Expression of *Ethylene Response Factor* Genes during Fresh-cut Lotus Root Storage and Browning

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ABSTRACT. *Ethylene response factor (ERF)* genes have been characterized in numerous plants in which they are involved in responses to biotic and abiotic stress, including cold and heat stress. Cool temperatures is one of the most effective storage methods for delaying browning of fresh-cut lotus (*Nelumbo nucifera*) root. In model plants, *ERF* genes have been identified as being responsive to cold and heat stress. Whether *ERF* is associated with lotus root browning in cooler temperatures has not been studied. In this research, low-temperature storage (4 °C) effectively delayed browning of fresh-cut lotus root. Using RNA sequencing, seven *Nelumbo nucifera ERF (NnERF)* genes were isolated and studied. Transcriptional analysis indicated *NnERF* genes responded differently to temperature. *NnERF3/4/5* were reduced continuously by a low temperature (4 °C) and *NnERF5* was the most strongly downregulated. In contrast, transcripts of *NnERF1/2/7* were increased at a low temperature (4 °C). The expression of *NnERF6* showed no obvious difference between the two different temperatures. It is proposed that *NnERF3/4/5* could be important candidates as regulators of fresh-cut lotus root browning. The roles of other members are also discussed.

ERF genes encode plant-specific transcription factors that are located downstream of the ethylene signaling pathway (Nakano et al., 2006). *ERFs* are members of one of the largest transcription factor families, which is considered a superfamily, and is involved in plant biological and abiotic stress responses (Licausi et al., 2013; Min et al., 2012; Müller and Munne-Bosch, 2015; Phukan et al., 2017). It has been reported that *CsAP2/ERF* (APETALA2/ERF) may play important roles in the abnormal temperature stress response in *Camellia sinensis* (Wu et al., 2015). *TERF2/LeERF2* enhance the freezing tolerance of *Nicotiana tabacum* and *Solanum lycopersicum* through ethylene biosynthesis and the ethylene signaling pathway (Zhang and Huang, 2010). *NnDREB2* (dehydration response element binding)/*ERF* might play an important role in the salt stress adaptation of plants by binding directly with the dehydration responsive element, regulating downstream gene expression in salt-resistant lotus (Cheng et al., 2015a). In fruits and vegetables, *ERF* genes are key targets for investigating the transcriptional regulatory roles in fruit and vegetable development and ripening (Karlova et al., 2011; Licausi et al., 2013; Mizoi et al., 2012; Xu et al., 2011).

Fresh-cut lotus root slices have received increasing attention because they are extremely convenient and highly edible (He et al., 2017; Sun et al., 2015; Zhang et al., 2013). However, it is well known that enzymatic browning is the most serious problem for fresh-cut lotus root during processing and storage, limiting the shelf life of lotus root slices (Conesa et al., 2007; Francis et al., 2012; Hodges and Toivonen, 2008). Phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) have been reported previously as being involved in enzymatic browning of many species (Banerjee

et al., 2015; Cheng et al., 2015b; Zheng et al., 2016; Zhou et al., 2003). Our previous research reported that *NnPAL1*, *NnPPOA*, and *NnPOD1-6* are the most important candidate genes for the browning of fresh-cut lotus root (cv. E Lian 6) during storage at different temperatures (Min et al., 2017). However, many aspects of transcriptional regulation in lotus root senescence and quality are yet to be explored.

