PLOS ONE

RESEARCH ARTICLE

DkNAC7, a novel high-CO_2/hypoxia-induced NAC transcription factor, regulates persimmon fruit de-astringency

Rong Jin[1,2☯], Qing-gang Zhu[1☯], Xin-yue Shen[1], Miao-miao Wang[1], Wajeeha Jamil[1], Donald Grierson[1,3], Xue-ren Yin[1,4]*, Kun-song Chen[1,4]

1 Zhejiang Provincial Key Laboratory of Horticultural Plant Integrative Biology, Zhejiang University, Zijingang Campus, Hangzhou, PR China, **2** Agricultural Experiment Station, Zhejiang University, Zijingang Campus, Hangzhou, PR China, **3** Plant & Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom, **4** The State Agriculture Ministry Laboratory of Horticultural Plant Growth, Development and Quality Improvement, Zhejiang University, Zijingang Campus, Hangzhou, PR China

☯ These authors contributed equally to this work.
* xuerenyin@zju.edu.cn

Citation: Jin R, Zhu Q-g, Shen X-y, Wang M-m, Jamil W, Grierson D, et al. (2018) *DkNAC7*, a novel high-CO_2/hypoxia-induced NAC transcription factor, regulates persimmon fruit de-astringency. PLoS ONE 13(3): e0194326. https://doi.org/10.1371/journal.pone.0194326

Editor: Jin-Song Zhang, Institute of Genetics and Developmental Biology Chinese Academy of Sciences, CHINA

Received: January 24, 2018

Accepted: February 28, 2018

Published: March 14, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This research was supported by the National Key Research and Development Program (2016YFD0400102 to KC), the National Natural Science Foundation of China (31722042; 31672204 to XY), the Natural Science Foundation of Zhejiang Province, China (LR16C150001 to XY), the Fundamental Research Funds for the Central

Abstract

Artificial high-CO_2 atmosphere (AHCA, 95% CO_2 and 1% O_2) has been widely applied as a postharvest de-astringency treatment for persimmon fruit. AHCA increases expression of transcription factors, including ethylene response factors (*DkERF*), that target de-astringency genes. Here, the promoter of *DkERF9*, a previously characterized AHCA-inducible and de-astringency regulator, was utilized to screen a cDNA library by yeast one hybrid assay. A novel NAC transcription factor, named *DkNAC7*, was identified. Dual-luciferase assay indicated that *DkNAC7* could not only trans-activate the promoter of *DkERF9*, but also activated the previously identified deastringency-related gene *DkPDC2*. Real-time PCR analysis showed that *DkNAC7* was up-regulated by AHCA treatment, in concert with the removal of astringency from persimmon fruit and subcellular localization showed *DkNAC7* was located in the nucleus. Thus, these results indicate that *DkNAC7* is a putative transcriptional activator involved in regulating persimmon fruit deastringency by trans-activition on both *DkERF9* and *DkPDC2*, which encodes pyruvate decarboxylase.

Introduction

Persimmon (*Diospyros kaki* L.) is a worldwide crop, which originated in Southeast Asia. Persimmon fruit can be divided into astringent and non-astringent types, but most native cultivars in China are of the astringent types [1]. These astringent persimmon fruit have the unique feature of accumulating abundant amounts of condensed tannins (CT) [2]. Astringent persimmon accumulates abundant CTs in fruit even at maturity and soluble CTs (SCT) cause astringency [1,3], which severely affects the persimmon industry and consumer acceptance.

A range of artificial technologies have been developed to remove astringency, including high-CO_2, ethylene and ethanol [4–8]. Among these, high CO_2 (usually > 90%) is the most

Universities, and the 111 Project (B17039 to XY). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: AbA, aureobasidin A; AD, pGADT7; ADH, alcohol dehydrogenase; cv, Cultivar; ERF, ethylene response factor; PDC, pyruvate decarboxylase; SCT, soluble condensed tannins.

widely used treatment, in which the O_2 level is reduced to 1%. In plants, hypoxia usually causes the accumulation of products from anaerobic metabolism [9], and these products (especially acetaldehyde) effectively reduce the SCTs and accelerate deastringency in persimmon fruit [5,7,10]. The activities of alcohol dehydrogenase (ADH, EC 1.1.1.1) and pyruvate decarboxylase (PDC, EC 4.1.1.1) and also their encoding genes (*DkADH1*, *DkPDC1*, *DkPDC2* and *DkPDC3*) have been shown to increase in amount during deastringency [7,11]. Transient over-expression of *DkPDC2* led to a lower level of SCTs in persimmon leaves [7], suggesting that *DkPDC2* is a key gene for the de-astringency program of persimmon fruit. These results confirmed that CO_2 driven astringency removal involves hypoxia-triggered acetaldehyde metabolism.

In the model plant *Arabidopsis*, a few ethylene response factors (*ERFs*) have been reported to be involved in the hypoxia response, including *HRE1*, *HRE2*, *RAP2.2* and *RAP2.12*. These *ERF* genes could transcriptionally regulate *ADH* and *PDC*, and result in hypoxia tolerance [12–15]. As stated above, persimmon fruit deastringency by high CO_2 treatment is considered to operate mainly through the hypoxia fermentation pathway. In persimmon, four *DkERF* were previously reported to be involved in persimmon fruit deastringency, including *DkERF9/10/19/22* [7,8]. Of these, *DkERF9* was characterized as an activator of the promoter of *DkPDC2*, a key gene for deastringency [7]. Due to the low oxygen in high CO_2 treatment, these *DkERFs* were previously termed as hypoxia responsive [8]. But, high CO_2 treatment is an atypical anoxia environment, with effects of both high CO_2 and low O_2, thus it could be induced either a high-CO_2 or hypoxia response.

