Biochemical and Biophysical Research Communications 454 (2014) 30–35

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Canola (*Brassica napus* L.) *NAC103* transcription factor gene is a novel player inducing reactive oxygen species accumulation and cell death in plants

Fangfang Niu, Boya Wang, Feifei Wu, Jingli Yan, Liang Li, Chen Wang, Yiqiao Wang, Bo Yang, Yuan-Qing Jiang *

State Key Laboratory of Soil Erosion and Dryland Farming on Loess Plateau, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 4 October 2014
Available online 18 October 2014

Keywords:
Brassica napus
Cell death
NAC
Reactive oxygen species
Transcription factor

ABSTRACT

NAC transcription factors are plant-specific and play important roles in many processes including plant development, response to biotic and abiotic stresses and hormone signaling. So far, only a few NAC genes have been identified to mediate cell death. In this study, we identified a novel NAC gene from canola (*Brassica napus* L.), *BnaNAC103* which induces reactive oxygen species (ROS) accumulation and cell death in *Nicotiana benthamiana* leaves. We found that *BnaNAC103* responded to multiple signalings, including cold, salicylic acid (SA) and a fungal pathogen *Sclerotinia sclerotiorum*. BnaNAC103 is located in the nucleus. Expression of full-length *BnaNAC103*, but not either the N-terminal NAC domain or C-terminal regulatory domain, was identified to induce hypersensitive response (HR)-like cell death when expressed in *N. benthamiana*. The cell death triggered by *BnaNAC103* is preceded by accumulation of ROS, with diaminobenzidine (DAB) staining supporting this. Moreover, quantification of ion leakage and malondialdehyde (MDA) of leaf discs indicates significant cell membrane breakage and lipid peroxidation induced by *BnaNAC103* expression. Taken together, our work has identified a novel NAC transcription factor gene modulating ROS level and cell death in plants.

1. Introduction

The NAC [no apical meristem (NAM), *Arabidopsis thaliana* transcription activation factor [ATAF1/2] and cup-shaped cotyledon (CUC2)] proteins constitute one of the largest transcription factor (TF) families and are plant-specific [1]. NAC TFs are characterized by a well-conserved N-terminal NAC domain and highly divergent C-terminus. Based on its motif distribution, the NAC domain, which comprises nearly 160 amino acid residues, can be divided into five subdomains (A–E) [2]. There are 105 putative NAC genes in *Arabidopsis*, and 140–151 in rice [3]. NAC TFs are demonstrated to be involved in many different biological processes, such as maintenance of the shoot apical meristem, regulating cell division and cell expansion in flower organs, and promoting lateral root development as reviewed in [1]. Many other NAC genes have been implicated in diverse cellular processes in various plant species, such as hormone signal pathways [4], leaf senescence [5–7] and abiotic stress signaling and tolerance [8,9]. In addition, a few NAC TFs have also been identified to positively or negatively regulate plant defense responses. For instance, *Arabidopsis* ATAF1 and ATAF2 are negative regulators of defense responses against bacterial and fungal pathogens [10,11]. *Arabidopsis NAC019* and *NAC055* mediate drought tolerance, but their overexpression also decrease resistance to *Botrytis cinerea* [12]. However, the molecular mechanisms wait to be elucidated.

In recent years, studies also indicate that NAC TFs play a role in endoplasmic reticulum (ER) or osmotic stress-induced cell death in *Arabidopsis*, rice and soybean, possibly through regulating vacuolar processing enzyme (VPE) or caspase-like protein activity [13–16]. However, whether there are other NAC genes regulating this process is not known yet.

Although members of NAC gene family in *Arabidopsis*, rice, and a few other species have been studied [2], little is known with canola. Canola is a very important oil crop in China and worldwide. However its yield is frequently influenced by environmental factors including drought, salinity, cold and fungal pathogens. Up to now, 11 NAC genes have been cloned and studied in canola response to stresses [17–19]. It is therefore essential to clone and

* Corresponding author. Fax: +86 29 87092262.
E-mail addresses: fangfangyouran@163.com (F. Niu), miao_kk@126.com (B. Wang), 283915941@qq.com (F. Wu), yanjingli512@163.com (J. Yan), 994051374@qq.com (L. Li), wangchen361@sina.cn (C. Wang), 859562712@qq.com (Y. Wang), yangwl@nwafu.edu.cn (B. Yang), jiangyq@nwafu.edu.cn (Y.-Q. Jiang).

http://dx.doi.org/10.1016/j.bbrc.2014.10.057
0006-291X/

characterize the NAC gene family in canola before stress/disease tolerant canola species can be developed. In our recent transcriptomic profiling studies in canola seedlings treated with a fungal pathogen *Sclerotinia sclerotiorum*, we identified several NAC genes induced by *S. sclerotiorum* (unpublished data). We therefore initiated a systemic identification and cloning of canola NAC genes through mining the public expressed sequence tag (EST) database. In this study, we reported the identification of a canola NAC gene, *BnaNAC103* that could elicit reactive oxygen species (ROS) accumulation and cell death when expressed in *Nicotiana benthamiana* leaves.

