Effects of Vacuum Packaging on *NAC* Gene Expression in Fresh-cut Lotus Root

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ABSTRACT. *NAC* transcription factors have been characterized in numerous plants, and the *NAC* gene has been shown to be involved not only in plant growth and development, but also in plant responses to abiotic and biological stresses, such as drought, high salinity, low temperature, and anaerobic/hypoxic stress. Creating an environment of anaerobic/hypoxic stress has been shown to be one of the effective storage methods for delaying the browning of fresh-cut lotus (*Nelumbo nucifera*) root. However, whether *NAC* is associated with lotus root browning under anaerobic stress has not been studied. In this study, vacuum packaging (VP; anaerobic/hypoxic stress) effectively delayed the browning of fresh-cut lotus root. The changes in the expressions of *NnPAL1*, *NnPPOA*, and *NnPOD2/3* were consistent with phenylalanine aminolase, polyphenol oxidase (PPO), and peroxidase (POD) enzyme activity changes and lotus root browning. Using RNA sequencing, five *NnNAC* genes were isolated and studied. Transcriptional analysis indicates that the *NnNAC* genes showed different responses to VP. The expressions of *NnNAC1/4* were inhibited by VP, which was consistent with the observed change in the degree of fresh-cut lotus root browning. However, *NnNAC2* messenger RNA (mRNA) levels were upregulated, and the expressions of *NnNAC3/5* showed no clear differences under different packaging scenarios. Thus, *NnNAC1/4* were identified as promising candidates for further transcriptional regulation analysis in lotus root to understand more fully the molecular mechanism of browning under anaerobic/anoxic stress.

With the increasing interest in healthy and nutritious diets, and continuing changes in consumer lifestyles, the consumption of fresh-cut fruits and vegetables has become increasingly popular (Chen et al., 2016; Sipahi et al., 2013). However, the

short shelf-lives and decline in the postprocessing of fresh-cut fruits and vegetables limit their production for retail. Lotus (*Nelumbo nucifera*) root is an important aquatic vegetable in China. Fresh-cut lotus root is a popular vegetable, but browning is one of the major factors limiting its quality for sale. Thus, browning control is a critical issue in the lotus root industry.

Artificial treatments have been developed successfully to delay the browning of fresh-cut lotus roots, including modified-atmosphere packaging (MAP) (Cheng et al., 2015), VP in low-temperature storage (Min et al., 2017, 2019; Xie et al., 2018), heat treatment (Tsouvaltzis et al., 2011), and so on. Among the various treatments, VP in low-temperature storage has been most successful in maintaining quality and prolonging the shelf life of fresh-cut lotus root (Aquino-Bolanos et al., 2000;

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Sharma and Rao, 2017; Toivonen and Brummell, 2008). Total phenolic content, oxygen, PPO, and POD are known to be the main factors that cause browning (Du et al., 2009; Walker and Ferrar, 1998). Our previous research reported that *NnPAL1*, *NnPPOA*, and *NnPOD2/3* are the most promising candidates for genes to target to control fresh-cut lotus root browning with MAP and VP in low-temperature storage (Min et al., 2017, 2018, 2019).

Although the anaerobic/hypoxic environment produced by VP creates a kind of unfavorable stress to plants, it has a remarkable effect on delaying fresh-cut lotus root browning. In our previous study, we explored the role of ethylene response factor (*ERF*) in delaying lotus root browning in VP under low temperatures and found that *NnERF4/5* could be important candidates for regulators of fresh-cut lotus root browning (Min et al., 2018). However, the relationship between other transcription factors and fresh-cut lotus root browning has rarely been reported. In addition to *ERFs*, *NAC* genes are among the important transcription factors reported to be involved in anaerobic/hypoxic responses, which indicate their potential to be involved in lotus root browning under VP. *NAC* (*NAM*, *ATAF1/2*, *CUC2*) transcription factors are a class of transcriptional regulators unique to higher plants. More than 100 *NAC* genes have been identified and characterized in the model plant *Arabidopsis thaliana* (Nuruzzaman et al., 2010). Among them, *ANAC019*, *ANAC055*, and *ANAC072* enhance tolerance to drought stress in *A. thaliana* (Tran et al., 2004), and *ANAC2* is involved in the response to plant hormones (He et al., 2005). In addition, *ANAC102* is induced by hypoxia stress (0.1% oxygen), and the expression of some genes related to hypoxia stress is enhanced in transgenic plants overexpressing *ANAC102*. After knocking out the *ANAC102* gene, the seed germination rate under hypoxia stress was shown to be significantly decreased (Christianson et al., 2009).

