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Analysis of transcriptome and phytohormone profiles reveal novel insight into ginger (*Zingiber officinale* Rose) in response to postharvest dehydration stress

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ABSTRACT

Dehydration stress is one of the severe postharvest problems of ginger. To better understand the molecular regulatory mechanism in ginger in response to postharvest dehydration stress, RNA-seq and phytohormone profiles analysis were performed in ginger rhizomes after 0 h, 2 h, 12 h, 24 h of postharvest water stress (with loss of 0 %, 1.7 %, 4.7 %, and 9.4 % of initial weight), respectively. The results indicated that postharvest dehydration stress contributes significantly to a loss of nutritious quality and storability in ginger rhizomes. Both the levels of abscisic acid (ABA) and salicylic acid (SA) markedly increased, however, auxin (indol-acetic acid, IAA), cytokinin (*tans*-zeatin, *tZ*), and gibberellin (GA_1 and GA_3) significantly decreased in ginger rhizomes under dehydration stress. Transcriptome analysis revealed a total of 1415, 2726, and 6641 genes were differently expressed after 2 h, 12 h, and 24 h of water-loss stress treatment compared with that in 0 h of ginger rhizomes, respectively. Additionally, 518 DEGs share similar expression patterns during 24 h of dehydration stress. These genes are mainly enriched in plant hormone signaling, phenylpropanoid biosynthesis, phenylalanine metabolism, fatty acid elongation, starch and sugar metabolism, and carotenoid biosynthesis. In addition, expression levels of MYB genes sharply increased in ginger rhizomes in response to water loss, which may function in regulation of lignin biosynthesis. These findings suggest that postharvest dehydration tolerance of ginger rhizomes may be mainly related to antagonistic regulation of endogenous phytohormones biosynthetic pathway and signaling, MYB transcription factors mediated lignin metabolism, antioxidant enzyme regulatory oxidative balance, and maintenance of energy supply. Our results provide new insights into molecular mechanism of ginger in response to postharvest dehydration which are of agricultural importance.

1. Introduction

Fruits and vegetables, after their harvest, are susceptible to fast deterioration resulting from various postharvest abiotic stress (e.g. cold/heat, low oxygen/light carbon dioxide, and dehydration) that eventually strongly declines their quality and commercial value (Chen et al., 2016). Among them, water loss or dehydration is one of the most unavoidable postharvest stress factors, which is particularly prone to induce undesirable quality traits including wilting, loss of colour, aroma and nutrients (Sagar and Kumar, 2010).

So far, the physiological and biochemical changes linked with postharvest water stress in fruits and vegetables have been deeply investigated. Several studies in grapes suggested that postharvest water loss significantly increases in soluble solids, ethanol, anthocyanin, and markedly induces senescence related hormones, such as ethylene and abscisic acid (Rizzini et al., 2009; Savoi et al., 2019). Water stress also sharply accelerated ripening of postharvest strawberry fruit accompanied with promotion of anthocyanin levels (Nunes et al., 2005), as well as in bell pepper fruit (Díaz-Pérez et al., 2007). Carrots that suffer with postharvest moisture loss become shriveled and are subjected to decay (Shibairo et al., 1997). Interestingly, the potential ability of prevention of moisture loss in different carrot cultivars mainly depends

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on specific surface area and relative electrolyte leakage from the roots (Shibairo et al., 1997). For litchi fruit, postharvest commercial value rapidly reduces due to the water loss-induced pericarp browning, which may result from both loss of the function of antioxidant and increase in the oxidation of phenolic (Jiang and Fu, 1999). Furthermore, water deficit stress, is an unavoidable postharvest problem for cut flowers (e.g. roses and chrysanthemum) during long distance shipping, which causes huge economical loss (Dai et al., 2012; Xu et al., 2013).

Additionally, molecular response to the dehydration has been extensively studied not only in model plants including Arabidopsis (Seki et al., 2002), rice (Rabbani et al., 2003), and maize (Zheng et al., 2004), but also in other horticultural crops including rose (Dai et al., 2012), chrysanthemum (Xu et al., 2013), grape (Rizzini et al., 2009; Savoi et al., 2019; Zenoni et al., 2016), and sweet orange (Romero et al., 2012). Several critical genes involved in water loss response have been comprehensively identified through microarray hybridization and transcriptome analysis, including 277 genes in Arabidopsis (Seki et al., 2002), 62 genes in rice (Rabbani et al., 2003), 123 genes in maize (Zheng et al., 2004), 3,513 uniESTs in rose (Dai et al., 2012), 8,558 unique transcripts in chrysanthemum (Xu et al., 2013), 2,933 genes in grape (Zenoni et al., 2016), and 1,471 genes in orange (Romero et al., 2012). These genes are classified into two groups, functional proteins and regulatory proteins respectively (Shinozaki and Yamaguchi-Shinozaki, 2007). Furthermore, biological function involved in regulation of dehydration tolerance of a few master genes from horticultural crops have been demonstrated, including a bHLH transcription factor gene, *VvbHLH,* in grape (Wang et al., 2016), *RhNAC2*, *RhNAC3*, and *RhFer1* from roses (Dai et al., 2012; Jiang et al., 2014; Liu et al., 2017), and *Hahb-4* from sunflower (Manavella et al., 2006).

Phytohormones also function in dehydration stress responses to balance plant growth and survival (Urano et al., 2017). Abscisic acid (ABA) enhances dehydration tolerance primarily through induction of protective protein expression, accumulation of metabolites, and regulation of stomata closure (Kim et al., 2010). Auxin may affect root architecture to enhance dehydration tolerance (Uga et al., 2013). Ethylene and gibberellin (GA) contribute to improving dehydration tolerance through inhibition of plant growth (Harb et al., 2010; Skirycz et al., 2011). In contrast, cytokinins negatively regulate dehydration stress via increasing the cell membrane integrity and ABA hypersensitivity (Nishiyama et al., 2011). However, further characterization of phytohormones metabolism as well as signaling networks in response to dehydration stress are required for precise clarification.

Ginger, one of the most common vegetables worldwide, has been used as a spice and natural additive for more than 2000 years (Bartley and Jacobs, 2000), and can also be utilized in several commercial value-added products like ginger tea, cookies, candy, bread, and ginger oil (El-Ghorab et al., 2010; Pruthi, 1992). However, dehydration stress is one of most severe postharvest problems in maintaining postharvest quality of ginger rhizomes during shipping and storage due to high water content in these vegetables (85 % in mature rhizomes, more than 90 % in immature rhizomes) (Kaushal et al., 2017; Zhang et al., 1994), which typically results in noticeable shriveling/desiccation (Kaushal et al., 2017), shortened shelf-life (Liu et al., 2016), and significant accumulation of crude fiber (Wang et al., 2011). Although application of chitosan, oligochitosan, and waxing significantly inhibited postharvest water loss in ginger rhizomes (Liu et al., 2016; Wu et al., 2019), phytohormones profiles and molecular regulatory mechanisms involved in response to postharvest dehydration stress in ginger rhizomes have not yet been illustrated.

