACA-GACGGA-3′) and TaNAC2-R (5'-: TCGGTCGGTCGATTCTACTA-3′) was used to amplify the ORF of *TaNAC2* that the length is 990 bp. The quality and integrity of the total RNA were determined by electrophoresis in a formamide denaturing gel, and the quantity was tested by using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA). One microgram of total RNA was used for cDNA production with a Verso cDNA kit (Thermo scientific) and oligo dT as a primer. The expression patterns of TaNAC2 under the different conditions as described above were detected by qRT-PCR by following the procedure described by Wang et al. [27], with a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The wheat elongation factor *TaEF-1a* gene (GenBank accession no. Q03033) was used as the internal reference in qRT-PCR. The *TaEF-1a* primer sequences were as follows: Rust-EF1-F: 5′-TTCGCCGTCCGTGATATGAGACAA-3'; Rust-EF1-R: 5′-ATGCGTAT-CATGGTGGTGGAGTGA-3'. qRT-PCR primers TaQNAC2-F:5′-GGAC-GACCTGCTCTACTCC -3′, TaQNAC2-R: 5′-CCCGTTTCCTATGCTGTATTT-3'. Reactions with no cDNA template were used as negative controls, we added the dd water to make the reaction volume same as the treatment wells. All reactions were performed in triplicate. The comparative $2^{-\Delta\Delta CT}$ method was used to quantify relative gene expression [28].