It has been reported that 1-methylcyclopropene (an ethylene inhibitor) treatment reduced rachis browning, whereas ethylene tended to enhance it in *Vitis vinifera* (Li et al., 2015). Ethylene can increase the activity of phenolase in fresh-cut *Colocasia esculenta* and accelerate the occurrence of browning during storage (Tan and Zeng, 2014). It is well known that the biological function of ethylene is realized through the ethylene signal transduction pathway. As the most important element in the ethylene signal transduction pathway, *ERF* plays an important role in the realization of ethylene function (Nakano et al., 2006). It has been reported that *DcERF1* and *DcERF2* may function in different ways in committing to the upregulation of *DcPAL3* promoter activity in anthocyanin-synthesizing *Daucus carota* cells (Kimura et al., 2008). In addition, carbon dioxide has been shown to downregulate expression of *ERF* genes in *Medicago truncatula* infested by *Acyrtosiphon pisum* and to reduce resistance against *A. pisum* in terms of less POD and PPO activity (Guo et al., 2014). This suggests that *ERF* may be involved in the synthesis of phenolic precursors through the transcriptional regulation of *PAL* in plants, or the transcriptional regulation of *PPO* and *POD* involved in plant stress responses. However, there is a lack of experimental evidence for this in relation to lotus root.

In this research, seven of 38 *NnERF* genes were isolated from lotus root based on differentially expressed genes in an RNA sequencing (RNA-Seq) database and the National Center for Biotechnology Information (NCBI) database (Supplemental Table 1). In the treatment, lotus root was stored at 4 and 20 °C, and *NnERF* transcripts were analyzed throughout the browning process. Some *NnERF* genes were found to correlate positively

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with lotus root browning. The possible roles of these and other *NnERF* genes are discussed.

Materials and Treatments

Lotus roots ('E Lian 5') were sourced from a commercial agricultural wholesale market (Si Ji Mei Agricultural Wholesale Market, Wuhan, China) in 2016 and then transported to the laboratory. The batch and treatment of 'E Lian 5' lotus roots were the same as that used in our previous report (Min et al., 2017). Before treatment, the lotus roots were stored at 4 °C for 24 h. They were washed with tap water to remove soil and were peeled. They were then cut into 5-mm-thick slices along the cross-section of the lotus root. After being transferred to clean water, and soaked for 2 min, these fresh-cut lotus root slices were dried with filter paper and packed in fruit and vegetable boxes (18.8 × 12 × 1.5 cm) (MS-08; SUNWAY KORDIS, Shanghai, China), with two pieces in each box, and transferred to the refrigerator (4 and 20 °C, respectively).

BROWNING DEGREE. The extraction and determination of degree of browning were carried out according to our previous report (Min et al., 2017). Flesh tissue (3.0 g) was homogenized with 30 mL distilled water at 4 °C, then centrifuged at 10,000 g_n for 5 min at 4 °C. The supernatant was collected and incubated in a 25 °C water bath for 5 min. Absorbance was measured using a spectrophotometer (GL-20G-II; Shanghai Anting, Shanghai, China) at 410 nm; browning degree (BD) was expressed as $A_{410} \times 10$.

TOTAL PHENOLIC CONTENT. Total phenolic content was measured according to our previous article (Min et al., 2017). A 3.0-g fresh sample of flesh tissue was homogenized with 30 mL 60% ethanol and centrifuged. The results are expressed as milligrams gallic acid equivalents per kilogram fresh weight, and all treatments were performed with three biological replicates.

PAL ACTIVITY. The extraction and assay of PAL activity were performed using a phenylalanine ammonia-lyase kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), as described previously (Min et al., 2017). Flesh tissue (0.1 g) was homogenized in 1 mL extracting solution in an iced mortar, and the solution was then centrifuged at 10,000 g_n for 10 min at 4 °C. The supernatant was collected as a crude PAL extract. The reaction mixture consisted of 20 μ L crude PAL extract, 780 μ L reagent 1 (the blank group for 800 μ L) and 200 μ L reagent 2. Afterward, the mixture was incubated at 30 °C for 30 min, and 40 μ L reagent 3 was added into the previous mixture and was left to stand for 10 min. The absorbance value of the sample at 290 nm was measured. Enzyme activity units (U) were defined spectrophotometrically as a change of 0.1 in absorbance per minute per gram fresh weight.