In this study, we did a systematic analysis of transcriptome and phytohormone profiles in ginger to better understand and dissect the complex mechanisms responsible for postharvest dehydration stress, including 1) measuring the effect of dehydration stress on postharvest quality and storability of ginger rhizomes, 2) uncovering antagonistic regulatory mechanisms of phytohormones in ginger in response to postharvest dehydration, 3) obtaining a deeper understanding of molecular mechanism regarding ginger responses to dehydration stress.

2. Materials and methods

2.1. Plant materials and postharvest dehydration stress treatments

Ginger plants (*Z. officinale* Ros. cv. zhugen) were grown in a greenhouse of Chongqing University of Arts and Sciences, with photoperiod of 12 h light/12 h dark and light intensity of 200 μE m^{-2} s^{-1} (Jiang et al., 2018). In this study, the immature ginger rhizomes (with more than 90 % of water content) were harvested, and delivered to the lab within 1 h. Rhizomes with a uniform size (about 500 g), and without any vascular discoloration were used (Liu et al., 2016). Before treatment, all ginger rhizomes were disinfected with 1 % (v / v) sodium hypochlorite for 2 min, washed with tap water and then air-dried (Liu et al., 2016). For postharvest dehydration stress treatment, ginger rhizomes were held in a growth chamber with 22 ± 2 °C and 65 % relative humidity conditions. Ginger rhizome samples were collected after 0 h, 2 h, 12 h and 24 h of postharvest dehydration stress. Three biological and technical replicates were performed. All samples were frozen in liquid nitrogen immediately and stored at −80 °C.

2.2. Determination of ginger rhizome quality

The phenotypes of ginger rhizomes were recorded and photographed at each time point. In addition, water loss ratio (WLR) was measured following previously established protocols (Liu et al., 2016; Wu et al., 2019). Soluble sugar content (SSC), soluble protein content (SPC), and lignin content (LC) in ginger rhizomes after 0 h, 2 h, 12 h and 24 h of water stress were performed by the plant soluble sugar content test kit, total protein quantitative assay kit (Nanjing Jiancheng Bioengineering Institute, China) (Wu et al., 2019), and lignin content test kit (Jiangsu Mei Biao Biological Technology Co., Ltd, China) according to the manufacturer's protocol.

2.3. SOD, POD and CAT activities, as well as ROS and MDA content

Activities of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as malondialdehyde (MDA) content and reactive oxygen species (ROS) content in ginger rhizomes after 0 h, 2 h, 12 h and 24 h of dehydration were performed through enzyme linked immunosorbent assay (ELISA) using superoxide dismutase assay kit, peroxidase assay kit, catalase assay kit, malondialdehyde assay kit, and reactive oxygen species assay kit (Jiangsu Mei Biao Biological Technology Co., Ltd, China) according to the manufacturer's protocol (Zhao et al., 2018).

2.4. LC–MS/MS analysis of endogenous phytohormones content

The ginger rhizomes after 0 h, 12 h and 24 h of postharvest water deprivation were collected for endogenous phytohormones profiles analysis. Briefly, the phytohormones in ginger rhizomes were analyzed using liquid chromatography-tandem mass spectrometry (LC–MS/MS) equipped with an electrospray ionization (ESI) interface. LC–MS/MS was performed by Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China). Briefly, the freeze-dried rhizomes were crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 15 min at 30 Hz. 100 mg of powder mixed with 1 mL of 70 % aqueous methanol overnight at 4 °C. Following centrifugation at 10,000 *g* for 10 min, the extracts were absorbed (CNWBON Carbon-GCB SPE Cartridge, 250 mg, 3 mL; ANPEL, Shanghai, China) and filtrated (SCAA-104, 0.22 μM pore size, ANPEL, Shanghai, China).

The sample extracts were analyzed using an LC-ESI-MS/MS system (HPLC, Shim-pack UFLC SHIMADZU CBM30A system; MS, Applied Biosystems 6500 Q TRAP). Metabolite profiling was carried out using a widely targeted metabolome method by Wuhan Metware

Biotechnology Co., Ltd. (Wuhan, China). Quantification of metabolites was carried out using a multiple reaction monitoring (MRM) method. For each time point, three biological replicates were performed.

2.5. RNA extraction, RNA-seq, and bioinformatics analysis

12 of ginger samples after 0 h, 2 h, 12 h and 24 h of postharvest dehydration stress were harvested for RNA-seq. Total RNA was extracted and purified from ginger rhizomes using an EasyPure Plant RNA kit (Huayueyang Biotechnology Co., Ltd., China). Afterwards, the concentrations were measured using NanoDrop 2000 spectophotometer (Thermo). 3 μg of total RNA were used for RNA library construction. RNA-seq was performed by ABOROAD (Beijing, China). In brief, ribosomal RNAs were removed by Epicentre Ribo-Zero™ Gold Kits (Epicentre, USA), and then the strand-specific RNA-sequencing libraries were constructed by NEBNext Ultra™ RNA Libray Prep Kit from Illumina (NEC, USA). Afterward, the second-stand cDNA were synthesized, and the fragments with excepted size were sequenced. The raw reads are available at the NCBI Sequence Read Archive (SRA) under accession number PRJNA592215.

To harvest high quality of sequences, raw sequences and low-quality read were trimmed. And then, these assembly sequences referred to as unigenes were annotated through BLASTX/BLASTP similarity search using Trinotate programs (20140717). Functions of all annotated unigenes were determined by Clusters of Orthologous Groups of proteins (COG), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes database (KEGG) (E-value $\leq 1e^{-5}$) (Zou et al., 2018). The tool of Reads Per Kilobase Millon Mapped Reads (RPKM) and Deseq were used to calculate, and to analyze unigenes expression differentiation, respectively. Expressed differential genes were defined in condition of $\log^2$Ratio ≥ 1 and q (adjusted P value) < 0.05 (Tang et al., 2018).

2.6. Real-time RT-PCR analysis

To figure out the optimal internal reference genes for this assay, the expression stabilities of 10 potential reference genes from three traditional housekeeping gene families including actin, tubulin, as well as ubiquitin were evaluated by NormFinder analysis according to previous description (Andersen et al., 2004; Meng et al., 2013). First the expression levels of those genes were estimated by the Ct values in ginger rhizomes treated with dehydration stress using for transcriptome sequencing. The results showed that the Ct values varied between 17 and 27 (Fig. S3A). In addition, genes with the lowest normalization factor (NF) are considered to be the most stable (Andersen et al., 2004; Meng et al., 2013). Among them, both *TUB2* and *ACT11* had the lowest of NF, which means those two genes are the most stable genes in ginger rhizome in response to postharvest dehydration stress (Fig. S3B). Due to higher expression levels of *TUB2* than that of *ACT11* in ginger rhizomes (average Ct values of *TUB2* and *ACT11* are 19.7 and 23.8, respectively), therefore *TUB2* was chose to be the internal reference gene in our case. Primers were designed by online software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC = BlastHome) (Supplemental Table S1).

