Transcriptomics and Metabolomics Reveal the Possible Mechanism by which 1-MCP Regulates the Postharvest Senescence of *Zizania latifolia*

Mohamed Hawali Bata Gouda\textsuperscript{1,a}, Sijia Peng (彭思佳)\textsuperscript{1,a}, Renying Yu (虞任莹)\textsuperscript{1}, Jianqi Li (李建奇)\textsuperscript{2}, Guihong Zhao (赵贵红)\textsuperscript{2}, Yuru Chen (陈育如)\textsuperscript{1}, Huibo Song (宋慧波)\textsuperscript{2,*} and Haibo Luo (罗海波)\textsuperscript{1,1*}

\textsuperscript{1} School of Food Science and Pharmaceutical Engineering, Nanjing Normal University, Nanjing 210023, China

\textsuperscript{2} College of Agricultural and Biological Engineering (College of Tree Peony), Heze University, Heze, 274015, China

* Correspondence to: Huibo Song, College of Agricultural and Biological Engineering (College of Tree Peony), Heze University, 2269 Daxue Road, Heze, 274015, China. E-mail address: Huibo Song 729061751@qq.com; Haibo Luo, School of Food Science and Pharmaceutical Engineering, Nanjing Normal University, 2 Xuelin Road, Nanjing, 210023, China. E-mail address: luohaibo_1216@126.com

\textsuperscript{a} Co-first authors.

© The Author(s) 2022. Published by Oxford University Press on behalf of Zhejiang University Press. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Abstract

To understand the mechanism governing the postharvest senescence of *Zizania latifolia*, and the regulatory mechanism induced by 1-methylcyclopropene (1-MCP) during storage at 25°C, physiobiochemical and conjoint analyses of the transcriptome and metabolome were performed. The results indicated that 1-MCP treatment engendered changes in the expression of genes and metabolites during the postharvest storage of *Z. latifolia*. The 1-MCP treatment maintained a good visual appearance, preserved the cell structure, and membrane integrity of *Z. latifolia* by keeping the expression of membranes-related lipolytic enzymes (and related genes) low and the amount of phosphatidylethanolamine high. Compared to the control group, 1-MCP treatment enhanced the activities of antioxidant enzymes, resulting in a decrease of reactive oxygen species (ROS) and malondialdehyde (MDA) contents, and thus inhibition of oxidative damage and loss of membrane integrity. In addition, 1-MCP treatment retarded the senescence of *Z. latifolia* by down-regulating the expression of ethylene biosynthesis-related genes and promoting up-regulation of brassinosteroid insensitive 1 (BRI1) kinase inhibitor 1, calmodulin (CaM), glutathione reductase, jasmonate amino acid synthase, and mitogen-activated protein kinase (MAPK)-related genes. Moreover, 1-MCP retarded *Z. latifolia* senescence by inducing the activity of ATP-biosynthesis related genes and metabolites. Our findings should facilitate future research on the postharvest storage of *Z. latifolia*, and could help delay senescence and prolong the storage time for commercial applications.

**KEYWORDS**

*Zizania latifolia*; Genes; Metabolites; Postharvest Senescence; Pathways
Graphical Abstract

(25 °C, 6 days of storage time)

Physiobiochemical analysis

Transcriptome analysis

Metabolome analysis

Joint transcriptome and metabolome analysis
Introduction

*Zizania latifolia* belongs to the *Oryzae* family and has been harvested for more than a thousand years. It is used as an aquatic crop around the world, especially in Asian countries. *Z. latifolia* is an important vegetable with high nutritional and economic value (Zhang et al., 2021). It is also used to prevent and treat metabolic diseases (Yan et al., 2018). However, its shelf-life and quality during postharvest storage are reduced due to respiratory disorders, shell etiolation, surface browning, transpiration, and tissue hollowness (Luo et al., 2019; Luo et al., 2012). Therefore, there is a need to better understand the physiological, biochemical and molecular processes that occur under postharvest senescence and effective development postharvest treatments that can delay senescence and quality deterioration.