Apart from *ERFs*, some other transcription factors were reported as high-CO_2/hypoxia responsive in persimmon fruit, such as *DkMYB6* [16] and *DkTGA1* [17]. *NAC* genes are the main transcription factors reported to be involved in the plant hypoxia response. In *Arabidopsis*, more than 100 *NAC* genes have been characterized [18] that share highly conserved consensus in the N-terminal region of a Petunia gene (NAM), *Arabidopsis* ATAF1/2 and CUC2 proteins [19]. Among these genes, hypoxia-responsive *NAC* genes have rarely been reported, and the results from studies on *ANAC102* also indicate that additional *NAC* genes might exist for the hypoxia response, as *ANAC102* knockout lines did not show altered *ADH* gene transcription in *Arabidopsis* [9]. In persimmon, six *NAC* genes have been characterized, among which *DkNAC1/3/5/6* were high-CO_2/hypoxia responsive, however their regulatory roles in persimmon deastringency remain unclear [20]. Thus, the potential role of *NAC* genes in regulating persimmon deastringency still lacks experimental evidence.

Here, a novel NAC transcription factor (*DkNAC7*) was obtained as a result of yeast one hybrid screening by using the promoter of *DkERF9* as bait and the regulatory role of *DkNAC7* in persimmon de-astringency was investigated using yeast one-hybrid assay, dual-luciferase, real-time PCR and subcellular localization.

Materials and methods

Plant materials and treatment

'Mopanshi' (astringent cultivar) persimmon (*D. kaki*) fruit were obtained from a commercial orchard at Fangshan (Beijing, China) in 2012. Fruit without disease or mechanical wounding were selected and treated with artificial high-CO_2 atmosphere (AHCA, 95% CO_2 and 1% O_2) or air in air-tight containers for 1 d. The physiological data and sampling information were described in Wang et al. [21].

RNA extraction and cDNA synthesis

Total RNAs were extracted from frozen fruit flesh (2.0 g) and the cDNA synthesis carried out according to the method used previously [6].

Gene isolation and sequence analysis

A NAC transcription factor was obtained based on the Matchmaker Gold Yeast One-hybrid Library Screening System (Clontech, USA), using the promoter of deastringency-related *DkERF9* [7,16] as the bait DNA sequence. The full-length *NAC* gene was isolated with a SMART RACE cDNA Amplification Kit (Clontech). The sequences of primers are described in Table 1. For phylogenetic analysis, the NAC genes in persimmon and methods were as described in Min *et al* [20]

Yeast one-hybrid assay (Y1H)

According to library screening results, the protein-DNA interaction was verified with DkNAC7-AD and *DkERF9* promoter, individually. Meanwhile, interaction between DkNAC7 and *DkPDC2* promoter was also investigated by Y1H. The promoter of *DkERF9* was constructed into pAbAi vector (primers are listed in Table 1). The *DkPDC2*-pAbAi was constructed by Min *et al.* [8]. The full-length sequence of transcription factor *DkNAC7* was subcloned into pGADT7 AD vector (AD) (primers are listed in Table 1).

Auto-activation and the interaction analysis were conducted according to the manufacturer's protocol.

Dual luciferase assay

Dual-luciferase assay was used as a rapid and efficient method to detect *in vivo* trans-activation or trans-repression effects of transcription factors [22]. Full-length *DkNAC7* was inserted into pGreen II 0029 62-SK vector (SK), using the primers listed in Table 1. The dual luciferase assay was carried out with *Nicotiana benthamiana* leaves, using the protocol described by Min *et al.* [7]. Three independent experiments (with minimum five replicates) were performed to verify the results.

Table 1. Sequences of the primers used for RACE, full-length amplification, real time PCR and vector construction.

Gene	Methods used	Primers (5'-3')
DkNAC7	3'RACE (Primary PCR)	CAAGCCCTTCCGATTCGATGCCAT
DkNAC7	3'RACE (Secondary PCR)	GGAAGACAACAGGAAAGGACAGGCC
DkNAC7	5'RACE (Primary PCR)	CTCGTCATCCTCCCATTCCTCCTCAAC
DkNAC7	5'RACE (Secondary PCR)	CTCGTGCATCACCCAGTTGGTCCTCT
DkNAC7	Full-length clone (FP)	CATCGGCGGTGACCAAAACGG
DkNAC7	Full-length clone (RP)	CACAAAGTCCCTAGATCTCAGA
DkNAC7	Y1H constructs (FP)	CGCGAATTCATGGGCCTCGATCCATCGTC
DkNAC7	Y1H constructs (RP)	GATGGATCCCTACCTCGATGCATTTCCCG
DkNAC7	SK vector construction (FP)	CGCGCGGCCGCATGGGCCTCGATCCATCGTC
DkNAC7	SK vector construction (RP)	GATGGATCCCTACCTCGATGCATTTCCCG
DkNAC7	Q-PCR (FP)	TGAGTTTCAAAATTGGGAGT
DkNAC7	Q-PCR (RP)	CCCTAGATCTCAGATGGTGA
DkNAC7	GFP vector construction (FP)	CGCGGTACCATGGGCCTCGATCCATCGTC
DkNAC7	GFP vector construction (RP)	CATGTCGACCCTCGATGCATTTCCCG
DkERF9	pAbAi vector construction (FP)	CGCGAGCTCAAATAATTTAATTAAAGATA
DkERF9	pAbAi vector construction (RP)	CGCGTCGACATACACAGGAAAACAGGATT

Note: underlined sequences show cutting sites for restriction enzymes

https://doi.org/10.1371/journal.pone.0194326.t001

Real-time PCR analysis

For real-time PCR, gene specific oligonucleotide primers were designed and are shown in Table 1. The quality and specificity of primers were checked by melting curve and PCR products resequencing. The *DkACT* was chosen as a housekeeping gene to monitor the abundance of mRNA [7].