To validate the RNA-seq data and to analyze MYB transcription factors (TFs) genes expression levels, 9 differentially expression genes (DEGs) and 12 MYB TFs were selected for real time RT-PCR analysis using the same samples with 0 h, 2 h, 12 h and 24 h of water stress for transcriptome sequencing. 0.5 μg of total RNA from treated ginger rhizomes was used for first-stand cDNA synthesis by HiScript® II Q Select RT Supermix for qPCR (Vazyme, China) following the manufacture's protocol. The melting peaks and dissociation curves were evaluated for the specificity. For qRT-PCR analysis, 20 μL of PCR mixture comprising with 10 μL of 2 × ChamQ™ SYBR qPCR Master Mix (Vazyme), 0.6 μL of each primer (10 μM), 2 μL of cDNA, and 6.8 μL of single distilled water (SDW) were performed on QuantStudio 3 and 5 Real-Time PCR Systems (Thermo Fisher), under the following reaction condition: initiated by 5 min at 95 °C, and then followed with 45 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s, and completed with a melting curve analysis program (Wu et al., 2017). Three biological replicates were performed for all qRT-PCR analysis. Relative expression level was calculated based on the $2^{-\Delta\Delta Ct}$ method.

2.7. Data analysis

Data was analyzed by one-way ANOVA and Duncan's multiple range test by SPSS version 16.0 (SPSS Inc., USA). The results are means of three biological replicates with standard deviations. Effects were indicated to significant differences according to Duncan's multiple range test ($P < 0.05$).

3. Results

3.1. The influence of postharvest dehydration stress on quality of ginger rhizomes

In order to measure the effect of postharvest dehydration on ginger rhizome quality, ginger rhizome phenotypes were examined at each time point. We found that shriveling of the rhizomes became noticeable after 24 h of dehydration treatment with loss of 9.4 % of the initial weight (Fig. 1, Table 1). Postharvest nutritional quality can be assessed by looking at the levels of soluble sugar content (SSC) and soluble protein content (SPC) (Liu et al., 2016). We measured these and found that after 24 h of postharvest dehydration stress, both the SSC and SPC markedly reduced from 1.3 % to 0.7 %, and from 11.1 g kg^{-1} to 5.4 g kg^{-1}, respectively (Table 1). In addition, lignin accumulation results in lignification, which significantly influences postharvest storability and quality of loquat fruit (Xu et al., 2015). In the present study, the levels of lignification content (LC) in ginger rhizomes significantly increased from 5.7 g kg^{-1} to 13.3 g kg^{-1} (Table 1). Thus, postharvest

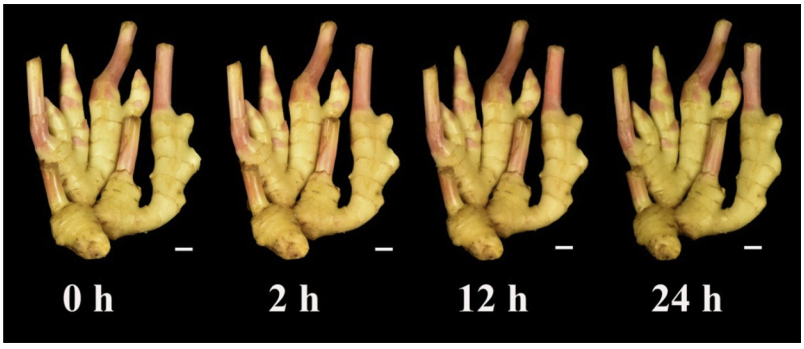

Fig. 1. The images of immature ginger rhizomes under postharvest dehydration stress. 0 h, 2 h 12 h and 24 h mean time course of dehydration treatment. Bar = 1 cm.

Table 1
Changes in water loss ratio (WLR), solution sugar content (SSC), solution protein content (SPC), and lignin content (LC) in ginger rhizomes after postharvest dehydration stress. 0 h, 2 h, 12 h, 24 h represent time of postharvest dehydration stress, respectively. The results are means of three biological replicates with standard deviations. Letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

Dehydration (h)	Water loss ratio (%)	SSC (%)	SPC (g kg^{-1})	LC (g kg^{-1})
0	—	1.3 ± 0.2a	11.1 ± 0.4a	5.7 ± 0.3a
2	1.7 ± 0.2a	1.1 ± 0.2b	9.7 ± 0.5b	6.8 ± 0.2a
12	4.7 ± 0.5b	0.9 ± 0.1c	7.8 ± 0.8c	8.6 ± 0.3b
24	9.4 ± 0.2c	0.7 ± 0.1d	5.4 ± 0.6d	13.3 ± 0.9c

dehydration stress significantly decreases the nutritional content, and enhances lignin accumulation of ginger rhizomes.

3.2. Antioxidant enzyme activities

Dehydration stress significantly increases production of reactive oxygen species (ROS), resulting in the loss of postharvest storability of vegetables and fruits (Chai et al., 2005). To investigate the effect of water stress on ginger postharvest storability, the ROS changes in ginger rhizomes were monitored. The content of ROS in ginger rhizomes strikingly accumulated after 2 h of dehydration treatment, and up to 15.2 ng kg^{-1} FW after 24 h of stress (Table 2). To counteract harmful effect of ROS, plants always increase antioxidant enzyme activities (Chai et al., 2005; Jin et al., 2006; Iturbe-Ormaetxe et al., 1998), thus the activity of antioxidant enzymes can be used to assess postharvest storability under dehydration stress. To test whether antioxidant enzymes activity changed in ginger rhizomes after postharvest dehydration stress, SOD, POD and CAT activities were measured. All of SOD, POD, and CAT activities significantly increased after water loss stress due to dehydration, and the activities were up to 15.7 (U kg^{-1} Pro × 10^6), 9.4 (U kg^{-1} Pro × 10), and 2.4 (U Kg^{-1} Pro × 10^4) after 24 h of treatment, respectively (Table 2). In addition, content of MDA was also detected to evaluate the level of membrane lipid peroxidation (Jin et al., 2006). We found that the MDA content of ginger rhizomes significantly accumulated after 12 h of dehydration and reached to 68.6 (mM kg^{-1} FW) after 24 h of stress (Table 2). Our results suggest that postharvest dehydration increases ROS generation resulting in loss of postharvest storability in ginger.