PPO ACTIVITY. The extraction and assay of PPO activity were performed as described previously (Min et al., 2017). Flesh tissue (3.0 g) was homogenized in 50 mL phosphate-buffered

Table 1. Sequences of the primers used for ethylene response factor (*NnERF*) partial coding sequences clone.

Gene	Primary PCR	Secondary PCR
<i>NnERF1</i>	GCTCATACCCAATTCCAGCG	ATGAGTGAGTGGTGCAGGAG
<i>NnERF2</i>	TGTGGCTGGGTACTTTCACT	AGCCCCGAAAGATGTACGTA
<i>NnERF3</i>	CCCACATCTTCCCTGCCTAC	GATGTATCTCCTCTCATTGGTG
<i>NnERF4</i>	TCCTCCGTCTATCTCCGTCA	TCACAGAGAGAGAGAGAGAGAGA
<i>NnERF5</i>	TGGAAGATTGAAGCGCCAAA	ACAGTACGCAGAGAGGGGAAA
<i>NnERF6</i>	CGACCCAGTTGACGATTTCC	CCGTACAGAACACATCAAGACA
<i>NnERF7</i>	CCCAGTTGCAGAGTTTGTT	GGCATGGCAAAGAACACAGA

Table 2. Sequences of the primers for real-time polymerase chain reaction (PCR) of the ethylene response factor of *Nelumbo nucifera* (*NnERF*) genes.

Gene	Primary PCR	Secondary PCR
<i>NnERF1</i>	TAGAACTCCTGCACCACTCA	TACCCATATCACCCGAACCA
<i>NnERF2</i>	GATCTTCTTGGTTGTGAGGGA	GCCCCGAAAGATGTACGTA
<i>NnERF3</i>	GCAGATCAGTGGCTCTTATTCT	TTCCCTTGTATGATGTATCTCCTC
<i>NnERF4</i>	CTCTCTCTCTCTCTGTGAAAA	CCCTCTTGCATCCTCAGATATAA
<i>NnERF5</i>	ATCATCTCTCTCTTCTCTGTTCC	CAAACCAAACCGAAACGAAAG
<i>NnERF6</i>	TATCCGTCTCTTCTCTAGTTA	CGTACAGAACACATCAAGACATG
<i>NnERF7</i>	CCGTTGAAGACATGGAGATATATC	CATGGCAAAGAACACAGAAGTT

Fig. 1. Effects of two different temperatures on browning degree of fresh-cut lotus root ('E Lian 5'). Browning degree was chosen as the main index of browning, with more serious browning indicated by greater index values. Fresh-cut lotus root was stored at 4 °C (circles) and 20 °C (squares), separately. Error bars represent the SE from three biological replicates. LSD = least significant difference.

saline (PBS; 0.05 mol·L⁻¹, pH 7.0) in an iced mortar, and the solution was then centrifuged at 3820 g_n for 15 min at 4 °C. The supernatant was collected as a crude PPO extract. The reaction mixture consisted of 1.0 mL 0.1 mol·L⁻¹ catechol and 1.5 mL 0.05 mol·L⁻¹ PBS (pH 7.0). Afterward, the mixture was incubated at 35 °C for 5 min, 1.0 mL crude PPO was added into the previous mixture, and the mixture of changes in the absorbance at 420 nm was measured. One unit of PPO activity was defined as a change of 0.001 at 420 nm in absorbance per minute.

POD ACTIVITY. POD was extracted and assayed spectrophotometrically according to previous work (Min et al., 2017). In a mortar, 5.0 g fresh-cut lotus root was homogenized with

Fig. 2. Effects of two different temperatures on total phenol content of fresh-cut lotus root ('E Lian 5'). Total phenol was the main substrate for enzymatic browning. Fresh-cut lotus root was stored at 4 °C (circles) and 20 °C (squares), separately. Error bars represent the SE from three biological replicates. LSD = least significant difference; FW = fresh weight.