2. Materials and methods

2.1. Plant materials and growth condition

Canola (double haploid DH12075) and *N. benthamiana* plants were grown in Pindstrup soil mix (Denmark) in a growth chamber at 22 °C with 14 h light/10 h dark, with a light intensity of ca. 100 μ Em^{-2} s^{-1}. The relative humidity was 60–70%.

2.2. RNA isolation and RT-PCR

Young leaves of canola seedlings were harvested for RNA isolation using the Plant RNA kit (Omega bio-tek, USA). First-strand cDNA synthesis and high-fidelity PCR amplification using PrimeSTAR HS DNA polymerase (TaKaRa, Japan) were performed as previously described [20]. Primers used were listed in Table S1. PCR products were purified and cloned into pJET1.2 vector supplied in CloneJET PCR cloning kit (Fermentas, USA) before sequenced. The sequence of *BnaNAC103* was deposited in GenBank under the accession number KF738277.

2.3. Phylogenetic tree reconstruction and bioinformatic analysis

The predicted amino acid sequences of *NAC* of canola and other species were aligned using ClustalX1.83 and then phylogenetic tree was reconstructed using the maximum parsimony (MP) algorithm implemented in MEGA6.06 (release 6140226). Motif analysis of BnaNACs was determined by using Prosite program (http://prosite.expasy.org/prosite.html). The respective domains of NAC proteins were aligned using ClutsalX1.83 and illustrated by Boxshade (http://www.ch.embnet.org/software/BOX_form.html).

2.4. Quantitative RT-PCR (qRT-PCR) assay

Seven-day-old canola seedlings grown vertically on 1/2 MS plate medium supplemented with 1% sucrose in a growth chamber under a photoperiod of 14 h light /10 h dark with a relative humidity of 60% were transferred onto a variety of stress medium plates containing different chemical solutions, including 200 mM NaCl (MP Biomedicals), 50 μM abscisic acid (±-ABA, Sigma, USA), 10 μM methyl viologen (MV, Sigma), 12.5% PEG8000 (MP Biomedicals), 2 mM SA (Sigma) and 25 μM 1-aminocyclopropane-1-carboxylic acid (ACC, Sigma). Control, cold and heat treatments were set up by transferring seedlings onto normal 1/2 MS medium plates and placed in the same growth chamber, 4 °C, 37 °C, respectively. For fungal pathogen *S. sclerotiorum* and its virulence factor oxalic acid (OA) treatments, 18-d old canola seedlings were used as described previously [20]. Whole seedlings were collected at 1 h and 24 h post treatments, flash frozen in liquid nitrogen and stored at −80 °C. Total RNA samples were isolated and the first-strand cDNAs were synthesized from 2.5 μg of total RNA as described previously [20]. Three independent biological replicates of each sample were prepared at different times.

Quantitative reverse transcriptase PCR (qRT-PCR) was carried out using 10-fold diluted cDNA and SYBR Premix Ex Taq II (TaKaRa, Japan) on the CFX96 real-time PCR machine (Bio-Rad, USA). Primers used for qRT-PCR were designed using PrimerSelect program (DNASTAR Inc. USA), which targeted mainly at 3′UTR with an amplicon size of 75–200 bp (Table S1). The specificity and amplification efficiency of each pair of primers were examined through both BLASTn search in NCBI database and by running standard curves with melting curves. Three independent biological replicates and two technical replicates for each biological replicate were run and the significance was determined through *t*-test of SPSS statistic software ($p \leqslant 0.05$).

2.5. Subcellular localization and confocal microscopy

To examine the localization of BnaNAC103 *in planta*, the coding region was amplified using *Pfu* polymerase (Bioer, China) with primers listed in the Table S1. After purification, PCR products were restricted and then fused upstream of green fluorescent protein gene (*GFP*) in the pYJGFP vector. Agroinfiltration of *N. benthamiana* leaves was performed as described previously [20]. Two days later, leaf discs were observed of GFP under confocal microscope LSM510 (Zeiss, Germany).

2.6. Agroinfiltration and physiological assay

The full-length coding region or different fragments of *BnaNAC103* were amplified by high-fidelity *Pfu* polymerase using primers listed in Table S1. After digestion, the PCR products were inserted downstream of a double CaMV 35S promoter in the binary vector pYJGFP. Recombinant plasmids were transformed into *Agrobacterium tumefaciens* GV3101 and infiltrated into the lower epidermal side of five-week-old leaves of *N. benthamiana* plants. For each construct, 15 independent leaves of five independent plants (three leaves per plant) were used for each time-point tested. After that, infiltrated plants were kept under normal growth condition with the phenotype observed and recorded daily. To quantify the degree of cell death, electrolyte leakage was measured according to [21]. Distribution of hydrogen peroxide (H_2O_2) was detected by 3,3′-diaminobenzidine (DAB, MP Biomedicals, USA) staining according to the previously described protocol [21].