3.3. The influence of dehydration stress on endogenous phytohormones content

Plants alter endogenous phytohormones metabolism to survive under abiotic stress (Urano et al., 2017). To understand the temporal regulation of endogenous phytohormones metabolism in ginger rhizomes under dehydration stress, we analyzed levels of the bioactive phytohormones IAA, GA_1, GA_3, GA_4, GA_7, iP, *t*Z, *c*Z, JA, SA, and ABA in ginger rhizomes following 0 h, 2 h, and 24 h of dehydration stress using LC–MS/MS. Among these endogenous phytohormones, levels of GA_4 and GA_7 were not detected. However, we did detect GA_1 and GA_3 though they significantly dropped after 12 h and 24 h of dehydration stress, respectively (Table 3). Nonetheless, JA, iP and *c*Z levels did not significantly change in response to dehydration stress. However, *t*Z content markedly decreased after 12 h of treatment, although no significant change was measured between 12 h and 24 h after treatment. Similarly, we also observed a similar trend in IAA levels as *t*Z. In contrast both SA and ABA content significantly accumulated after 12 h of treatment, reaching a maximal value of about 105.7 (ng kg^{-1} FW × 10^2) and 36.9 (ng kg^{-1} FW × 10^3) after 24 h of treatment, respectively (Table 3). These results indicate that plant hormone ABA and SA may play antagonistic role with cytokinin, auxin and gibberellin in the regulation of dehydration tolerance of ginger rhizomes.

Table 3
The content of phytohormones in ginger rhizomes after postharvest dehydration stress. 0 h, 2 h, 12 h, and 24 h represent time of postharvest dehydration stress, respectively. ND means no detected. The results are means of three biological replicates with standard deviations. Letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

Phytohormones	Dehydration (h)		
	0	12	24
IAA (ng kg^{-1} FW × 10)	58.2 ± 5.7a	32.3 ± 5.5b	29.9 ± 4.3b
GA1 (ng kg^{-1} FW × 10)	72.4 ± 8.6a	48.3 ± 5.3b	33.0 ± 3.2c
GA3 (ng kg^{-1} FW × 10^2)	21.6 ± 2.4a	22.8 ± 1.5a	16.5 ± 1.4b
GA4	ND	ND	ND
GA7	ND	ND	ND
iP (ng kg^{-1} FW)	41.6 ± 4.1a	36.7 ± 3.6a	36.8 ± 4.0a
***t*Z** (ng kg^{-1} FW)	41.9 ± 2.8a	21.4 ± 1.7b	23.9 ± 2.7b
***c*Z** (ng kg^{-1} FW)	27.3 ± 3.2a	28.5 ± 5.2a	22.3 ± 3.7a
JA (ng kg^{-1} FW × 10^2)	24.4 ± 1.9a	27.0 ± 1.8a	26.5 ± 2.9a
SA (ng kg^{-1} FW × 10^2)	46.0 ± 1.2a	86.6 ± 4.7b	105.7 ± 9.2c
ABA (ng kg^{-1} FW × 10^3)	3.8 ± 0.4a	6.0 ± 0.5b	36.9 ± 2.9c

3.4. Transcriptional and functional enrichment analysis of ginger rhizomes in response to postharvest dehydration stress

To deepen understanding of transcriptome profiling in ginger rhizomes in response to dehydration stress, RNA-seq assay was used. This RNA-seq data was re-confirmed using qRT-PCR of 8 random selected genes. The results showed that RNA-seq and qRT-PCR data had similar expression pattern and a positive correlation ($R^2 = 0.92$). This indicates that the RNA-seq data is highly reliable. (Supplemental Fig. S1).

A total of 1415, 4726, and 6641 genes were differentially expressed (DEGs) following 2 h, 12 h, and 24 h of dehydration stress versus that in 0 h of control, respectively. Of these genes, 604, 2621 and 2921 genes were up-regulated, however, 811, 2105 and 2720 genes were down-regulated (Fig. 2A; Supplementary Table 2), thereby indicating that DEGs gradually increased alongside prolonging dehydration stress. In addition, a total of 518 DEGs share similar expression pattern during 24 h of stress treatment (Fig. 2B). This indicates that these 518 DEGs may play a critical role in regulation of dehydration tolerance in ginger rhizomes.

To illustrate how the various up-regulation and down-regulation of

Table 2
SOD, POD, and CAT activities, MDA and ROS content in ginger rhizomes after postharvest dehydration stress. 0 h, 2 h, 12 h, 24 h represent time of postharvest dehydration stress, respectively. The results are mean of three biological replicates with standard deviations. Letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

Dehydration (h)	SOD (U kg^{-1} Pro × 10^6)	POD (U kg^{-1} Pro × 10)	CAT (U Kg^{-1} Pro × 10^4)	MDA (mM kg^{-1} FW)	ROS (ng kg^{-1} FW)
0	4.7 ± 0.4a	3.5 ± 0.2a	0.9 ± 0.04a	42.6 ± 0.5a	7.4 ± 0.7a
2	6.4 ± 0.6b	4.4 ± 0.3b	1.1 ± 0.06ab	46.2 ± 0.5a	9.2 ± 0.8b
12	9.4 ± 0.8c	5.9 ± 0.4bc	1.5 ± 0.1b	55.0 ± 0.6b	12.9 ± 1.4c
24	15.7 ± 1.6d	9.4 ± 0.6d	2.4 ± 0.2c	68.6 ± 1.9c	15.2 ± 1.7d