Fig. 3. Effects of two different temperatures on phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) activity of fresh-cut lotus root ('E Lian 5'). PAL, PPO, and POD have been proposed as being involved in enzymatic browning. Fresh-cut lotus root was stored at 4 °C (circles) and 20 °C (squares), separately. Error bars represent the SE from three biological replicates. LSD = least significant difference.

5.0 mL 0.2 mol·L⁻¹ PBS (pH 7.0) 1 mol·L⁻¹ polyethylene glycol, 4% (w/v) polyvinyl polypyrrolidone, and 1% (w/v) nonionic surfactant (Triton X-100; AMRESCO, Solon, OH) in an ice bath. The homogenates were then centrifuged at 15,290 g_n for 30 min at 4 °C. The supernatant was collected for the POD activity assay. The reaction cuvette contained 3.0 mL 25 mmol·L⁻¹ guaiacol, 0.5 mL enzyme solution (the blank group for PBS), and 0.2 mL 5 mol·L⁻¹ dissolved hydrogen peroxide. The change of the mixture in absorbance at 470 nm was recorded once every 10 s. An enzyme activity unit was defined spectrophotometrically as an increase of 0.01 at 470 nm in absorbance per minute per gram fresh weight.

RNA EXTRACTION AND COMPLEMENTARY DNA SYNTHESIS. Total RNA was prepared according to a method used previously (Min et al., 2015). Traces of contaminating genomic DNA in total RNA were removed with TURBO Dnase (Ambion, Austin, TX). A total of 1.0 µg DNA-free RNA was used for complementary DNA (cDNA) synthesis using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's protocol. For each sampling point, three biological replicates were used for RNA extraction.

GENE ISOLATION AND SEQUENCE ANALYSIS. The *ERF* genes were isolated based on the RNA-Seq and NCBI databases. RNA-Seq isolation was performed by the Beijing Genome Institute (Shenzhen, China) as reported previously (Min et al., 2014). The sequences of primers used for gene cloning are listed in Table 1. Phylogenetic trees of *ERF* genes were calculated and generated using FigTree (version 1.3.1; University of Edinburgh, Edinburgh, UK). The amino acid sequences of homologous genes of different species were obtained from the NCBI database.

OLIGONUCLEOTIDE PRIMERS AND REAL-TIME POLYMERASE CHAIN REACTION ANALYSIS. Oligonucleotide primers for real-time polymerase chain reaction (PCR) analysis were designed using primer 3 (Min et al., 2017). The specificity of primers was determined by melting curves and PCR product resequencing as described in Min et al. (2012). The sequences of oligonucleotide primers are listed in Table 2. Real-time PCR was carried out with a Ssofast EvaGreen Supermix kit (Bio-Rad Laboratories) and a CFX96 (Bio-Rad Laboratories) optics module for gene expression studies according to our previous reports (Min et al., 2015, 2017).

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

Results and Discussion

LOTUS ROOT BROWNING AND TOTAL PHENOL CHANGES. BD remained almost constant during storage at 4 °C, whereas the 20 °C temperature storage caused a rapid increase in BD from 0.236 at 0 d to 1.584 at 5 d (Fig. 1). Phenols increased ≈ 3 -fold in the 20 °C sample after 5 d of storage. However, the total phenol content of the samples at 4 °C showed no obvious change, only a slight increase (Fig. 2).

BD value was the main index for evaluating browning. By analyzing the change in BD in storage at two different temperatures, these results confirmed our previous results (Min et al., 2017) and those of others (Kang et al., 2012; Kwon and Baek, 2014). Thus, this is further support that low-temperature storage is effective in delaying the browning of fresh-cut lotus root ('E Lian 5'). When comparing the total phenol content of fresh-cut lotus root ('E Lian 5') in storage at two different temperatures, the value of total phenols was greater at 20 °C, which is consistent with previous reports (Abohatem et al., 2011; Min et al., 2017).