2.7. Lipid peroxidation assay and DNA ladder detection

For malondialdehyde (MDA) content determination, nearly 100 mg of leaf discs were homogenized in 4 mL of 0.1% trichloroacetic acid (TCA). After centrifuging the extract at 10,000×*g* for 15 min, the supernatant was collected and 1 mL of supernatant was mixed with 2 mL of 20% TCA and 2 mL of 0.5% thiobarbituric acid (TBA). Then the mixture was heated at 95 °C for 30 min in a fume hood and later cooled on ice. The absorbance of supernatant at 532 nm and 600 nm was read. *A*600 is the nonspecific absorbance and is subtracted from the values for *A*532.

DNA ladders were detected by extracting total DNA from homogenized leaf discs. Samples were incubated for 5 min at 65 °C in DNA extraction buffer (2%(w/v) CTAB, 1.4 M NaCl, 20 mM EDTA (pH8.0), 100 mM Tris–HCl (pH8.0), 0.2% β-mercaptoethanol) and mixed with an equal volume of chloroform/isoamyl alcohol (24:1, vol/vol). The mixture was centrifuged for 10 min at 12,000×*g*. The supernatant was precipitated with a 0.7 volume of isopropanol, washed with 70% ethanol, and dissolved in Tris-EDTA buffer containing RNase A (40 μg/ml). Eight micrograms of DNA samples from each genotype were separated on a 1.5% agarose gel in 1 × Tris-Acetate-EDTA, stained with ethidium bromide, and visualized under UV light in GelDoc imager system (Bio-Rad).

3. Results and discussion

3.1. Identification, cloning and sequence analysis of NAC103 gene from canola

In a recent systematic identification and study of NAC transcription factor gene family in canola, we identified and cloned over 40 *BnaNAC* (for *Brassica napus* NAC, to differentiate it from *Brassica nigra*) genes through mining the public expressed sequence tag (EST) database and RT-PCR, and *BnaNAC103* is one of them (unpublished data). To facilitate comparisons between species, we followed the established nomenclature of *Arabidopsis NACs* when naming the canola *NAC* genes. Sequence analysis of *BnaNAC103* indicated that its translated protein contains 346 amino acids, which bore domains and motifs that were typical of NAC proteins (Figs. 1A and S1). Further comparison of BnaNAC103 gene with *Arabidopsis* counterpart demonstrated that it showed an identity of 75.4%, with similarity 76.7% at the nucleotide level. At the amino acid level, the identity was 65.2% with the similarity 78.7%. Although *Arabidopsis NAC103* gene was recently demonstrated to be regulated by bZIP60 transcription factor and participate in unfolded protein response (UPR) [22], whether *ANAC103* or its orthologous *BnaNAC103* regulates cell death is unknown yet.

We further examined conserved domains and motifs within the BnaNAC103 protein. From this alignment (Fig. S1), it is clear that BnaNAC103 protein possessed a ca. 160 amino acid amino-terminal NAC domain, which is further divided into five highly conserved subdomains (A–E) [2,3]. These five subdomains are interspersed by short and variable sequences. In subdomain D, there is a short sequence containing conserved basic amino acids, which is the nuclear localization signal (NLS). To infer the phylogenetic relationships of canola NAC103 to other representative NAC proteins from crops and model plants, a maximum parsimony (MP) tree was reconstructed with amino acid sequences of NAC