Real-time PCR reactions were carried out on a CFX96 instrument (Bio-Rad). The PCR protocols were according to our previous reports, using Ssofast EvaGreen Supermix Kit (Bio-Rad) [6]. The relative expression of this *NAC* gene was calibrated with values for day 0 fruit set as 1.

Subcellular localization analysis

35S-*DkNAC7*-GFP was transiently expressed in tobacco leaves by *Agrobacterium*-mediated infiltration (GV3101) according to previous reports [23,24]. The green fluorescent protein (GFP) fluorescence in tobacco leaves was imaged 2 d after infiltration using a Zeiss LSM710NLO confocal laser scanning microscope. Primers used for GFP construction are described in Table 1.

Statistical analysis

Least Significant Difference (LSD) test was used to compare the statistical significance differences among treatments by using DPS 7.05 or Student's *t*-test. The figures were drawn with Origin 8.0.

Results and discussion

Y1H based library screening discovered a novel *NAC* gene, which targeted the *DkERF9* promoter

In our previous reports, *DkERF9* transcription factor was shown to be involved in persimmon de-astringency via regulation of the *DkPDC2* promoter [7]. In order to obtain further information about the transcriptional regulatory mechanism controlling persimmon fruit deastringency, Y1H based screening was employed to screening the potential interacting transcription factors, using the *DkERF9* promoter as bait. A total of 150 PCR products were obtained, among which only one NAC transcription factor gene was characterized. Individual verifications with Y1H indicated that the NAC transcription factor could bind to *DkERF9* promoter (Fig 1). As

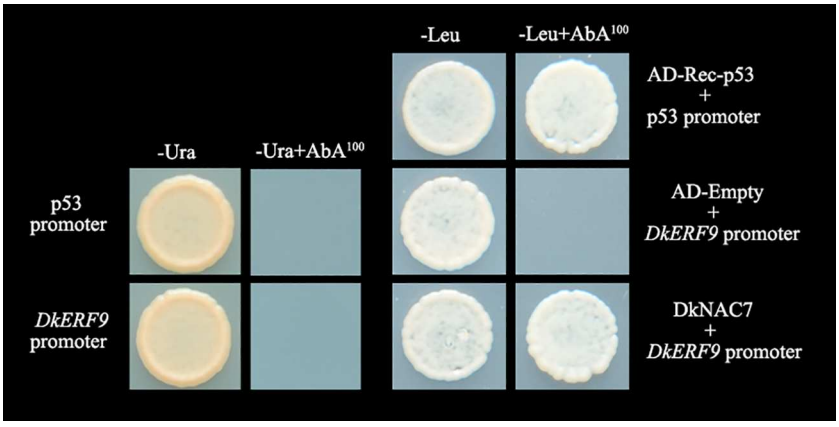

Fig 1. Protein-DNA interaction between DkNAC7 and the promoter of *DkERF9* using yeast one hybrid analysis. Interaction was confirmed on SD medium lacking Leu in the presence of aureobasidin A (-Leu+AbA^{100}). AD-Rec-p53 and p53-AbAi were used as a positive control; AD-empty and pDkERF9-AbAi were used as a negative control.