Previous studies reported that 1-methylcyclopropene (1-MCP) treatment could maintain postharvest quality and extend the shelf-life of fruit and vegetables, including pak choi (Song et al., 2020), celery (Massolo et al., 2019), chive (Dai et al., 2021), broccoli (Yuan et al., 2010), green bell pepper (Cao et al., 2012), tomato (Min et al., 2018), kiwifruit (Ali et al., 2020), apple (Lv et al., 2020), pear (Cheng et al., 2019), and peach (Qian et al., 2021). By interacting with ethylene receptors, 1-MCP regulates the level of ethylene to avoid ethylene-dependent reactions (Massolo et al., 2019). In addition, studies have demonstrated that 1-MCP treatment inhibited the production of ethylene in apples by down-regulating the expression of ethylene biosynthesis-related genes (Yang and Song, 2012). A 1-MCP treatment delayed the ripening and senescence of climacteric and non-climacteric crops, such as guava, *joanna-red* plums, and eggplant, by inhibiting ethylene production as well as the activities of phenylalanine ammonia lyase (PAL) and peroxidase (POD), and decreasing the reactive oxygen species (ROS) and malondialdehyde (MDA) contents (Song et al., 2020; Singh and Pal, 2008).

“Omnics” technologies have been used to shed light on the fundamental processes involved in the senescence of horticultural products by collecting information at the DNA, RNA, protein, and metabolite levels (Witzel et al., 2015). Transcriptomic technology has been used to explore changes
in salad leaves 24 hours after stress treatment (Cavaiuolo et al., 2017) and to identify the genes regulated in the avocado-chitosan-Colletotrichum interaction system (Xoca-Orozco et al., 2017). In a previous study, proteomic techniques were used to understand the molecular mechanism underlying the senescence of Z. latifolia (Luo et al., 2019). Transcriptomic, proteomic, and metabolomic techniques have been used to study the mechanism regulating the peel ripening of harvested bananas (Zhang and Hao, 2020) and the cellular response to environmental stress in Brassicaceous vegetables (Witzel et al., 2015). Moreover, integrated metabolomic and transcriptomic analyses of plant organs have been well documented and can provide phenotypic data informing transcriptome analyses (Ying et al., 2019). Transcriptomic and metabolic techniques have been integrated to study the mechanism underlying the postharvest senescence of citrus fruit (Ding et al., 2015), fruit development and flesh coloration in Prunus mira (Ying et al., 2019), leaf yellowing in Pak choi (Song et al., 2020) and the salinity adaptation mechanism in sugar beet roots (Liu et al., 2020), as well as the seed germination mechanism in Punica granatum (Fu et al., 2021). However, to the best of our knowledge, there is no information regarding the use of transcriptomics and metabolomics during postharvest storage of Z. latifolia. Thus, in this study, we combined transcriptomic profiling and metabolomics to improve our knowledge of the molecular mechanism underlying Z. latifolia postharvest senescence and the regulatory effect of 1-MCP during storage at room temperature.

This study investigated the effect of 1-MCP on changes in overall appearance, cell ultrastructure, and physiological and biochemical parameters such as the antioxidant system and lipid metabolism during postharvest storage at room temperature (25 °C). Thereby shedding light on the mechanism of Z. latifolia postharvest senescence based on a combined analysis of the transcriptome and metabolome. The results improve our understanding of the mechanism by which 1-MCP treatment regulates the postharvest senescence of Z. latifolia, and should facilitate efforts to maintain quality and extend shelf-life.
Materials and methods

Plant material

Fresh *Z. latifolia* stems were hand-harvested from commercial farmland in Yixing, Jiangsu Province, China. They were selected based on similarity in size, color, and absence of visible defects and then arbitrarily divided into two 15 kg groups of vegetables. The first group of vegetables was treated with 10 µL L⁻¹ 1-MCP (AgroFresh, Philadelphia, PA, USA) in a sealed chamber at room temperature (25 °C) for 20 h. The second group (CK) was subjected to the same conditions without exposure to 1-MCP. Following treatment, the chambers were opened and all groups of vegetables were stored at room temperature for 6 d. Samples were taken out at 0 (before treatment, CK0), 1 (CK1, 1-MCP1), 3 (CK3, 1-MCP3), and 6 (CK6, 1-MCP6) d, and manually peeled to remove roots. Afterward, about 5 cm of the stem was removed from each end of individual vegetables with a sharp stainless-steel knife; the remainder was used for index analysis, or directly frozen in liquid nitrogen and stored at −80 °C until further analysis.