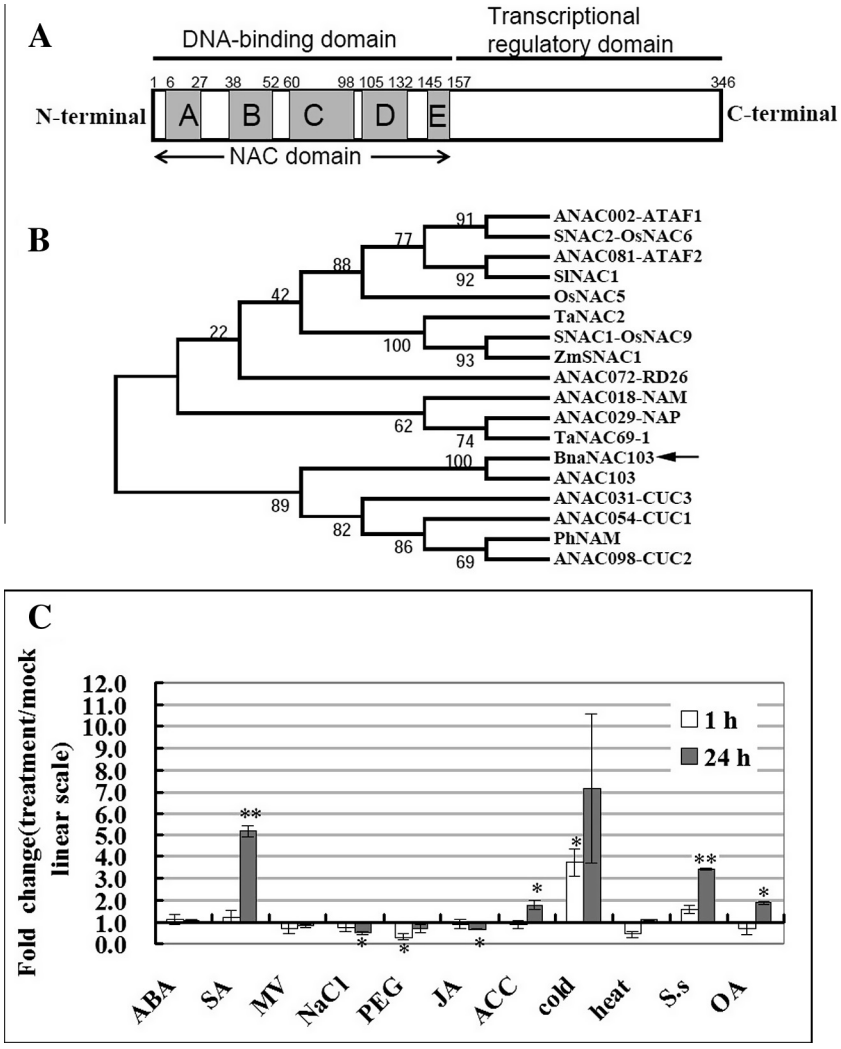

Fig. 1. Domain organization, phylogenetic analysis and expression profiling of canola *NAC103*. (A) Schematic representation of BnaNAC103 protein. A highly conserved NAC domain is located at the N-terminal which is further divided into five conserved subdomains (A–E; shown in gray boxes). This region holds DNA-binding (DB) ability. The C-terminal region is more diverged and serves as a potential transcriptional regulatory (TR) domain. (B) Phylogenetic relationship of BnaNAC103 with those from representative species. The tree was reconstructed with amino acid sequences through the maximum parsimony method implemented in MEGA6.06 with BnaNAC103 indicated by an arrow. The numbers on the nodes are percentages from a bootstrap analysis of 500 replicates. Os, *Oryza sativa*; Ta, *Triticum aestivum*; Sl, *Solanum lycopersicum*; Zm, *Zea may*; Ph, *Petunia hybrida*. ANAC029-NAP, At1g69490; TaNAC69-1, AY625682; ANAC018-NAM, At1g52880; ANAC002-ATAF1, At1g01720; SNAC2-OsNAC6, Os01g66120; ANAC081-ATAF2, At5g08790; SlNAC1, NP_001234482; OsNAC5, Os11g08210; SNAC1-OsNAC9, Os03g60080; ZmSNAC1, JQ217429; TaNAC2, AAU08786; ANAC072-R26, At4g27410; ANAC054-CUC1, At3g15170; PhNAM, X92205; ANAC098-CUC2, At5g53950; BnaNAC103, KF738277; ANAC103, At5g64060; ANAC031-CUC3, At1g76420. (C) qRT-PCR analysis of *BnaNAC103* in response to various treatments. Data is the mean of three biological replicates ±S.E. Asterisks denote significant differences (compared to 1) by Student *t*-test analysis ($p \leqslant 0.05$).

proteins (Fig. 1B). It can be seen that the BnaNAC103 was clustered with ANAC103, which promoted us to further investigate its role in plants.

3.2. BnaNAC103 responded to multiple to stress treatments

To elucidate of the functions of *BnaNAC103* in the context of abiotic and biotic stresses, the expression pattern of it was studied through quantitative real-time PCR (qRT-PCR). We subjected canola seedlings to moderate stress treatments and measured the response at two time-points to better monitor the transcript changes of *BnaNAC103*. As a result, we found that expression of *BnaNAC103* was significantly up-regulated by SA, ACC, cold, OA and *S. sclerotiorum* treatments at 24, 24, 1, 24, 24 h time-point, respectively (Fig. 1C). In contrast, salt, dehydration and JA treatments significantly down-regulated *BnaNAC103* expression at 24, 1, and 24 h time-point, respectively. The other stress or hormone treatments did not significantly change the expression of *BnaNAC103* transcript level. Taken together, these data indicate that BnaNAC103 gene participates in transduction of multiple stresses, and therefore may play a role in the cross-talk of multiple stresses, including both abiotic and biotic stresses.