https://doi.org/10.1371/journal.pone.0194326.g001

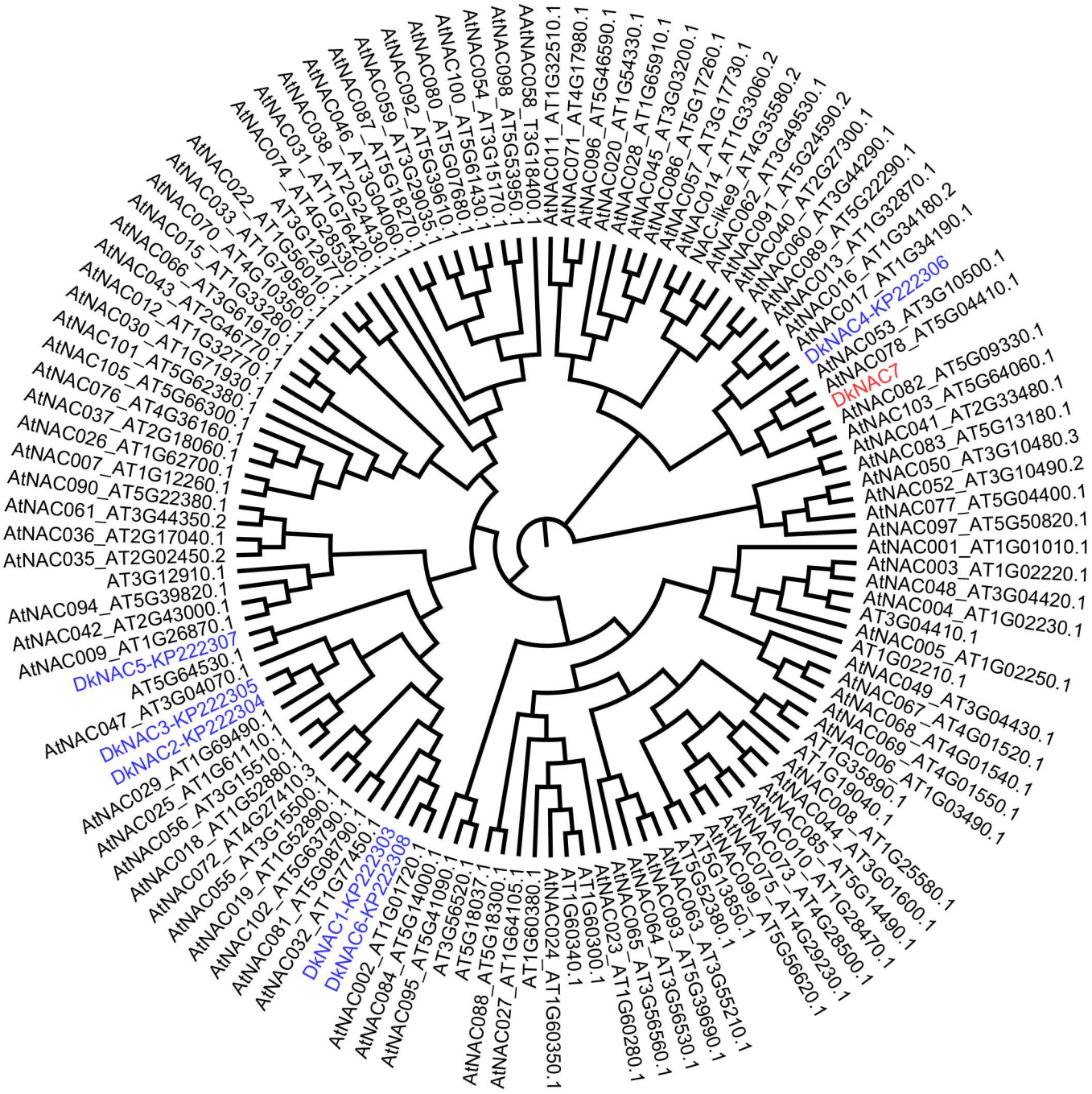

Fig 2. Phylogenetic analysis of DkNAC7 and NAC members from persimmon and *Arabidopsis*. Persimmon *DkNAC* is highlighted in red (*DkNAC7*, newly isolated) and blue (previously reported). The amino acid sequences of NAC transcription factors were obtained from the *Arabidopsis* Information Resource or National Center for Biotechnology Information, and their accession numbers are included in the diagram. The phylogenetic tree was constructed with Figtree (v 1.3.1).

https://doi.org/10.1371/journal.pone.0194326.g002

six *DkNAC* genes were reported previously in persimmon fruit [20], this NAC transcription factor was named as *DkNAC7* (GenBank no.MG792350) (Fig 1).

Phylogenetic tree analysis indicated that *DkNAC7* was close to *DkNAC4*, but not the other five previously reported *DkNAC* genes (Fig 2) [20]. Compared to *Arabidopsis* NAC transcription