PAL, PPO, AND POD ACTIVITY CHANGES. The effects of the two different temperatures on PAL, PPO, and POD activity clearly differed during storage (Fig. 3). PAL activity of the samples at the two different temperatures showed varying

degrees of increase, from an initial value of 5.594 to 9.284 U/g (4 °C) and 21.683 U/g (20 °C) in 5 d. The PPO activity of the samples at 4 °C showed a slow increase from an initial value of 0.367 to 0.633 U/g in 5 d. However, PPO activity increased rapidly at 20 °C from an initial value of 0.367 to 1.271 U/g in 5 d. POD activity of the samples at 4 °C showed a slow increase, from an initial value of 0.034 to 0.080 U/g in 5 d. However, POD activity increased rapidly at 20 °C from an initial value of 0.034 to 0.336 U/g in 5 d.

As mentioned previously, PAL, PPO, and POD have been proposed to be involved in enzymatic browning in lotus root (Hu et al., 2014; Min et al., 2017; Zhang et al., 2013). By analyzing the change in PAL, PPO, and POD enzymatic activity in storage at two different temperatures, it is clear that the greater the temperature, the faster the increase in PAL, PPO, and POD activity, which was in agreement with previous results (Min et al., 2017).

PAL, PPO, AND POD GENE EXPRESSION. There was a strong differential response to temperature for two *PAL*, two *PPO*, and seven *POD* genes. Expression of *NnPAL1* was particularly responsive to the 20-°C temperature induced from 0 d, reaching a peak at 3 d and maintaining a high level thereafter. *NnPAL2* expression increased gradually during storage and peaked at 5 d (Fig. 4). The influence of temperature on the expression of the two *PPO* genes was similar to the *PAL* genes (Fig. 5), being

Fig. 4. Messenger RNA amounts from phenylalanine ammonia lyase (*PAL*) genes in response to different temperature treatments. Fresh-cut lotus root was stored at 4 °C (white) and 20 °C (black), separately. Zero-day sample values were set at 1. Error bars represent the SE from three biological replicates. LSD = least significant difference.

Fig. 5. Messenger RNA amounts from polyphenol oxidase (*PPO*) genes in response to different temperature treatments. Fresh-cut lotus root was stored at 4 °C (white) and 20 °C (black), separately. Error bars represent the SE from three biological replicates. LSD = least significant difference.

Fig. 6. Messenger RNA amounts from peroxidase (*POD*) genes in response to different temperature treatments. Fresh-cut lotus root was stored at 4 °C (white) and 20 °C (black), separately. Error bars represent the SE from three biological replicates. LSD = least significant difference.

induced at 20 °C and reaching a peak at 3 d. The seven *POD* genes exhibited different expression patterns. Transcripts of *NnPOD2*, *NnPOD3*, and *NnPOD4* were induced continuously by a 20-°C temperature and peaked on 5 d, with messenger RNA (mRNA) increasing in abundance ≈ 200 -, 1400-, and 1500-fold after storage at 20 °C, respectively. *NnPOD1*, *NnPOD5*, and *NnPOD7* were induced to varying degrees by the two different temperatures during storage, albeit in varying degrees. Unlike the other *NnPOD* genes, the expression of *NnPOD6* was increased with storage at 4 °C (Fig. 6).

Two *NnPAL*, two *NnPPO*, and seven *NnPOD* genes were isolated in our earlier work (Min et al., 2017). In this study, *NnPAL1/2*, *NnPPOA/C*, and *NnPOD2-4* were substantially more responsive to high temperature than the other gene family members. However, the genes used in the current study were somewhat different from our previous work (Min et al., 2017), in which we found that the upregulation of *NnPAL1*, *NnPPOA*, and *NnPOD1-6* at high temperatures coincided with an increase in fresh-cut lotus root BD, which may be because of the differences in raw materials. Considering our previous study and our current results, *NnPAL1*, *NnPPOA*, and *NnPOD2-5*

should be considered important candidates for fresh-cut lotus root browning, and further functional analysis needs to be performed.