3.3. BnaNAC103 localizes in nuclei

To investigate the subcellular localization of canola NAC103 protein, coding region of it was fused upstream of GFP and expressed in leaves of *N. benthamiana*. We found that in leaf cells expressing fusion proteins of BnaNAC103-GFP, the GFP signals were present in the nucleus (Fig. 2A), which is in agreement with its role of transcription factor. As a control, we also examined the subcellular localization of the GFP protein in leaf cells, and GFP signals were present obviously both in cytosol and nuclei (Fig. 2B).

3.4. Expression of BnaNAC103 induce ROS accumulation and cell death

In the aforementioned GFP subcellular assay in *N. benthamiana*, we observed that expression of *BnaNAC103* in leaves led to hypersensitive response (HR) symptoms within 2 d after infiltration. HR is a common feature of plant immune responses and a type of programmed cell death (PCD). Activation of cell death is one of the aspects of plant defense responses where ROS play a crucial role [23]. Hence, we performed DAB staining of the agroinfiltrated leaves and identified evident staining, indicative of ROS accumulation (data not shown). To further explore the role of *BnaNAC103* in cell death, the coding region, as well as the N-terminal NAC domain and C-terminal regulatory domain sequence of *BnaNAC103* were cloned into a binary vector individually under the driving of CaMV 35S promoter (Fig. 3B). Then we performed agroinfiltration of them into *N. benthamiana* leaves and evaluated the phenotype. Interestingly, expression of full-length *BnaNAC103* indeed caused pathogen-independent cell death compared with GFP vector control, beginning 48 h post-infiltration (hpi) and proceeded as expected (Fig. 3A). We observed that the water-soaking symptom appeared as early as 48 hpi in *BnaNAC103*-expressing leaves compared to that of *GFP* control. To explore the role of ROS during cell death, we performed DAB staining and the results showed there was strong staining in sites expressing *BnaNAC103* beginning at 48 hpi and stronger stainings were observed at 96 and 144 hpi, but not in sites expressing *GFP* control or truncated *BnaNAC103* (Fig. 3A). Moreover, we examined the electrolyte leakage of leaf discs taken from leaves expressing *BnaNAC103* or its truncated versions and the *GFP* alone. The results showed that a significant increase in ion leakage was visible 2 d after agroinfiltration of *BnaNAC103* in contrast to that of leaves expressing either the N-terminal NAC domain, C-terminal regulatory domain or the *GFP* alone (Fig. 3C), which further demonstrates that hypersensitive response-like cell death associated with hydrogen peroxide production was triggered by high expression of full-length *BnaNAC103*.

Lipid peroxidation is a process caused by accumulation of intracellular ROS, which remove electrons from the lipids in the cell membranes thereby damaging the cells. During the process of lipid peroxidation, the malondialdehyde (MDA) is formed by the decomposition of polyunsaturated fatty acids. To further examine the role of *BnaNAC103* in cell death, we measured the content of MDA in

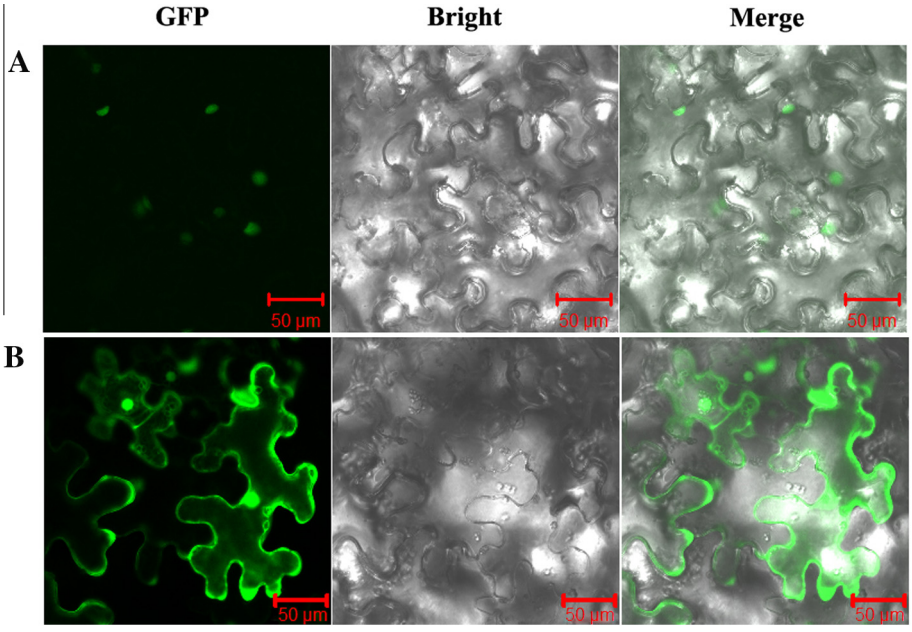

Fig. 2. Subcellular localization of BnaNAC103 protein in *N. benthamiana* cells using green fluorescence protein (GFP). Panels A–B represented BnaNAC103 and GFP alone, respectively. In each panel, the extreme left is GFP fluorescence, the middle bright field and the right an overlay of the two images as indicated on the top of the picture. Bar = 50 μm.