Determination of color

The CIE L, a, and b values of *Z. latifolia* was measured using a portable colorimeter (CR-200; Minolta, Osaka, Japan) at three sides (top, middle, and bottom) of eight samples. The whiteness was represented by L value (L = 0, black; L = 100, white); a value described the reddishness (a+ = red; a− = green); b value indicated the yellowness (b+ = yellow; b− = blue).

The formula stated by Luo et al. (2012) was used for the determination of the total color difference (ΔE). \( \Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2} \) (where \( L_0, a_0, \) and \( b_0 \) are the readings at the beginning of storage, and \( L, a, \) and \( b \) are the individual readings at each storage time point thereafter).
Ultrastructure visualization

The ultrastructure of *Z. latifolia* cell was visualized using the method of Li et al. (2009) with some modification. Pieces of *Z. latifolia* were collected from the cut surface of three *Z. latifolia* per treatment. The pieces were resuspended in pH 7.2 phosphate-buffered saline (PBS) containing 5 mM cerium chloride (CeCl₃) and incubated at 28 °C for 1.5 h after pretreatment. Cells were collected by centrifugation and the supernatant containing residual CeCl₃ was discarded. The cells were resuspended in 3 % glutaraldehyde for more than 4 h and pelleted by centrifugation. After fixing with osmium tetroxide and embedding with epoxy resin, the material was sliced and observed with a transmission electron microscope at an acceleration potential of 80 kV.

Determination of ascorbic acid (AsA) content

The 2,6-dichlorophenol indophenol method reported by da Silva et al. (2017) was used to measure the AsA content of *Z. latifolia* samples. The results are expressed as grams of AsA per kilogram on a fresh weight basis.

Determination of superoxide anions (\(\text{O}_2^-\)) and hydrogen peroxide (\(\text{H}_2\text{O}_2\))

The production rate of \(\text{O}_2^-\) and the content of \(\text{H}_2\text{O}_2\) were measured according to the method of Bata et al. (2021) with few modifications. A 2 g of ground *Z. latifolia* sample was mixed with 5 ml of 65 mM potassium phosphate buffer solution (pH 7.8), 1 mL of 10 mM hydroxylamine hydrochloride and ethylenediamine tetra acetic acid (0.1 M). The mixture was then centrifuged for 15 min (10 000 \(\times\) g, 4 °C). 2 mL of 7 mM \(\alpha\)-naphthylamine and 2 mL of 17 mM p-amino-benzenesulphonic acid were added to 2 mL of supernatant obtained after the centrifugation. The reaction mixture was kept at 37 °C for 15 min in a water bath and mixed with 3 mL of anhydrous ether. After the second centrifugation, the water phase was eliminated and the absorbance (\(A_{530}\)) value was measured. \(\text{O}_2^-\) production rate was calculated using a standard curve and expressed on a fresh weight basis.
Next, 6 mL of acetone pre-cooled at 4 °C were added to 2 g of ground *Z. latifolia* sample for \( \text{H}_2\text{O}_2 \) determination. After centrifugation (10 000 \( \times \) g for 15 min at 4 °C) of the mixture, 2 mL of the supernatant were mixed with 0.2 mL of concentrated ammonia and 0.1 mL of 5 % titanium sulfate for a second centrifugation at 3 000 \( \times \) g for 10 min. The precipitate was cleansed thrice with acetone and dissolved in 5 mL of 2 M sulfuric acid. The volume was adjusted to 10 mL after dissolution and then cooled down. The \( A_{415} \) value was measured and is expressed in mM kg\(^{-1}\) of the fresh sample.