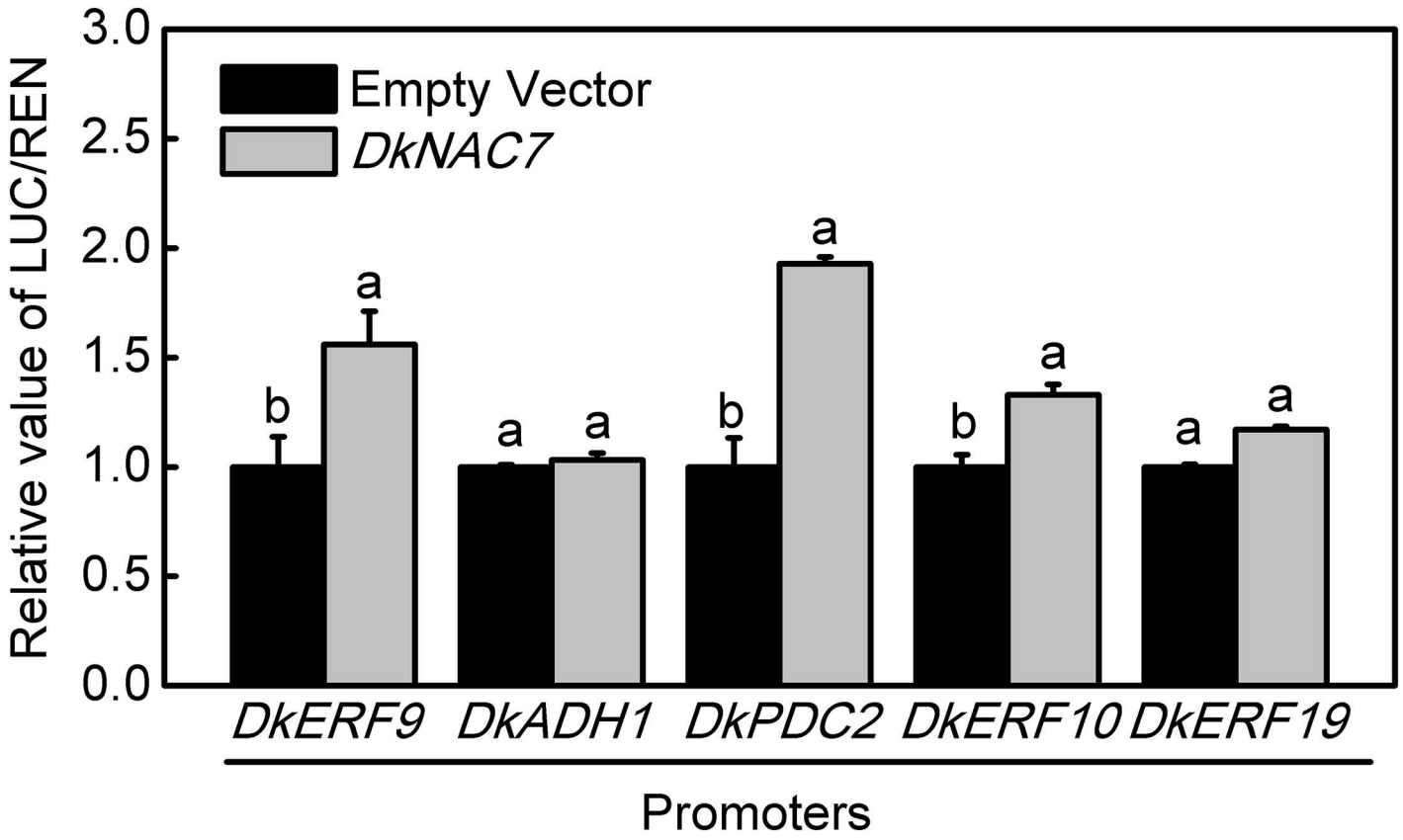

Fig 3. Regulatory effects of DkNAC7 on the promoters of deastringency-related genes (*DkERF9/10/19*, *DkADH1*, *DkPDC2*) using the dual-luciferase assay. The ratio of LUC/REN of the empty vector (SK) plus promoter was used as calibrator (set as 1). Error bars indicate SEs from five replicates. Different letters above the columns indicate significant differences ($P<0.05$).

https://doi.org/10.1371/journal.pone.0194326.g003

factors, *DkNAC7* was close to *AtNAC078*, which was reported to regulate flavonoid biosynthesis under high light in *Arabidopsis* [25], while it was not clustered with *ANAC102*, which was shown to be induced by low oxygen (0.1%) in *Arabidopsis* [9].

In vivo regulatory roles of DkNAC7 on deastringency related genes (*DkERF*, *DkADH1* and *DkPDC2*)

Further investigations on the possible transcriptional regulatory linkage between *DkNAC7* and deastringency related genes were carried out. Three previously studied *DkERF* genes (*DkERF9/10/19*) and two structural genes (*DkADH1* and *DkPDC2*) were selected for test. Dual luciferase assay indicated that *DkNAC7* could significantly trans-activate the promoters of *DkERF9* and *DkPDC2* with 1.55 and 1.92-fold enhancement, respectively (Fig 3). The effect of DkNAC7 on the *DkERF10* promoter also reached statistical significance, but the response was very limited (about 1.32-fold) and the *DkNAC7* gene had no significant effects on the promoters of *DkADH1* and *DkERF19* (Fig 3).

In persimmon, twenty-two ethylene response factors (*DkERFs*) were differently expressed in response to high CO_2 treatment. Of these 22 genes, only four *ERFs* (*DkERF9/10/19/22*) were fund to be involved in persimmon de-astringency [7,8], via the interaction with promoters of de-astringent related target genes (e.g. *DkADH1*, *DkPDC2* and *DkPDC3*). Furthermore, a MYB transcription factor (*DkMYB6*) and a bZIP transcription factor (*DkTGA1*)

were also characterized as regulators of persimmon fruit astringency removal, respectively [16,17]. Thus, these finding with *DkNAC7* unveiled a new transcription factor that participates in regulation of persimmon fruit deastringency. Furthermore, *DkNAC7* is not closely related to the low oxygen-induced *ANAC102* gene [9] from phylogenetic result (Fig 2) which suggests that more than one various type NAC transcription factor may contribute to the hypoxia response.

Cascade regulations of *DkNAC7-DkERF9-DkPDC2*

Comparing the effects *DkNAC7* and the previously characterized TFs on the *DkPDC2* promoter, *DkNAC7* was shown to have only a relatively limited action, which was only slightly stronger than *DkTGA1* [17]. Y1H analyses indicated that *DkNAC7* cannot bind to and is therefore an indirect regulator for hypoxia responsive *DkPDC2* (S1 Fig). As the present results indicated that interaction between DkNAC7 and *DkERF9* promoter (Fig 1) and our previous study indicated that DkERF9 could physically bind to *DkPDC2* promoter [17]. Thus, it could be proposed that a regulatory cascade involving *DkNAC7-DkERF9-DkPDC2* contributes to persimmon fruit deastringency. The regulatory roles of NAC transcription factors in hierarchical interactions with *ERFs* have also been reported in other fruits, for instance *MdNAC029/MdNAP*, an apple NAC gene, was reported to directly repressed the expression of two *ERF* genes (*MdCBF1* and *MdCBF4*) by binding to their promoters, thus negatively regulating cold tolerance via the *CBF*-dependent pathway [26]. These finding from persimmon not only partial explain the transcriptional regulations during deastringency, but also provided a new example of NAC-ERF regulation. Moreover, since the NAC-ERF cascade contributes to persimmon deastringency (high-CO_2/hypoxia response) and apple cold tolerance, this raises the question whether other NAC-ERF may be involved in abiotic stress responses.