In addition, some studies have also shown that there is a correlation between *NAC* and phenylalanine ammonia lyase (*PAL*), *PPO*, and *POD* enzymes, and coding genes. *NAC* and the *PAL* gene showed overlapping expression patterns in the response of *Citrus sinensis* to cold exposure (Crifò et al., 2012). The expressions of *NAC* and *PAL* were upregulated simultaneously during gibberellic acid (GA3) treatment in GA3-induced xylem development in *Betula* (Guo et al., 2015). Overexpression of *ThNAC13* in *Tamarix hispida* induced *POD* activities, and *ThNAC13* induced the expression of *PODs* in transgenic *A. thaliana* (Wang et al., 2017b). *DgNAC1*-overexpressed transgenic *Chrysanthemum indicus* showed greater activities of superoxide dismutase, *POD*, and catalase under salt stress (Wang et al., 2017a). This suggested that *NAC* transcription factors have the potential to be involved in lotus root browning, but experimental evidence is lacking in lotus root.

In our study, five *NnNAC* genes were isolated from lotus root based on the RNA sequencing (RNA-Seq) database and a National Center for Biotechnology Information (NCBI) database. The effect of VP on browning, total phenol, *PPO*, *PAL*, and *POD* enzyme activity; and *PAL*, *PPO*, *POD*, and *NAC* gene expression changes were analyzed. Some *PAL*, *PPO*, *POD*, and *NnNAC* genes were found to correlate positively with lotus root browning, and the possible roles of these and other *NnNAC* genes are discussed here.

Materials and Methods

SAMPLE PREPARATION AND TREATMENTS. Lotus roots (cv. Wuzhi 2) were purchased from the Caidian District, Wuhan, China, in 2016 and were shipped immediately to the laboratory. Lotus roots that were not wounded and were of uniform size were selected for treatment. After the lotus roots were stored at 4 °C for 24 h, they were rinsed gently with tap water by hand to remove any silt. Next, the lotus roots were peeled and cut into 5-mm-thick slices. The slices of lotus root were divided into two groups for different treatments: a) VP and b) atmospheric pressure packaging (AP). Finally, the samples were stored at 4 ± 1 °C for 35 d, and sampling was performed every 7 d. Fifty kilograms lotus root was purchased for each experiment and ≈20 kg sliced root was packaged. The size of the vacuum packages was 200 × 280 mm and the material was polyethylene. The size of atmospheric pressure packages was 180 × 120 × 25 mm and the materials were polypropylene pallet and polyvinyl chloride plastic wrap. One hundred fifty replications samples were available for each treatment, and 20 replications samples were used during each 7-d sampling time. The experiment repeated three times over time.

BROWNING DEGREE (BD) AND TOTAL PHENOLIC CONTENT. The BD and total phenolic content were determined according to the methods described in our previous study (Min et al., 2017). Briefly, the BD was determined as follows: Lotus root tissue (3.0-g slices) was homogenized with 30 mL distilled water at 4 °C and then centrifuged for 5 min at 10,000 g_n. Next, the supernatant was collected and incubated for 5 min in a water bath at 25 °C. The absorbance was measured at 410 nm using a spectrophotometer, and the BD was expressed as A₄₁₀ × 10.

The total phenolic content was determined as follows: Lotus root tissue (3.0-g slices) was homogenized with 30 mL ethanol (60%) and then centrifuged for 5 min at 10,000 g_n. The supernatant (10 mL) was collected and mixed with 40 mL 60% ethanol to obtain an extract. The extract (0.125 mL) was mixed with 0.625 mL distilled water, and then 0.125 mL Folin phenol reagent was added. After fully mixing the solution, the mixture was left at room temperature for 3 min. Then, 1.25 mL Na₂CO₃ (7%) and distilled water (1.0 mL) were added. The mixture was incubated at 25 °C in the dark for 90 min, after which the absorbance was measured at 760 nm using a spectrophotometer. The standard curve of gallic acid was used to quantify the total phenol content. The result was the expression of milligrams gallic acid equivalents per kilogram fresh weight, and all treatments were repeated three times.