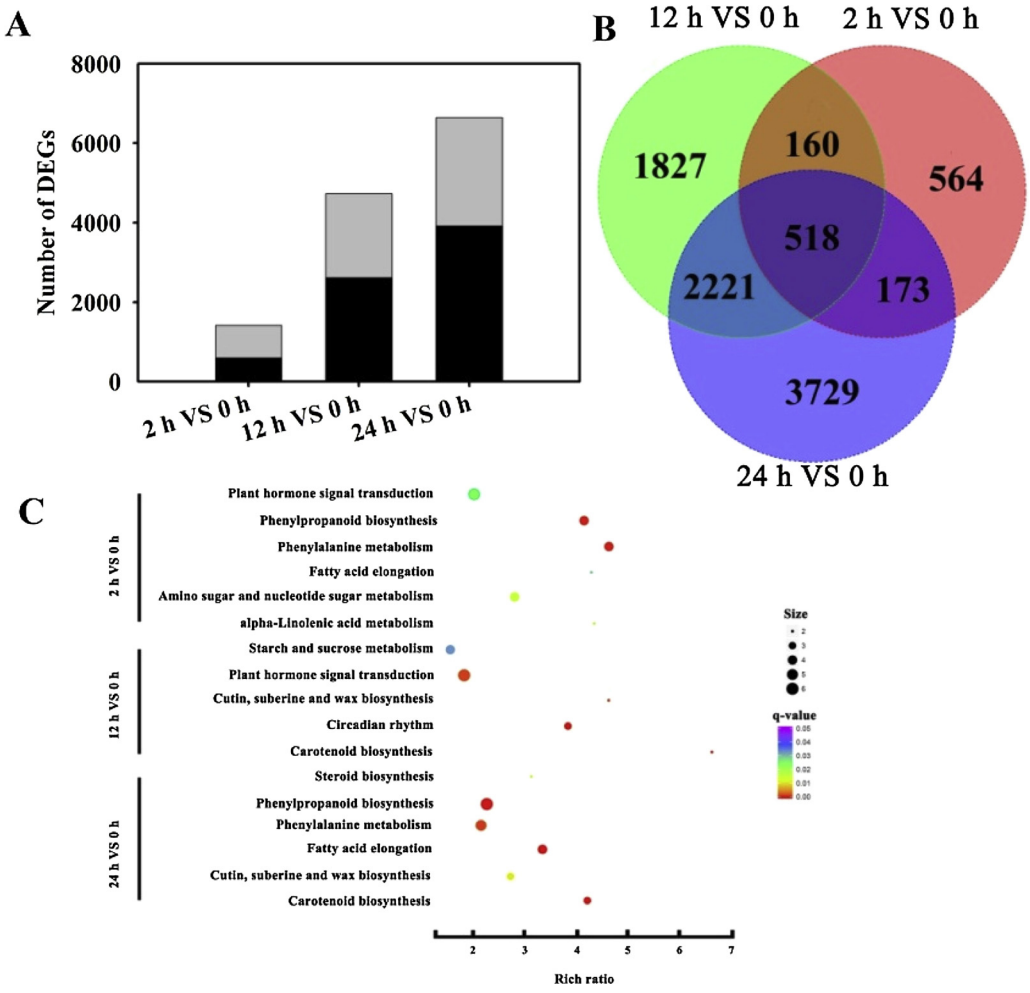

Fig. 2. Identification of differentially expression genes (DEGs) in ginger rhizomes in response to postharvest dehydration stress. (A) Number of DEGs in ginger rhizomes after 2 h, 12 h, 24 h of postharvest dehydration stress versus that in 0 h of control, respectively; Black means the number of significant up-regulated genes, grey means significant down-regulated genes. (B) Venn diagram of DEGs after 2 h, 12 h, 24 h of postharvest dehydration stress versus that in 0 h of control, respectively. (C) Functional enrichment analysis of DEGs in ginger rhizomes after 2 h, 12 h, 24 h of postharvest dehydration stress versus that in 0 h of control. Each point represents one Go enrichment item; the color of each point represents enrichment degree; the size of each point represents the number of this GO enrichment item. q-value indicates the adjusted *p*-value.

DEGs affect water stress, we grouped and analyzed genes based on their respective metabolic pathways using KEGG pathways database (Fig. 2C). After 2 h of dehydration stress, these DEGs, as early response genes, were mainly enriched in plant hormone signal transduction, phenylpropanoid biosynthesis, phenylalanine metabolism, and amino sugar and nucleotide sugar metabolism in ginger rhizomes. Interestingly, DEGs function in starch and sucrose metabolism, plant hormone signal transduction, and carotenoid biosynthesis were significantly enriched in the ginger rhizomes treated with 12 h of dehydration. Notably, after 24 h of water loss stress, the expression of these late response genes playing roles in phenylpropanoid biosynthesis, phenylalanine metabolism, fatty acid elongation, cutin, suberine, and wax biosynthesis, and carotenoid biosynthesis sharply changed (Fig. 2C). These data indicate that ginger rhizomes respond to postharvest dehydration stress mainly through antagonistic regulation of endogenous phytohormones biosynthetic pathways and signaling, maintenance of oxidative balance, and energy supply.

3.5. Involvement of genes in endogenous phytohormone biosynthesis and signaling in ginger rhizomes response to postharvest dehydration stress

Since the content of several endogenous phytohormones in ginger rhizomes significantly changed after postharvest dehydration stress, including ABA, SA, GAs, Auxin, and CTKs (Table 3), to further explore genes involved in these phytohormones biosynthesis and signaling, we screened key genes in RNA-seq data. We found increased expression of genes encoding vital enzymes in ABA and SA biosynthetic pathways, such as phytoene synthase (*PSY*), zeaxanthin epoxidase (*ZEP*), violaxanthin de-epoxidase (*VDE*), 9-*cis*-epoxycarotenoid dioxygenase (*NCED*), short-chain dehydrogenase/reductase (*SDR*), phenylalanine ammonia lyase (*PAL*) and isochorismate synthase (*ICS2*). Additionally, we also found an increase in positive regulators of signaling such as ABA receptors (*PYL12-like*) *ABI5*, *TRAB1*, pathogenesis-related proteins genes (*PR*), and WRKY transcription factor (*WRKY70*), suggesting that both biosynthesis and response pathway of ABA and SA are activated in response to dehydration (Fig. 3A, B). This is further supported by the reduction in negative regulator of ABA signaling, protein phosphatase 2C, *PP2C* (Fig. 3A). Interestingly, expression levels of three ABA

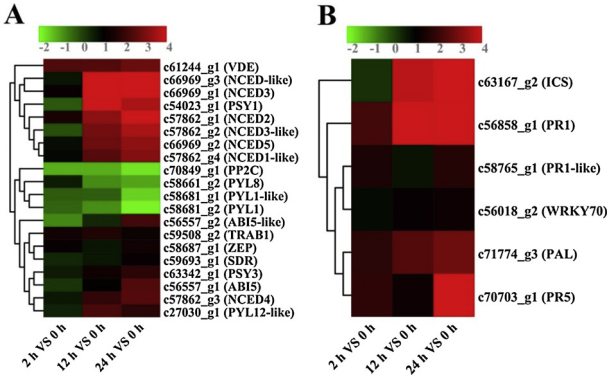

Fig. 3. Heatmap showing different expression level of DEGs associated with ABA and SA biosynthesis and signaling pathway. Red or green mean up-regulated or down-regulation, respectively. The value was showed by Log^2Ratio. (A) DEGs associated with ABA biosynthesis and signaling pathway; (B) DEGs associated with SA biosynthesis and signaling pathway.