Expression and subcellular localization analyses for *DkNAC7*

The above-mentioned regulatory effects of *DkNAC7* on deastringency related genes encouraged us to study the response of *DkNAC7* to deastringency treatment. From previous results, AHCA treatment (also called CO_2 treatment or high CO_2 treatment: 95% CO_2 and 1% O_2) was very effective in removing astringency in various persimmon [5,7,21]. Therefore, using previously described materials [21], the expression of *DkNAC7* was analyzed. The *DkNAC7* gene exhibited a sharp increase in expression in response to AHCA treatment, with the highest level at 1 d (Fig 4). After removal of CO_2 treatment, transcripts of *DkNAC7* decreased concomitantly, but remained statistically significantly higher than in control fruits. Such expression was similar to most of the previously identified deastringency related transcription factors. Furthermore, subcellular localization analysis of *DkNAC7* in tobacco leaves using GFP tagging, showed strong signals in the nucleus (Fig 5).

Taken all the results of Y1H, dual-luciferase assay, expression and subcellular localization together, we propose *DkNAC7* as a novel regulator of persimmon fruit deastringency, acting via direct regulation of the *DkERF9*. Again, *DkNAC7* was not closest homolog to the low oxygen-induced *ANAC102* gene [9], indicating either potential differences between species or organs, or the complexity of NAC-regulatory roles. Another possible explanation would be the differences between experimental treatments, as in model plant or crops, anoxia treatments were generally low O_2, but AHCA treatment in persimmon involves high CO_2 and low O_2. Thus, the deastringency related *DkNAC7*, as well as the previously characterized transcription factors, could be termed as high-CO_2/hypoxia responsive.

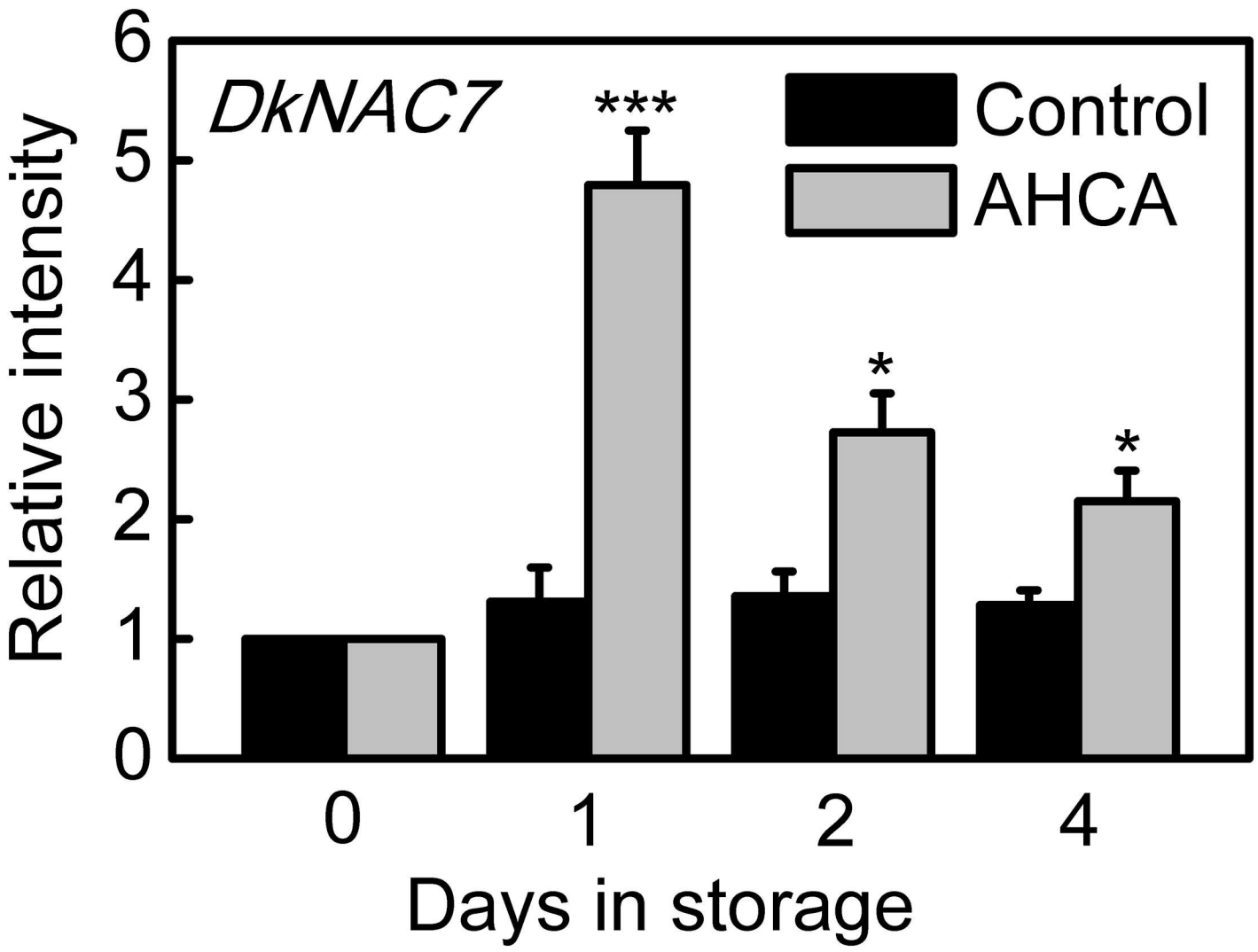

Fig 4. Expression of *DkNAC7* in response to AHCA treatment (95% CO_2, 1% O_2, 1 day). Relative mRNA abundance was evaluated by real-time PCR. Day 0 fruit values were set as 1. Error bars represent ± SE from three replicates ($^{*}p < 0.05$; $^{***}p < 0.001$).