PAL, PPO, AND POD ACTIVITY. *PAL*, *PPO*, and *POD* were extracted and analyzed according to the method described in our previous study (Min et al., 2017). The *PAL* enzyme was extracted as follows: In an ice bath, 0.1 g lotus root was added to 1 mL reagent 1 and centrifuged for 10 min at 10,000 g_n at 4 °C. The supernatant was then collected as a crude extract. The *PAL* enzyme activity unit (U) was defined by spectrophotometry at 290 nm, and 1 U was defined spectrophotometrically as a change of 0.1 in absorbance per min.

The *PPO* enzyme was extracted as follows: Fresh-cut lotus root (3.0 g) was homogenized with 50 mL phosphate buffered saline (0.05 mol·L⁻¹, pH 7.0) in an ice mortar and then centrifuged at 3820 g_n for 15 min at 4 °C. The *PPO* enzyme activity unit was defined by spectrophotometry at 420 nm; 1 U was described as the amount of enzyme leading to a change in absorbance of 0.001 per min.

The POD enzyme was extracted as follows: Fresh-cut lotus root was homogenized in 5.0 mL 0.2 mol·L⁻¹ extraction buffer [pH 7.0, 1 mol·L⁻¹ polyethylene glycol, 4% (w/v) polyvinyl pyrrolidone, 1% (w/v) nonionic surfactant] (Triton X-100; Amresco, Solon, OH) and the solution was centrifuged at 15,290 g_n for 30 min at 4 °C. The POD enzyme activity unit was defined by spectrophotometry at 470 nm; 1 U was defined spectrophotometrically as an increase of 0.01 in absorbance per min.

RNA EXTRACTION AND COMPLEMENTARY DNA (cDNA) SYNTHESIS. Total RNA was prepared according to the method described in our previous study (Min et al., 2017). TURBO Dnase (Ambion, Austin, TX) was used to remove the DNA traces in the total RNA that contaminated the genome. The iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) was used for cDNA synthesis of 1.0 mg RNA. At each sampling point, three biological replicates were used for RNA extraction experiments.

GENE ISOLATION AND SEQUENCE ANALYSIS. The *NAC* genes were isolated based on the RNA-Seq and NCBI databases. RNA sequencing was conducted by the Beijing Genome Institute (Shenzhen, China) according to the method described previously (Min et al., 2014). The sequences of primers for gene cloning are listed in Table 1. Phylogenetic trees of *NAC* genes were generated using ClustalX (version 1.81; Min et al., 2018) and calculated using FigTree (version 1.3.1; University of Edinburgh, Edinburgh, UK). The deduced amino acid sequences of homologous genes of *A. thaliana* were obtained from The Arabidopsis Information Resource.

OLIGONUCLEOTIDE PRIMERS AND REAL-TIME POLYMERASE CHAIN REACTION (PCR) ANALYSIS. Oligonucleotide primers were designed using primer 3 for real-time PCR analysis (Min et al., 2017). The specificity of primers was determined by melting curves and PCR product resequencing, as described in our previous study (Min et al., 2012). The sequences of oligonucleotide primers are listed in Table 2.

Ssofast EvaGreen Supermix kit (Bio-Rad Laboratories) and CFX96 fluorescence PCR were used for real-time PCR analysis for gene expression study according to the method described in our previous work (Min et al., 2017).

STATISTICAL ANALYSIS. Figures were drawn using Origin software (version 8.0; OriginLab, Northampton, MA). Statistical analysis of differences was conducted using the least significant difference via DPS7.05 software (Zhejiang University, Hangzhou, China).

Results and Discussion

BD AND TOTAL PHENOL CONTENT. The fresh-cut lotus root used in our research had a mean BD value of 0.2150 at day 0 (Fig. 1). VP promoted an increase in the BD value to 0.29 after 35 d, whereas BD showed a greater growth in AP (0.61 at day

Table 1. Sequences of the primers used for *NAC* genes partial coding sequences clone.