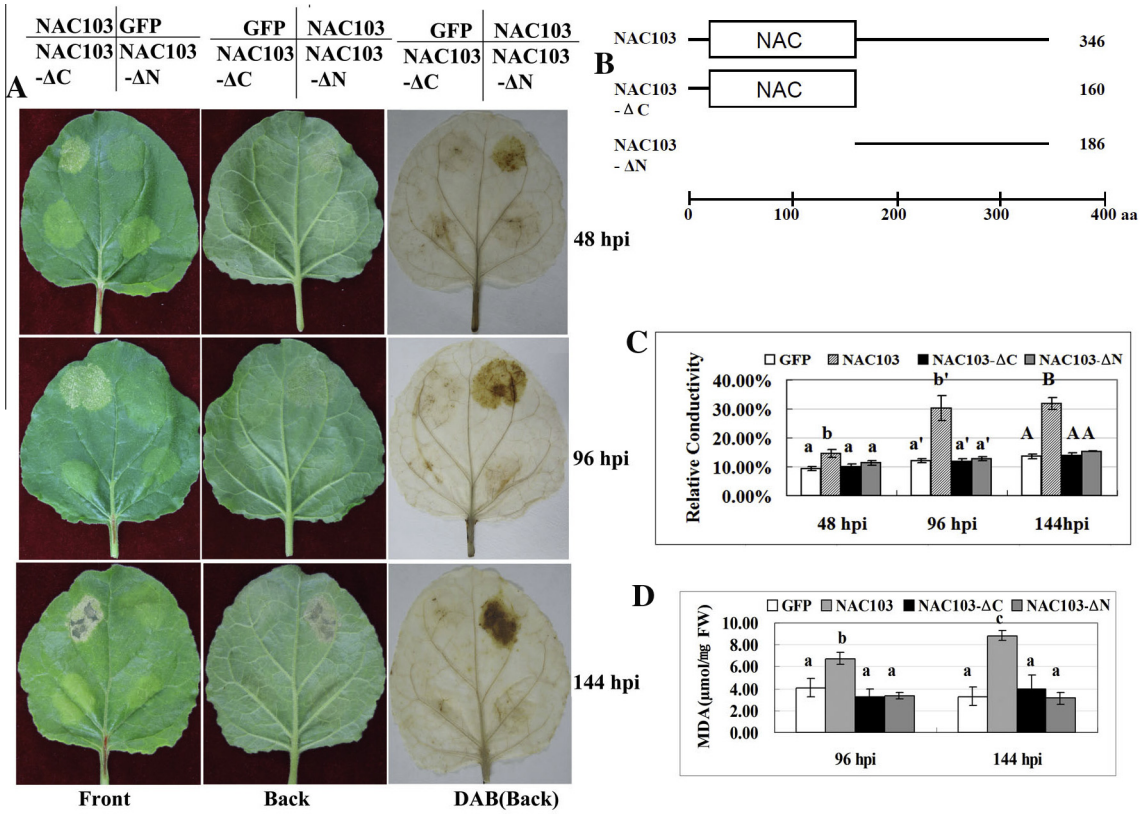

Fig. 3. Overexpression of *BnaNAC103* induced pathogen-independent cell death in *N. benthamiana* leaves. (A) Symptoms of *N. benthamiana* leaf areas expressing *BnaNAC103* gene 48, 96 and 144 h post-infiltration (hpi). The left, middle and right panels represent the front, back sides and DAB staining, respectively. Leaves were infiltrated with agrobacteria carrying individual plasmids. (B) Schematic representation of domain structures of BnaNAC103 protein. The DNA-binding NAC domain (open boxes) is located at the N-terminal region and the transcriptional regulatory domain is located at the C-terminal region. The numbers of total amino acid residues of full-length protein or truncated fragments are shown at the right side of each structure. Numbers at the bottom row indicate residue positions. (C) Measurement of electrolyte leakage in leaf discs expressing *BnaNAC103*, its truncated versions and *GFP* at 48, 96 and 144 hpi. (D) Quantification of MDA contents in leaf discs expressing *BnaNAC103*, its truncated versions and *GFP* at 96 and 144 hpi. Values represent the means of three independent assays for each time-point ±S.E. Identical and different letters represent non- and significant differences ($p \leqslant 0.05$).

leaves. The data showed that in leaf discs expressing the full-length *BnaNAC103*, the MDA concentration was significantly higher than that in either the *GFP* control, the truncated N- or C-terminal fragment of *BnaNAC103*, at both 96 and 144 hpi (Fig. 3D). This further indicates that the aforementioned cell death is associated with ROS-induced cell membrane breakage.