https://doi.org/10.1371/journal.pone.0194326.g004

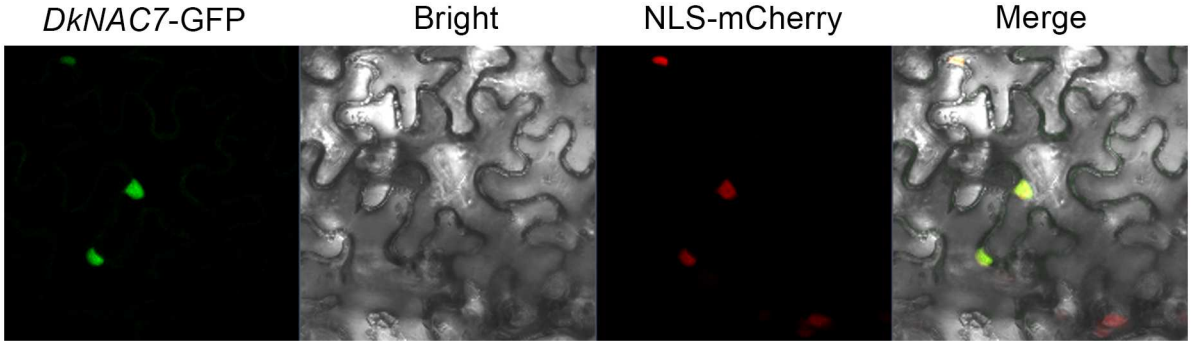

Fig 5. Subcellular localization of *DkNAC7*-GFP in tobacco leaves transformed by agroinfiltration. GFP fluorescence of DkNAC7 is indicated. Bars = 25 μm.

https://doi.org/10.1371/journal.pone.0194326.g005

Supporting information

S1 Fig. Yeast one-hybrid analysis of DkNAC7 binding to promoter of *DkPDC2*.
(TIF)

Acknowledgments

This research was supported by the National Key Research and Development Program (2016YFD0400102), the National Natural Science Foundation of China (31722042; 31672204), the Natural Science Foundation of Zhejiang Province, China (LR16C150001), the Fundamental Research Funds for the Central Universities, and the 111 Project (B17039).

Author Contributions

Data curation: Rong Jin.

Methodology: Qing-gang Zhu, Xin-yue Shen.

Supervision: Qing-gang Zhu, Xue-ren Yin, Kun-song Chen.

Writing – original draft: Miao-miao Wang, Wajeeha Jamil.

Writing – review & editing: Donald Grierson, Xue-ren Yin.

References

1. Akagi T, Ikegami A, Tsujimoto T, Kobayashi S, Sato A., Kono A., et al. DkMyb4 is a Myb transcription factor involved in proanthocyanidin biosynthesis in persimmon fruit. Plant Physiol. 2009; 4, 2028–2045.
2. Luo C, Zhang QL, Luo ZR. Genome-wide transcriptome analysis of Chinese pollination-constant nonastringent persimmon fruit treated with ethanol. BMC Genomics 2014; 15, 112. https://doi.org/10.1186/1471-2164-15-112 PMID: 24507483
3. Yonemori K, Suzuki Y. Differences in three-dimensional distribution of tannin cells in flesh tissue between astringent and non-astringent type persimmon. Acta Hortic. 2008; 833, 119–124.
4. Ikegami A, Eguchi S, Kitajima A, Inoue K, Yonemori K. Identification of genes involved in proanthocyanidin biosynthesis of persimmon (*Diospyros kaki*) fruit. Plant Sci. 2007; 172, 1037–1047.
5. Salvador A, Arnal L, Besada C, Larrea V, Quiles A, Pérez-Munuera I. Physiological and structural changes during ripening and deastringency treatment of persimmon fruit cv. 'Rojo Brillante'. Postharvest Biol Technol. 2007; 46, 181–188.
6. Yin XR, Shi YN, Min T, Luo ZR, Yao YC, Xu Q, et al. Expression of ethylene response genes during persimmon fruit astringency removal. Planta. 2012; 235, 895–906. https://doi.org/10.1007/s00425-011-1553-2 PMID: 22101946
7. Min T, Yin XR, Shi YN, Luo ZR, Yao YC, Grierson D, et al. Ethylene-responsive transcription factors interact with promoters of *ADH* and *PDC* involved in persimmon (*Diospyros kaki*) fruit de-astringency. J Exp Bot. 2012; 63, 6393–6405. https://doi.org/10.1093/jxb/ers296 PMID: 23095993
8. Min T, Fang F, Ge H, Shi YN, Luo ZR, Yao YC, et al. Two novel anoxia-induced ethylene response factors that interact with promoters of deastringency-related genes from persimmon. PLOS One 2014; 9, e97043. https://doi.org/10.1371/journal.pone.0097043 PMID: 24805136
9. Christianson JA, Wilson IW, Llewellyn DJ, Dennis ES. The low-oxygen-induced NAC domain transcription factor *ANAC102* affects viability of *Arabidopsis* seeds following low-oxygen treatment. Plant Physiol. 2009; 149, 1724–1738. https://doi.org/10.1104/pp.108.131912 PMID: 19176720
10. Matsuo T, Ito S. On mechanisms of removing astringency in persimmon fruits by carbon dioxide treatment. I. Some properties of the two processes in the de-astringency. Plant Cell Physiol. 1997; 18, 17–25.
11. Tamura F, Tanabe K, Itai A, Hasegawa M. Characteristics of acetaldehyde accumulation and removal of astringency with ethanol and carbon dioxide treatments in 'Saijo' persimmon fruit. J. Jpn. Soc. Hortic. Sci. 1999; 68, 1178–1183.
12. Licausi F, van Dongen JT, Giuntoli B, Novi G, Santaniello A, Geigenberger P, et al. *HRE1* and *HRE2*, two hypoxia-inducible ethylene response factors, affect anaerobic responses in *Arabidopsis thaliana*. Plant J. 2010; 62, 302–315. https://doi.org/10.1111/j.1365-313X.2010.04149.x PMID: 20113439