Gene	Primary PCR	Secondary PCR
<i>NnNAC1</i>	CTGAGGCCCTGAAGTTACA	GATGGAGCCACAACAACCTG
<i>NnNAC2</i>	TCCACGGAAAGCTTACTCCT	GCAAAACGTTTCATGCATCTCT
<i>NnNAC3</i>	CAGGGACAGAAGATGGGTGA	CAGGGGTGGTACTAGCCTTT
<i>NnNAC4</i>	GGGGCTGACAAACCCATTAG	CTGGCTCTTGTTCTCTTCTG
<i>NnNAC5</i>	CTTCTAGCTGCGTCCACTCT	TCACAATCGCCAAGTTGTCC

Five *NAC* complementary DNAs (*NnNAC1*-XP_010243336.1, *NnNAC2*-XP_010249131.1, *NnNAC3*-XP_010275272.1, *NnNAC4*-XP_010257746.1, and *NnNAC5*-XP_010269977.1) were isolated from lotus root.

Table 2. Sequences of the primers for real-time polymerase chain reaction (PCR) of *NAC* genes.

Gene	Primary PCR	Secondary PCR
<i>NnNAC1</i>	GGTTTGATTACCGGGAAAACC	AGAAAACACCAGTGAGCTTT
<i>NnNAC2</i>	TGATTCCAACCGCGAAGAAA	CTCGTAAAGCTCAACTACTGGAA
<i>NnNAC3</i>	TAGTACCACCCCTGAGATGTC	TACTTGAGATACTAGGAGGGGTG
<i>NnNAC4</i>	TGTACAGAAGAGAACACAAGAGC	GGGGAGGTGATTTGGAAAAGAAA
<i>NnNAC5</i>	GCCTACTAGTTATCCTGGTGTC	GTGGTACTTGAATTTCTGCTC

Five *NAC* expressions were analyzed: *NnNAC1*-XP_010243336.1, *NnNAC2*-XP_010249131.1, *NnNAC3*-XP_010275272.1, *NnNAC4*-XP_010257746.1, and *NnNAC5*-XP_010269977.1.

Fig. 1. Effects of two different packaging methods on browning degree of fresh-cut 'Wuzhi 2' lotus root. Browning degree was chosen as the main index of fruit and vegetable browning. Fresh-cut lotus root was packaged by vacuum packaging [VP (circles)] and atmospheric pressure packaging [AP (squares)] separately. Error bars represent SE from three biological replicates. LSD = least significant difference.

35) (Fig. 1). As shown in Fig. 2, the effects of the two different packaging methods on the total phenol content were clearly different during storage. Slower changes were found in the samples subjected to VP, changing from 96.89 mg·kg⁻¹ at day 0 to 108.98 mg·kg⁻¹ at day 35. However, the samples with AP showed faster growth, from 96.89 mg·kg⁻¹ at day 0 to 159.25 mg·kg⁻¹ at day 35.

BD value was chosen as the main index for evaluating browning. By determining the BD in storage after using two different packaging methods, it was clear that the BD of the samples subjected to VP (anaerobic stress) was significantly less than that of the control group. These results confirmed and extended our previous finding (Min et al., 2019).

PAL, PPO, AND POD ACTIVITIES. PAL, PPO, and POD enzyme activities under VP and AP were significantly different

Fig. 2. Effects of two different packaging methods on total phenol content of fresh-cut ‘Wuzhi 2’ lotus root. The total phenol was the main substrate for enzymatic browning in fruits and vegetables. Fresh-cut lotus root was packaged by vacuum packaging [VP (circles)] and atmospheric pressure packaging [AP (squares)] separately. Error bars represent SE from three biological replicates. FW = fresh weight; LSD = least significant difference.

during storage. As shown in Fig. 3, the PAL activity of the samples undergoing VP and AP showed different magnitudes of change, from the initial value of 12.802 to 12.225 U/g (VP) and 26.354 U/g (AP) at day 35.

The PPO activity of VP increased slowly, from the initial value of 0.396 to 0.604 U/g at day 35. However, PPO activity increased rapidly under AP, from the initial value of 0.396 to 1.18 U/g at day 35. The POD activity of the samples of VP increased slowly, from the initial value of 0.044 to 0.085 U/g at day 35, but POD activity increased rapidly when subjected to AP, from the initial value of 0.044 to 0.146 U/g at day 35.