DNA fragmentation occurs in naturally senescent leaves or during programmed cell death. Therefore, we further examined the change in nuclear DNA in leaf discs expressing *BnaNAC103* gene, its truncated versions or *GFP* gene alone. It can be seen that nuclear DNA extracted from leaf discs expressing full-length *BnaNAC103* showed evident degradation (became a smear) in the agarose gel (Fig. 4, lanes 2 and 4), whereas those from the *GFP* gene alone, the N-terminal or C-terminal *BnaNAC103* did not (Fig. 4, lanes 1, 3, 5 and 6). Overall, this assay supports that there was internucleosomal degradation of genomic DNA in leaf cells undergoing cell death.

In the present study, we described the characterization of canola *NAC103* gene, which responded to multiple stress and hormone treatments as well as caused ROS accumulation and cell death in *N. benthamiana* leaves. These data suggest that NAC103 TF may participate in the cross-talk of different signaling pathways under abiotic and biotic stress conditions. More importantly, we identified that *BnaNAC103* as novel regulator of ROS level and cell death in leaves of *N. benthamiana* (Fig. 3). In addition, quantification of MDA content supports that *BnaNAC103* expression indeed induced lipid peroxidation as a result of ROS accumulation. A

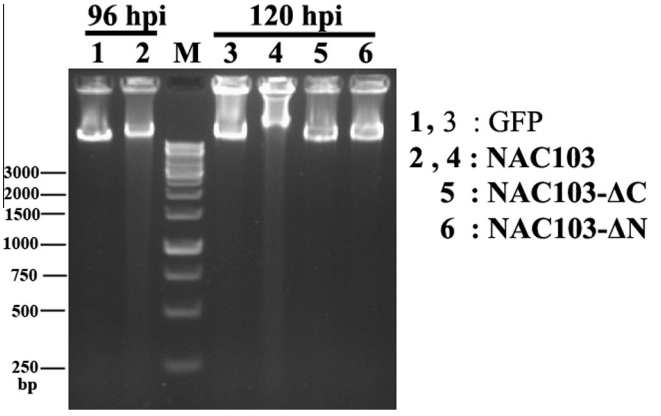

Fig. 4. DNA ladder assay of cell death induced by expression of *BnaNAC103* gene. Genomic DNA was extracted from leaf discs expressing full-length, N- or C-terminal *BnaNAC103* gene or GFP gene (control) at specified time points. After electrophoresis, DNA was visualized with EB staining.

bioinformatic analysis and literature search indicated that it is a novel cell death-mediating member of NAC TF family in plants. How *BnaNAC103* elicited ROS accumulation and cell death is still an outstanding question for further research. Besides, how *BnaNAC103* modulates plant immunity against the devastating necrotrophic fungal pathogen *S. sclerotiorum* is another interesting question.

Acknowledgments

This work was supported by the Young Talent funding provided by the Institute of Soil and Water Conservation, Norwest A&F University, and the National Natural Science Foundation of China [31270293 to Y-Q.J]. We would like to acknowledge prof. Michael K. Deyholos (University of British Columbia, Canada) for providing the canola seeds and, prof. Lili Huang for providing *S. scleortiorum* strain of SX09-904.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.057.