ERF GENE ISOLATION AND PHYLOGENETIC ANALYSIS. Seven *ERF* cDNAs (*NnERF1*-XM_010280223.1, *NnERF2*-XM_010276487.1, *NnERF3*-XM_010252213, *NnERF4*-XM_010263237.1, *NnERF5*-XM_010264002.1, *NnERF6*-XM_010247968.1, and *NnERF7*-XM_010243726.1) were cloned from RNA collected from lotus root ('E Lian 5') using the RNA-Seq and NCBI databases, all of which were partial coding sequences. These were aligned with the *Arabidopsis thaliana* *ERF* family. The amino acid sequences were obtained from the Arabidopsis Information Resource and NCBI databases. The subfamily name was based on a previous report (Nakano et al., 2006).

Phylogenetic analysis of the amino acid sequences showed that *NnERF* coding sequences could be divided into different groups (Fig. 7). *NnERF1* belongs to the subfamily III group; the *ERF* genes in this type have been reported to play an important role in cold response (Klay et al., 2014). *NnERF2* is closely related to subfamily VIII; the *ERF* genes in this type have been reported to play an important role in ethylene response and plant development (Pirrello et al., 2012). *NnERF3* is similar to subfamily V; the *ERF* genes in this type have been

reported to play an important role in ethylene and jasmonic acid response (Asahina et al., 2011). *NnERF4* and *NnERF6* in one group are similar to subfamily I; the *ERF* genes in this type have been reported to play an important role in abiotic responses, including cold and heat tolerance (Figuerola-Yañez et al., 2016). *NnERF5* is similar to subfamily IX; the *ERF* genes in this type have been reported to play an important role in regulating the plant defense response (Oñate-Sánchez et al., 2007). *NnERF7* is similar to subfamily II; the *ERF* genes in this type have been reported to be negative transcriptional regulators in defense responses to cold and drought stress in arabidopsis (Dong and Liu, 2010).

ERF GENE EXPRESSION. The seven *ERF* genes exhibited different expression patterns (Fig. 8). Transcripts of *NnERF3/4/5* were reduced continuously by the low temperature (4 °C). The expression of *NnERF3* peaked after 3 d, and *NnERF5* was the most strongly upregulated at 20 °C compared with at 4 °C, with its mRNA increasing in abundance ≈ 150 -fold after storage. The downregulation by low temperature (4 °C) of *NnERF3/4/5* is similar to the response of *ERF* genes in other plants, such as arabidopsis (Lim et al., 2007), *Cicer arietinum*,

Fig. 7. Phylogenetic tree of ethylene response factor (*ERF*) genes. Lotus root *NnERF* genes are highlighted in red. The amino acid sequences of the *Arabidopsis ERF* family were obtained from The Arabidopsis Information Resource (TAIR).

and *Cajanus cajan* (Agarwal et al., 2016). In contrast, transcripts of *NnERF1/2/7* increased at the low temperature (4 °C). Unlike the other *NnERF* genes, the expression of *NnERF6* showed no clear difference between the different temperatures (Fig. 8).

One of most interesting results is that *NnERF 3/4/5* were downregulated at the low temperature (4 °C), and this occurred concomitantly with the decrease in *NnPAL1*, *NnPPOA*, and *NnPOD2/3/4/5* gene expression and BD. This indicates that, in lotus root, the increased expression of *NnERF 3/4/5* was concurrent with an increase in browning, although the specific relationship between *ERF* and browning is unclear (and should be studied further).

The expression pattern of *NnERF4* further supports the prediction of phylogenetic analyses that the *ERF* genes in subfamily I have been reported to play an important role in cold and heat tolerance (Figueroa-Yañez et al., 2016). In addition, *NnERF3* and *NnERF5* were also observed to decrease expression in response to the low temperature (4 °C). However, *NnERF3* and *NnERF5* belong to subfamilies V and IX, respectively. Taken together, these results may indicate that more

subfamilies could be involved in the cold and heat response to browning in lotus root.