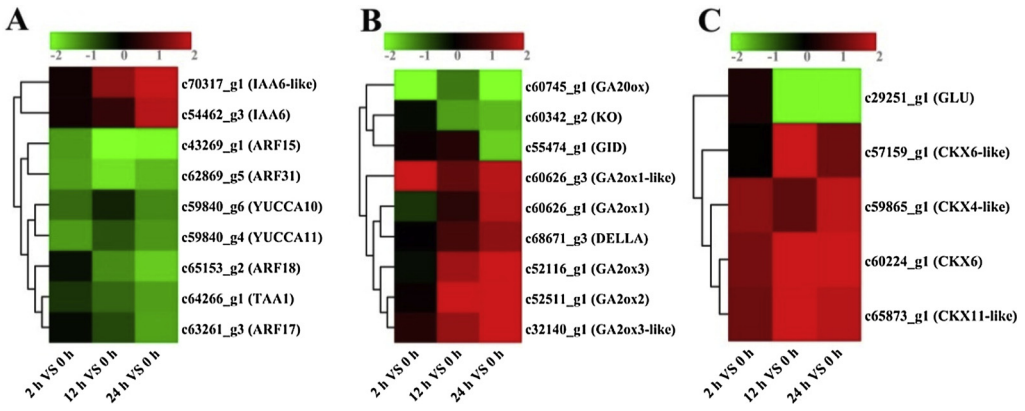

Fig. 4. Heatmap showing different expression level of DEGs associated with auxin, gibberellin and cytokinin biosynthesis and signaling pathway. Red or green mean up-regulated or down-regulation, respectively. The value was showed by Log^2Ratio. (A) DEGs associated with auxin biosynthesis and signaling pathway; (B) DEGs associated with GA biosynthesis and signaling pathway; (C) DEGs associated with cytokinin biosynthesis and signaling pathway.

receptors (*PYL1*, *PYL1-like* and *PYL8*) significantly decreased under dehydration stress (Fig. 3A). In addition, both *ABI5* and *ABI5-like* expression levels sharply reduced after 2 h and 12 h of dehydration, while they markedly increased after 24 h of stress treatment (Fig. 3A). These results suggest that both biosynthesis and ABA/SA response pathways have been activated to "resist" dehydration.

Contrarily, we observed a reduction in expression levels of genes function in auxin, cytokinin, and gibberellin biosynthesis and signaling in ginger rhizomes in response to water loss stress. This was supported by the reduction in expression of genes playing critical roles in biosynthesis of auxin, cytokinin and gibberellin, including *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS* 1 (*TAA1*), *YUCCA*, β-glucosidase gene (*GLU*), ent-kaurene oxidase (*KO*), and GA 20-oxidase (*GA20ox*). However, there was an increased expression of genes involved in gibberellin inactivation pathway and degradation of cytokinin, encompassing GA 2-oxidase (*GA2ox*) and cytokinin dehydrogenase (*CKX*) (Fig. 4A–C). Furthermore, we observed repression of positive regulators of gibberellin and auxin signaling like GA receptor (*GID*) and auxin response factor (*ARF*), and induction of negative regulator of auxin and gibberellin, such as *AUX/IAA* and *DELLA* (Fig. 4A, B). These results suggest that both biosynthesis and response pathway of auxin, cytokinin and gibberellin are also involved in playing a role of improving postharvest dehydration tolerance in ginger, through the antagonism of ABA and SA.

3.6. Induction genes of lignin biosynthesis and regulation-related in ginger rhizomes under postharvest dehydration stress

In ginger rhizomes, crude fiber always increases with aging (Aggarwal et al., 2002), or during postharvest storage through unclear mechanisms (Wang et al., 2011). The crude fiber was constituted with cellulose, lignin, and hemicellulose (Williams and Olmsted, 1935). Our results found that the content of lignin significantly increased in ginger rhizomes after postharvest dehydration (Table 1), which may contribute to understanding of crude fiber accumulation during postharvest storage.

To further uncover the molecular mechanism involved in regulation of lignin biosynthesis in ginger rhizomes after postharvest dehydration, we screened genes linked to lignin biosynthesis from RNA-seq data. We found that the expression level of phenylalanine ammonia lyase (*PAL*), laccase-3-like (*LAC3-like*), and shikimate O-hydroxycinnamoyltransferase (*HCT*) significantly increased in response to postharvest dehydration stress (Fig. 5), which could explain lignin accumulation in ginger under water loss stress.

Additionally, regulation of lignin biosynthetic genes is primarily done through binding of MYB transcription factors to AC-rich elements (Zhao and Dixon, 2011). In our RNA-seq data, a total of 95, 199, and 260 transcription factors (TFs) were differently expressed in ginger rhizomes, respectively (Supplemental Fig. S2), mainly belonging to

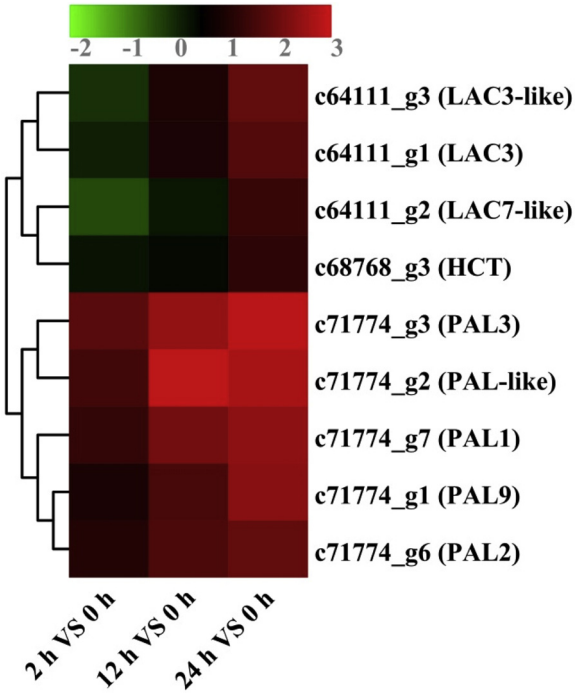

Fig. 5. Heatmap showing different expression level of DEGs associated with lignin biosynthesis pathway. Red or green mean up-regulated or down-regulation, respectively. The value was showed by Log^2Ratio.

bHLH, AP2/ERF, MYB, HB, and WRKY transcription factor families. Among them, the expression of 11, 19 and 31 MYB TFs significantly changed after 2 h, 12 h, 24 h of dehydration stress, respectively (Supplemental Fig S2). To screen MYB TFs involved in regulation of lignin biosynthesis in ginger rhizomes under water stress, a total of 12 differently expressed MYB genes expression levels, under dehydration stress in ginger rhizomes, were measured using qRT-PCR (Fig. 6). Among these MYB TFs genes, expression level of *c66024_g6*, *c68070_g1*, *c56414_g1*, *c55854_g1*, *c49329_g1*, and *c53852_g2* significantly increased in ginger rhizomes after 2 h stress and continued to increase after 12 h and/or 24 h treatment. These results suggest postharvest dehydration stress causes up-regulation of MYB transcription factors which enhance lignin biosynthetic gene expression.