References

[1] A.N. Olsen, H.A. Ernst, L.L. Leggio, K. Skriver, NAC transcription factors: structurally distinct, functionally diverse, Trends Plant Sci. 10 (2005) 79–87.
[2] S. Puranik, P.P. Sahu, P.S. Srivastava, M. Prasad, NAC proteins: regulation and role in stress tolerance, Trends Plant Sci. 17 (2012) 369–381.
[3] M. Nuruzzaman, R. Manimekalai, A.M. Sharoni, K. Satoh, H. Kondoh, H. Ooka, S. Kikuchi, Genome-wide analysis of NAC transcription factor family in rice, Gene 465 (2010) 30–44.
[4] X.J. He, R.L. Mu, W.H. Cao, Z.G. Zhang, J.S. Zhang, S.Y. Chen, AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development, Plant J. 44 (2005) 903–916.
[5] Y. Guo, S. Gan, AtNAP, a NAC family transcription factor, has an important role in leaf senescence, Plant J. 46 (2006) 601–612.
[6] T. Kjaersgaard, M.K. Jensen, M.W. Christiansen, P. Gregersen, B.B. Kragelund, K. Skriver, Senescence-associated barley NAC (NAM, ATAF1,2, CUC) transcription factor interacts with radical-induced cell death 1 through a disordered regulatory domain, J. Biol. Chem. 286 (2011) 35418–35429.
[7] A. Wu, A.D. Allu, P. Garapati, H. Siddiqui, H. Dortay, M.I. Zanor, M.A. Asensi-Fabado, S. Munne-Bosch, C. Antonio, T. Tohge, A.R. Fernie, K. Kaufmann, G.P. Xue, B. Mueller-Roeber, S. Balazadeh, JUNGBRUNNEN1, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in *Arabidopsis*, Plant Cell 24 (2012) 482–506.
[8] H. Hu, M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang, L. Xiong, Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 12987–12992.
[9] J.S. Jeong, Y.S. Kim, M.C. Redillas, G. Jang, H. Jung, S.W. Bang, Y.D. Choi, S.H. Ha, C. Reuzeau, J.K. Kim, OsNAC5 overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field, Plant Biotechnol. J. 11 (2012) 101–114.
[10] C. Delessert, K. Kazan, I.W. Wilson, D. Van Der Straeten, J. Manners, E.S. Dennis, R. Dolferus, The transcription factor ATAF2 represses the expression of pathogenesis-related genes in *Arabidopsis*, Plant J. 43 (2005) 745–757.
[11] X. Wang, B.M. Basnayake, H. Zhang, G. Li, W. Li, N. Virk, T. Mengiste, F. Song, The *Arabidopsis* ATAF1, a NAC transcription factor, is a negative regulator of defense responses against necrotrophic fungal and bacterial pathogens, Mol. Plant Microbe Interact. 22 (2009) 1227–1238.
[12] Q. Bu, H. Jiang, C.B. Li, Q. Zhai, J. Zhang, X. Wu, J. Sun, Q. Xie, C. Li, Role of the *Arabidopsis thaliana* NAC transcription factors ANAC019 and ANAC055 in regulating jasmonic acid-signaled defense responses, Cell Res. 18 (2008) 756–767.
[13] J.A. Faria, P.A. Reis, M.T. Reis, G.L. Rosado, G.L. Pinheiro, G.C. Mendes, E.P. Fontes, The NAC domain-containing protein, GmNAC6, is a downstream component of the ER stress- and osmotic stress-induced NRP-mediated cell-death signaling pathway, BMC Plant Biol. 11 (2011) 129.
[14] T. Kaneda, Y. Taga, R. Takai, M. Iwano, H. Matsui, S. Takayama, A. Isogai, F.S. Che, The transcription factor OsNAC4 is a key positive regulator of plant hypersensitive cell death, EMBO J. 28 (2009) 926–936.
[15] G.C. Mendes, P.A. Reis, I.P. Calil, H.H. Carvalho, F.J. Aragao, E.P. Fontes, GmNAC30 and GmNAC81 integrate the endoplasmic reticulum stress- and osmotic stress-induced cell death responses through a vacuolar processing enzyme, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 19627–19632.
[16] Z.T. Yang, M.J. Wang, L. Sun, S.J. Lu, D.L. Bi, L. Sun, Z.T. Song, S.S. Zhang, S.F. Zhou, J.X. Liu, The membrane-associated transcription factor NAC089 controls ER-stress-induced programmed cell death in plants, PLoS Genet. 10 (2014) e1004243.
[17] D. Hegedus, M. Yu, D. Baldwin, M. Gruber, A. Sharpe, I. Parkin, S. Whitwill, D. Lydiate, Molecular characterization of *Brassica napus* NAC domain transcriptional activators induced in response to biotic and abiotic stress, Plant Mol. Biol. 53 (2003) 383–397.
[18] L. Ying, H. Chen, W. Cai, BnNAC485 is involved in abiotic stress responses and flowering time in *Brassica napus*, Plant Physiol. Biochem. 79 (2014) 77–87.
[19] H. Zhong, Q.Q. Guo, L. Chen, F. Ren, Q.Q. Wang, Y. Zheng, X.B. Li, Two *Brassica napus* genes encoding NAC transcription factors are involved in response to high-salinity stress, Plant Cell Rep. 31 (2012) 1991–2003.
[20] W. Liang, B. Yang, B.J. Yu, Z. Zhou, C. Li, M. Jia, Y. Sun, Y. Zhang, F. Wu, H. Zhang, B. Wang, M.K. Deyholos, Y.Q. Jiang, Identification and analysis of MKK and MPK gene families in canola (*Brassica napus* L.), BMC Genom. 14 (2013) 392.
[21] Y. Sun, C. Wang, B. Yang, F. Wu, X. Hao, W. Liang, F. Niu, J. Yan, H. Zhang, B. Wang, M. Deyholos, Y.-Q. Jiang, Identification and functional analysis of mitogen-activated protein kinase kinase kinase (MAPKKK) genes in canola (*Brassica napus* L.), J. Exp. Bot. 65 (2014) 2171–2188.
[22] L. Sun, Z.T. Yang, Z.T. Song, M.J. Wang, L. Sun, S.J. Lu, J.X. Liu, The plant-specific transcription factor gene NAC103 is induced by bZIP60 through a new cis-regulatory element to modulate the unfolded protein response in *Arabidopsis*, Plant J. 76 (2013) 274–286.
[23] N.S. Coll, P. Epple, J.L. Dangl, Programmed cell death in the plant immune system, Cell Death Differ. 18 (2011) 1247–1256.