It has been suggested in previous work that PAL, PPO, and POD mainly involve enzymatic browning of lotus root (Soliva-Fortuny and Martn-Belloso, 2003; Zheng et al., 2016). Changes in PAL, PPO, and POD enzymatic activity in storage under two different packaging methods were analyzed. These results showed that PAL, PPO, and POD activity were inhibited during VP, which occurred in parallel with less significant browning during storage, which was consistent with previous reports (Min et al., 2019),

***NnPAL*, *NnPPO*, and *NnPOD* GENE EXPRESSION.** There was an obvious differential response to different packaging methods for the two *PAL*, two *PPO*, and seven *POD* genes. As shown in Fig. 4, compared with AP, *NnPAL1* expression in ‘Wuzhi 2’

Fig. 3. Effects of two different packaging methods on phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) activity of fresh-cut ‘Wuzhi 2’ lotus root. PAL, PPO, and POD have been proposed as being involved in enzymatic browning. Fresh-cut lotus root was packaged by vacuum packaging [VP (circles)] and atmospheric pressure packaging [AP (squares)] separately. Error bars represent SE from three biological replicates. LSD = least significant difference.

Fig. 4. Messenger RNA amounts from phenylalanine ammonia lyase (*PAL*) genes in response to different packaging methods. Fresh-cut ‘Wuzhi 2’ lotus root was packaged by vacuum packaging [VP (black)] and atmospheric pressure packaging [AP (white)] separately. Zero-day sample values were set at 1. Error bars represent SE from three biological replicates. LSD = least significant difference.

was inhibited by VP, and the expression of *NnPAL2* was induced by VP. The expressions of *NnPPOA* and *NnPPOC* were inhibited by VP during the storage process, of which *NnPPOA* was more obviously inhibited by VP (Fig. 5). Moreover, the relative expression was less than that of AP, which was consistent with changes in the PPO enzyme activity. The expressions of *NnPOD2* and *NnPOD3* in VP decreased by more than 50 times. The expressions of *NnPOD1* and *NnPOD5* were also inhibited by VP during the early stage, but the inhibition multiples were not as obvious as those of *NnPOD2* and *NnPOD3* (Fig. 6). *NnPOD4* and *NnPOD6* were induced by VP, especially *NnPOD6* (Fig. 6). *NnPOD7* was downregulated during storage, but there was no significant difference between the two groups (Fig. 6). In our previous study, *NnPAL1*, *NnPPOA*, and *NnPOD2/3* of fresh-cut lotus root (‘Elian 5’) with high-concentration CO₂ MAP were also more obviously downregulated than other genes (Xie et al., 2018).

The experimental results based on two cultivars showed that *NnPAL1*, *NnPPOA*, and *NnPOD2/3* were significantly down-regulated by VP (low oxygen). In addition, changes in the expressions of *NnPAL1*, *NnPPOA*, and *NnPOD2/3* were consistent with PAL, PPO, and POD enzyme activity changes. Previous studies and the current results further prove that

Fig. 5. Messenger RNA amounts from polyphenol oxidase (*PPO*) genes in response to different packaging methods. Fresh-cut ‘Wuzhi 2’ lotus root was packaged by vacuum packaging [VP (black)] and atmospheric pressure packaging [AP (white)] separately. Error bars represent SE from three biological replicates. LSD = least significant difference.

NnPAL1, *NnPPOA*, and *NnPOD2/3* may be important candidates for genes to target to control fresh-cut lotus root browning (Min et al., 2017, 2018, 2019).

NAC GENE ISOLATION AND PHYLOGENETIC ANALYSIS. Five *NAC* cDNAs (*NnNAC1*-XP_010243336.1, *NnNAC2*-XP_010249131.1, *NnNAC3*-XP_010275272.1, *NnNAC4*-XP_010257746.1, and *NnNAC5*-XP_010269977.1) were isolated from ‘Wuzhi 2’ lotus root using the RNA-Seq and NCBI databases. To study the phylogenetic relationship between the *NAC* proteins in lotus root and *A. thaliana*, a phylogenetic tree was constructed based on their translated amino acid sequences using ClustalX 2.1 and FigTree 3.1 software. As shown in Fig. 7, *NnNAC1/3/4* are closer to *ANAC063*, and this type of *NAC* gene plays an important role in drought resistance and salt tolerance (Yokotani et al., 2009). *ONAC063* expression was not induced by high-salinity in *Oryza sativa* roots, and the seeds of *ONAC063*-expressing transgenic *A. thaliana* showed enhanced tolerance to high salinity and osmotic pressure.