4. Discussion

Postharvest dehydration or water loss of vegetables and fruits is usually accompanied by physiological and biochemical changes, ultimately resulting in huge economical loss (Chen et al., 2016). In our study, noticeable shriveling/desiccation phenotypes of ginger rhizomes

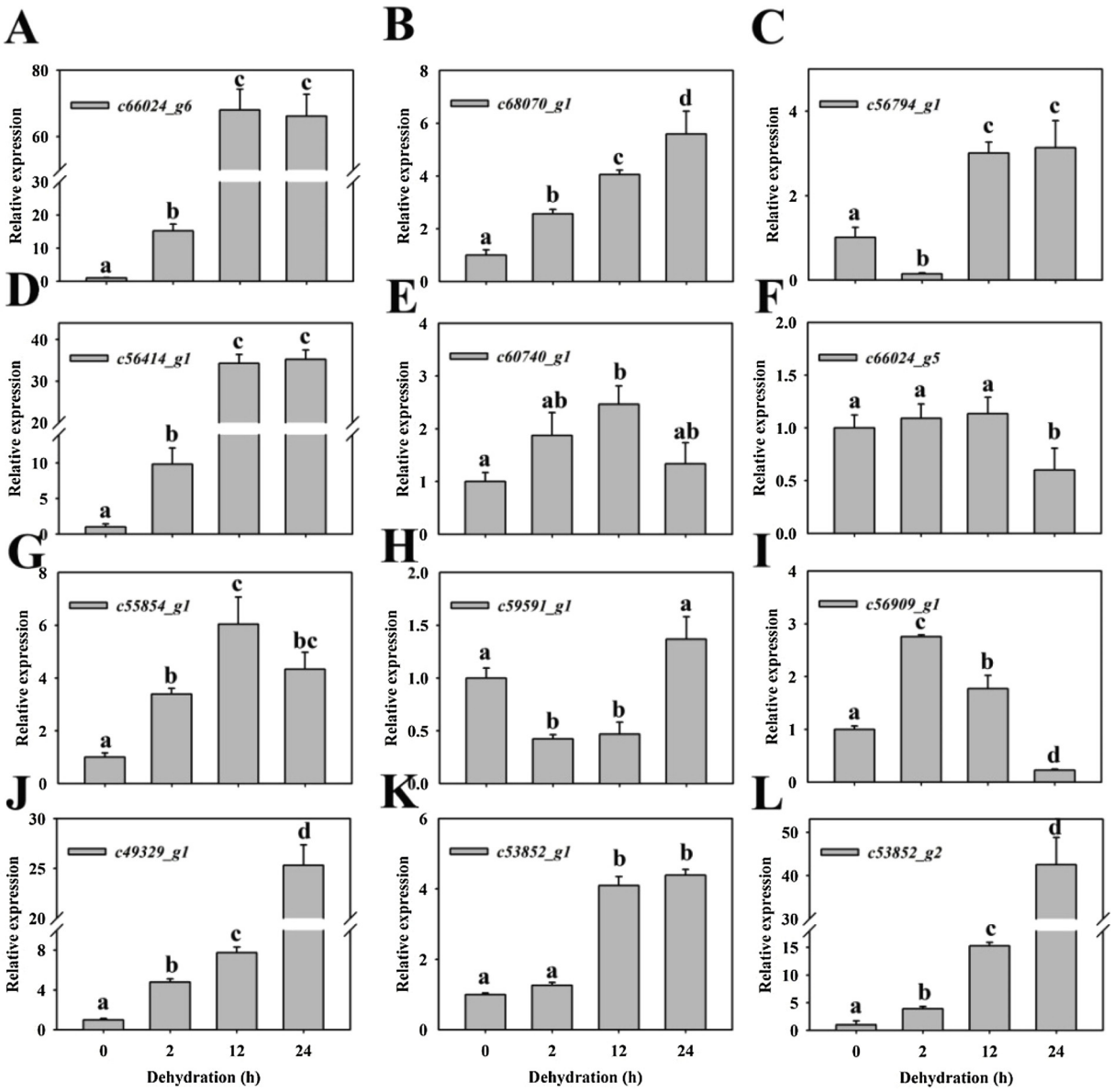

Fig. 6. Expression level of MYB transcription factors in ginger rhizomes in response to postharvest dehydration stress. 2 h, 12 h, 24 h represent time of postharvest dehydration stress, respectively. The results are the means of three biological replicates with standard deviations. Letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

were observed after 24 h of postharvest dehydration stress with 9.4 % of water loss ratio (Fig. 1, Table 1). This was similar to a previous report which stated that shriveling of the ginger rhizomes becomes noticeable after the loss of 10 % of the initial harvest weight (Kaushal et al., 2017). In addition, several basic postharvest quality parameters (SSC and SPC) of ginger significantly decreased under dehydration stress (Table 1). These results were supported by previous reports regarding the reduction of SSC or TA in several vegetables during postharvest storage, including sugarbeet roots (Lafta and Fugate, 2009), and ginger rhizomes (Liu et al., 2016). Lignin accumulation results in lignification, and significantly influences crops postharvest quality (Xu et al., 2015). In the present study, we found that the level of LC markedly accumulated in ginger rhizomes after dehydration stress (Table 1). It was also reported that elevation of lignin content was found in other crops containing etiolated asparagus spears (Hennion et al., 1992), green asparagus (Liu and Jiang, 2006), and loquat fruit (Xu et al., 2015) during postharvest storage. Interestingly, in order to prevent water loss, lignin was also significantly accumulated in carrots under dehydration and wounding stress (Becerra-Moreno et al., 2015). Therefore, our results may supply novel evidence for dehydration regulation of lignin accumulation during fruits or vegetables postharvest storage.

The reactive oxygen species (ROS) induced by water loss/dehydration can result in severe damage to lipid, proteins and DNA, and loss of postharvest storability (França et al., 2007). To maintain balance of oxidative stress, up-regulation of antioxidant enzyme system is an effective strategy. This view was supported by research of banana plantlets (Chai et al., 2005), maize (Jiang and Zhang, 2002), and roses (Jin et al., 2006). In the present study, we found SOD POD, and CAT activities significantly increased, as well as accumulation of ROS and MDA, in ginger rhizomes after postharvest dehydration stress (Table 2). This indicates oxidative damage is a master factor caused by postharvest dehydration resulting in loss of postharvest storability in ginger.

Phytohormones play crucial roles in the regulation of plant growth, development, and adaptation response in aversive environments, including drought (Ross et al., 2011; Santner and Estelle, 2009). ABA is considered one of the main plant signals which confers an enhanced dehydration tolerance (Duan et al., 2007) as found in citrus fruit (Romero et al., 2012), radiata pine (De Diego et al., 2012) and

Arabidopsis (Urano et al., 2017). In this study, we found that in ginger rhizomes after postharvest dehydration stress, ABA content significantly increased (Table 3), providing evidence for ABA accumulation in response to dehydration stress. On the other hand, GA_1, GA_3, and *tZ* content markedly decreased in ginger rhizomes in response to postharvest dehydration stress (Table 3). Similarly, in mature Arabidopsis leaves, the levels of CK and GA also clearly reduced after moderate dehydration stress (Urano et al., 2017), suggesting that these hormones play an antagonistic role to ABA during post-harvest dehydration stress. These data indicate that plant-kingdom conserved phytohormones response to dehydration also exists in ginger.