In addition to the three previously mentioned *NnERF* genes, *NnERF1/2/7* expression also exhibited interesting features. Unlike the positively regulated *ERF* genes, *NnERF1/2/7* mRNA levels were associated negatively with browning, which was upregulated by the low temperature (4 °C) in storage. It is well known that the processing of fresh-cut fruits and vegetables promotes faster physiological deterioration, biochemical change, and microbial degradation of the product, which may result in degradation of the color, texture, and flavor (Toivonen and Brummell, 2008). These results indicate that *NnERF1/2/7* might be involved in senescence-related or corruption processes for fruits and vegetables, but not browning. Similar results have also been reported for other fruits, such as *Actinidia sinensis*, in which *AdERF9* and *EILs* suppress the promoter activity of ripening-related genes and regulate fruit-ripening and -softening processes (Yin et al., 2010). It has also been reported that *GmERF113*, a novel glycine max *ERF* transcription factor, may play a crucial role in the defense against *Phytophthora sojae* infection (Zhao et al., 2017).

Fig. 8. Messenger RNA amounts from ethylene response factor (*ERF*) genes in response to different temperature treatments. Fresh-cut lotus root was stored at 4 °C (white) and 20 °C (black), separately. Error bars represent the SE from three biological replicates. LSD = least significant difference.

Conclusions

The degree of browning; PAL, PPO, and POD enzyme activity; and gene expression assay results in the current study showed that storage at a low temperature is effective in delaying lotus root browning. Seven *NnERF* genes were isolated and the transcriptional levels of *NnERF3/4/5* were associated with fresh-cut lotus root browning. *NnERF3/4/5* are prime candidates for additional transcriptional regulatory analysis in lotus root to understand the molecular control of browning.

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Supplemental Table 1. Information for all the ethylene response factors (*ERFs*) from the RNA-seq and National Center for Biotechnology Information database.

Gene ID	RPKM at 20 °C	RPKM at 4 °C
<i>NnERF1</i> -XM_010280223.1	31.31653549	2.035658
<i>NnERF2</i> - XM_010276487.1	1.109555535	—
<i>NnERF3</i> -XM_010252213	241.4901856	21.80209
<i>NnERF4</i> -XM_010263237.1	25.85743426	1.369193
<i>NnERF5</i> -XM_010264002.1	54.3935825	1.393153
<i>NnERF6</i> -XM_010247968.1	10.16833966	—
<i>NnERF7</i> -XM_010243726.1	0.423318324	—
<i>XM_010265290.1</i>		
<i>XM_010247014.1</i>		
<i>XM_010264001.1</i>		
<i>XM_010266949.1</i>		
<i>XM_010265165.1</i>		
<i>XM_010243704.1</i>		
<i>XM_010272292.1</i>		
<i>XM_010274498.1</i>		
<i>XM_010260532.1</i>		
<i>XM_010247969.1</i>		
<i>XM_010262090.1</i>		
<i>XM_010263926.1</i>		
<i>XM_010276018.1</i>		
<i>XM_010267377.1</i>		
<i>XM_010247418.1</i>		
<i>XM_010247244.1</i>		
<i>XM_010265251.1</i>		
<i>XM_010254050.1</i>		
<i>XM_010264640.1</i>		
<i>XM_010267180.1</i>		
<i>XM_010263467.1</i>		
<i>XM_010271692.1</i>		
<i>XM_010277121.1</i>		
<i>XM_010266854.1</i>		
<i>XM_010276486.1</i>		
<i>XM_010260875.1</i>		
<i>XM_010267406.1</i>		
<i>XM_010266127.1</i>		
<i>XM_010246498.1</i>		
<i>XM_010266126.1</i>		
<i>XM_010268255.1</i>		

RPKM = reads per kilobase per million reads.