NnNAC2 belongs to *SENU5* in Group I. It has been reported that this type of *NAC* gene plays an important role in high salt conditions, drought, and high-temperature stress (Dong, 2014). *NnNAC5* belongs to the subfamily of *ANAC011* in Group I. It has been reported that this type of *NAC* gene plays an important role in the reaction of ethylene and jasmonic acid (Kang et al.,

Fig. 6. Messenger RNA amounts from peroxidase (*POD*) genes in response to different packaging methods. Fresh-cut 'Wuzhi 2' lotus root was packaged by vacuum packaging [VP (black)] and atmospheric pressure packaging [AP (white)] separately. Error bars represent SE from three biological replicates. LSD = least significant difference.

2014). Collectively, these data suggest that *NnNAC1-5* may be involved in diverse functions.

NAC GENE EXPRESSION. The five *NAC* genes exhibited different expression patterns (Fig. 8). The expressions of

NnNAC1/4 were continuously inhibited by VP, with their mRNA decreasing in abundance ≈ 12 - and 60-fold, respectively. In contrast, transcripts of *NnNAC2* increased under VP storage at day 21. Unlike the other *NnNAC* genes, the

Fig. 7. Phylogenetic tree of *NAC* genes. Lotus root *NnNAC* genes are highlighted in red in the online article. The amino acid sequences of the *Arabidopsis thaliana* *NAC* family were obtained from The Arabidopsis Information Resource (TAIR) and National Center for Biotechnology Information (NCBI) databases.

expressions of *NnNAC3/5* showed no clear difference between the different packaging methods.

One of most interesting results is that *NAC1/4* were down-regulated by anaerobic or hypoxic environments (VP). Phylogenetic analysis showed that *ANAC063*, which is closer to *NAC1/4*, is not only responsive to drought resistance, but also to salt tolerance (Yokotani et al., 2009), which tells us that *NAC*

exhibit diversity in nonbiological stress responses. And the *NAC1/4* expression change pattern occurred concomitantly with increase in *NnPAL1*, *NnPPOA*, and *NnPOD2/3* gene expressions and BD. This indicates that, in lotus root, the expression of *NAC1/4* correlated highly with browning, and the relationships of *NAC1/4* and *NnPAL1*, *NnPPOA*, and *NnPOD2/3* should be the subjects of further research.

Conclusions

The BD; PAL, PPO, and POD enzyme activities; and gene expression assay results in our study show that VP was an effective method for delaying fresh-cut lotus root browning. Five *NnNAC* genes were isolated, and fresh-cut lotus root and *NnNAC1/4* correlated highly with fresh-cut lotus root browning. Finally, *NnNAC1/4* were identified as major candidates for further transcriptional regulation analysis in lotus root to understand more fully the molecular mechanism of browning under anaerobic/hypoxic stress.

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Fig. 8. Messenger RNA amounts from *NAC* genes in response to vacuum packaging (VP) and atmospheric pressure packaging (AP) treatments. Fresh-cut 'Wuzhi 2' lotus root was stored by VP (circles) and AP (squares) at 4 C separately. Fresh-cut lotus root was packaged by VP (black) and AP (white) separately. Error bars represent SE from three biological replicates. LSD = least significant difference.

In addition to the two aforementioned *NnNAC* genes, *NnNAC2* expression also showed interesting features. Unlike the positively regulated *NAC* genes, *NnNAC2* mRNA levels were inversely related to browning, which was upregulated by the anaerobic or hypoxic environments (VP) during the storage period.

It is well known that the processing of fresh-cut fruits and vegetables promotes faster physiological and biochemical changes and microbial growth, which may reduce the quality of their flavor and texture (Toivonen and Brummell, 2008). In addition, fresh-cut peppers in VP were found to have more noticeable ethanol and acetaldehyde contents (Gonzlez-Aguilar et al., 2004). Therefore, *NnNAC2* may be related to quality loss in fresh-cut fruits and vegetables, especially with respect to flavor. In similar studies, *DkNAC1/3/5/6* were found to be associated positively with the persimmon destringency process, which involves acetaldehyde and ethanol synthesis (Min et al., 2015).

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