Notably, the increase in JA content was recognized as a concomitant in the ABA levels and was necessary for the induction of ABA signaling pathway (López-Ráez, 2016). However, JA content did not significantly change in ginger rhizomes treated with dehydration (Table 3). Additionally, it has been previously reported that SA is reduced under dehydration stress in radiata pine (De Diego et al., 2012) and Arabidopsis (Urano et al., 2017), while IAA was not found to significantly change in Arabidopsis under drought stress (Urano et al., 2017). Interestingly, we found significantly increased amount of SA and decreased content of IAA in ginger rhizomes under water loss stress (Table 3). This suggests that the role of SA and IAA in postharvest dehydration in ginger could have different regulatory roles compared to Arabidopsis (Urano et al., 2017) and radiata pine (De Diego et al., 2012) and may serve as an important area for future study.

Fascinatingly, expression levels of genes involved in phytohormones biosynthesis and signaling also significantly altered in our data, such as *PLYs*, *ZEP*, *PSY*, and *NCED5*. These results were in accordance with the previous findings that conferring of drought tolerance was observed by phytoene synthase (*CpPSY3*) from grapefruit (Cidade et al., 2012), zeaxanthin epoxidase (*MsZEP*) from alfalfa (*Medicago sativa*) (Zhang et al., 2016), ABA receptors (*PYLs*) and 9-cis-epoxycarotenoid dioxygenase (*NCED5*) from Arabidopsis (Frey et al., 2012; Mega et al., 2019), and GA-inactivating enzyme (*GA2ox*) in Maize (Shan et al., 2013). Notably, some different findings also exist in our data compared to previous reports. For example, previously it was reported that overexpression of *AtYUC6* in potato establishes enhanced drought tolerance through regulated ROS homeostasis and increase in auxin content (Cheol Park et al., 2013), suggesting that *YUCCA* genes may play a positive role in regulation of drought stress in Arabidopsis. While reduced genes expression of *YUCCA* and *TAA1* was observed in ginger rhizomes in response to dehydration stress (Fig. 4A), implying that *YUCCA* and *TAA1* may play an adverse role in controlling dehydration in ginger. Additionally, drought induced elevation of CK content in roots of tobacco mainly by decrease of cytokinin oxidase/dehydrogenase (CKX) activity (Novakova et al., 2006). However, the expression of *CKX* clearly increased in ginger rhizomes after water stress (Fig. 4C). Therefore, our results provide new insights into phytohormones cross-talk regulation of dehydration tolerance through significant changes of expression levels of genes involved in plant hormones biosynthesis and signaling in ginger. However, further studies need to figure out how these complex phytohormones cross-talk play a role in regulation of ginger dehydration tolerance.

Furthermore, expression level of genes associated with lignin biosynthesis pathway from RNA-seq data significantly increased, including *PAL* (Raes et al., 2003), *LAC3-like* (Leonowicz et al., 2001) and *HCT* (Sonbo et al., 2009) (Fig. 5). These results were supported by previous reports that activation of *PAL* was observed when carrots were treated with water stress during storage (Becerra-Moreno et al., 2015). Similarly, the expression levels of genes playing critical roles in lignin biosynthesis pathway were significantly up-regulated in grapevine berries under postharvest dehydration process, comprising cinnamate 4-hydroxylase (*C4H*), 4-hydroxycinnamate: CoA ligase (*4CL*), cinnamoyl CoA reductase (*CCR*), caffeoyl CoA 3-*O*-methyltransferase (*CCoAOMT*) (Zenoni et al., 2016). This may explain the significant accumulation of lignin content in ginger in response to dehydration (Table 1). In addition, we found that 6 MYB TFs family genes, including *c66024_g6*, *c68070_g1*, *c56414_g1*, *c55854_g1*, *c49329_g1*, and *c53852_g2* significantly continued to increase in expression level in ginger rhizomes during the dehydration stress (Fig. 6). We speculate these MYB TFs may play critical roles in regulation of lignin biosynthesis by modulating the expression of *PAL*, *LAC3-like*, and *HCT* in ginger rhizomes during dehydration stress. This was supported by previous studies that AtMYB85, AtMYB58, and AtMYB63 in Arabidopsis were the first true lignin-specific transcription factors (Zhou et al., 2009). Furthermore, PtMYB1, PtMYB4, and PtMYB8 from *Pinus taeda* (Bomal et al., 2008); EgMYB2 from *E. gunnii* (Goicoechea et al., 2005); AmMYB308 from *Antirrhinum majus* (Tamagnone et al., 1998); and ZmMYB31 and ZmMYB42 from *Zea mays* (Sonbol et al., 2009) were also reported as lignin transcriptional regulatory factors.

5. Conclusions

In the present study, our results revealed that postharvest dehydration stress significantly decreases the nutritional content of ginger rhizomes (SSC and SPC) and enhances lignin accumulation. Dehydration stress also increases ROS generation which results in significant decreasing of postharvest storability of ginger rhizomes. In addition, ginger rhizomes increase dehydration tolerance mainly through antagonistic regulation of phytohormones biosynthesis and signaling. Furthermore, postharvest dehydration stress induces lignin accumulation mainly via MYB TFs - mediated regulation pathway. Therefore, our results provide not only new insights into horticultural crops in response to postharvest dehydration stress, but also the foundation of useful gene resources for the development of new ginger varieties with increased water loss tolerance through genetic engineering in the future.

Authors statement

Honghai Li and Zhengguo Li: substantial contribution to conception and design; **Honghai Li and Ning Tang**: substantial contribution to acquisition of data; **Ran Liu and Zhao Jin**: substantial contribution to analysis and interpretation of data; **Lin Wu**: drafting the article; **Lin Wu and Yiqing Liu**: critically revising the article for important intellectual content; **Yiqing Liu and Zhengguo Li**: final approval of the version to be published.

We would like to submit the enclosed manuscript entitled “Analysis of transcriptome and phytohormone profiles reveal novel insight into ginger (*Zingiber officinale* Rose) in response to postharvest dehydration stress”, which we wish to be considered for publication in Postharvest Biology and Technology. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Declaration of Competing Interest

We would like to submit the enclosed manuscript entitled “Analysis of transcriptome and phytohormone profiles reveal novel insight into ginger (*Zingiber officinale* Rose) in response to postharvest dehydration stress”, which we wish to be considered for publication in Postharvest Biology and Technology. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2019.111087.

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