13. Hinz M, Wilson IW, Yang J, Buerstenbinder K, Llewellyn D, Dennis ES, et al. *Arabidopsis RAP2.2*: an ethylene response transcription factor that is important for hypoxia survival. Plant Physiol. 2010; 153, 757–772. https://doi.org/10.1104/pp.110.155077 PMID: 20357136

14. Yang CY, Hsu FC, Li JP, Wang NN, Shih MC. The AP2/ERF transcription factor AtERF73/HRE1 modulates ethylene responses during hypoxia in Arabidopsis. Plant Physiol. 2011; 156, 202–212. https://doi.org/10.1104/pp.111.172486 PMID: 21398256

15. Papdi C, Pérez-Salamó I, Joseph MP, Giuntoli B, Bögre L, Koncz C, et al. The low oxygen, oxidative and osmotic stress responses synergistically act through the ethylene response factor VII genes *RAP2.12*, *RAP2.2* and *RAP2.3*. Plant J. 2015; 82, 772–784. https://doi.org/10.1111/tpj.12848 PMID: 25847219

16. Fang F, Wang MM, Zhu QG, Min T, Grierson D, Yin XR, et al. *DkMYB6* is involved in persimmon fruit deastringency, via transcriptional activation on both *DkPDC* and *DkERF*. Postharvest Biol Technol. 2016; 111, 161–167.

17. Zhu QG, Wang MM, Gong ZY, Fang F, Sun NJ, Li X, et al. Involvement of *DkTGA1* transcription factor in anaerobic response leading to persimmon fruit postharvest de-astringency. PLOS One. 2016; 11, e0155916. https://doi.org/10.1371/journal.pone.0155916 PMID: 27196670

18. Nuruzzaman M, Manimekalai R, Sharon AM, Satoh K, Kondoh H, Ooka H, et al. Genome-wide analysis of NAC transcription factor family in rice. Gene. 2010; 465, 30–44. https://doi.org/10.1016/j.gene.2010.06.008 PMID: 20600702

19. Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. Genes involved in organ separation in *Arabidopsis*: An analysis of the *cup-shaped cotyledon* mutant. Plant Cell. 1997; 9, 841–857. https://doi.org/10.1105/tpc.9.6.841 PMID: 9212461

20. Min T, Wang MM, Wang HX, Liu XF, Fang F, Grierson D, et al. Isolation and expression of *NAC* genes during persimmon fruit postharvest astringency removal. Int J Mol Sci. 2015; 16, 1894–1906. https://doi.org/10.3390/ijms16011894 PMID: 25599529

21. Wang MM, Zhu QG, Deng CL, Luo ZR, Sun NJ, Grierson D, et al. Hypoxia-responsive *ERFs* involved in postdeastringency softening of persimmon fruit. Plant Biotechno. J. 2017; 15,1409–1419.

22. Yin XR, Allan AC, Chen KS, Ferguson IB. Kiwifruit *EIL* and *ERF* genes involved in regulating fruit ripening. Plant Physiol. 2010; 153, 1280–1292. https://doi.org/10.1104/pp.110.157081 PMID: 20457803

23. Xu Q, Yin XR, Zeng JK, Ge H, Song M, Xu CJ, et al. Activator-and repressor-type MYB transcription factors are involved in chilling injury induced flesh lignification in loquat via their interactions with the phenylpropanoid pathway. J Exp Bot. 2014; 65, 4349–4359. https://doi.org/10.1093/jxb/eru208 PMID: 24860186

24. Li SJ, Yin XR, Wang WL, Liu XF, Zhang B, Chen KS. Citrus CitNAC62 cooperates with CitWRKY1 to participate in citric acid degradation via up-regulation of *CitAco3*. J Exp Bot. 2017; 68, 3419–3426. https://doi.org/10.1093/jxb/erx187 PMID: 28633340

25. Morishita T, Kojima Y, Maruta T, Nishizawa-Yokoi A, Yabuta Y, Shigeoka S. Arabidopsis NAC transcription factor, ANAC078, regulates flavonoids biosynthesis under high-light. Plant and Cell Physiol. 2009; 50, 2210–2222.

26. An JP, Li R, Qu FJ, You CX, Wang XF, Hao YJ. An apple NAC transcription factor negatively regulates cold tolerance via CBF-dependent pathway. J Plant Physiol. 2017; 221, 74–80. https://doi.org/10.1016/j.jplph.2017.12.009 PMID: 29253732