**Determination of the MDA content**

The method of Song *et al.* (2020) was modified and utilized for determination of the MDA content. Initially, 5 mL of 5 % TCA was added to 2 g of ground *Z. latifolia* and centrifuged at 10 000 \( \times \) g (10 min; 4 °C). Afterward, 2 mL of the crude enzyme solution and 0.67 % thiobarbituric acid (TBA) were mixed, while in the control only distilled water was added to the supernatant. The mixture was heated at 100 °C for 30 min, cooled, and then centrifuged at 10 000 \( \times \) g for 10 min at 4 °C. The absorbance values were measured at 450, 532, and 600 nm and the below formula was used to calculate the MDA content:

\[
C (\mu\text{M kg}^{-1}) = [6.45(A_{532} - A_{600}) - 0.56A_{450}] \times V_t / (V_s \times \text{FW}),
\]

Where \( C \) is the MDA content; \( A_{450}, A_{532}, \) and \( A_{600} \) are the absorbance values at 450, 532, and 600 nm, respectively; \( V_t \) is the total volume of the extract solution; \( V_s \) is the volume of the extract solution contained in the mixture; and \( \text{FW} \) is the fresh weight of samples.

**Determination of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities**

Ground *Z. latifolia* was added to different amounts of pre-cooled (at 4 °C) phosphate buffer to prepare the crude extract for determination of antioxidant enzymes (SOD, CAT, and APX) activities as described in our previous investigation (Bata *et al.*, 2021). For SOD activity, the absorbance was determined at 560 nm after exposure to light. One unit of SOD activity was defined as the amount of
enzyme that inhibited 50 % of the photoreduction of nitroblue tetrazolium at 560 nm per gram of fresh weight. The CAT activity was recorded at $A_{240}$ and expressed as a unit of enzyme activity per gram of fresh weight. APX activity was measured at $A_{290}$ every 30 s and recorded continuously for 3-5 min. it was expressed as the change in $A_{290}$ per minute per gram of fresh weight.

**Determination of phospholipase D (PLD), lipase and lipoxygenase (LOX) activities**

The determination of PLD, Lipase, and LOX activities were carried out based on the method of Song *et al.* (2020). PLD and lipase activities were measured at $A_{520}$. A 0.01 change in the absorbance value per minute was outlined as a unit of enzyme activity. For the determination of LOX activity, the change in absorbance was recorded at $A_{243}$ every 30 s for 3-5 min. A 0.01 change in the absorbance value per minute was defined as a unit of LOX enzyme activity.

**Transcriptome data analysis**

**Extraction of RNA and cDNA synthesis**

Total RNA was extracted from *Z. latifolia* samples. An interruption reagent was added to disaggregate mRNA into short fragments after digesting the DNA with DNase. The interrupted mRNA was used as a template to synthesize one-strand cDNA with six-base random primers, and a two-strand synthesis reaction system was then prepared to synthesize two-strand cDNA. The uracil-DNA glycosylase (UNG) enzymatic method was used to digest one strand containing dUTP, leaving only the first cDNA strand with different linkers in the linking strand. The first strand of cDNA was purified, repaired, and connected to the sequencing adapter. The constructed library was sequenced by an HiSeq™ 2500 system (Illumina, San Diego, CA, USA) after being qualified by an Agilent 2100 bioanalyzer system (Agilent, Santa Clara, CA, USA).

**Bioinformatics analysis process**

Raw reads or raw data were obtained using the Illumina sequencing platform and then subjected to a quality control (QC) procedure. Clean reads were then obtained by filtering and...
compared to the reference sequence using Bowtie 2 software. The reads on the reference sequence were then counted to determine whether the comparison result passed a second alignment QC process. The transcript expression was then quantified using the fragments per kilobase per million reads method (Trapnell et al., 2010). According to the DESeq software package (http://bioconductor.org/packages/release/bioc/html/DESeq/html), a negative binomial distribution test based on the Bowtie 2 results was used to calculate the differential expression of transcripts and differentially expressed genes (DEGs). The DEGs were selected using a threshold of ≥ 2-fold and p-value <0.05. In addition, Gene Ontology (GO) enrichment analysis of the differentially expressed transcripts was performed and combined with the GO annotation results. The number of differential transcripts included in each GO was counted, and a hypergeometric distribution test method was used to calculate significantly differentially enriched transcripts. Afterward, pathway analysis of the differential transcripts was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2008).

**Metabolome data analysis**

**Sample preparation**

80 mg of *Z. latifolia* sample was transferred to a 1.5 mL Eppendorf tube. Two small steel balls were added to the tube. 20 μL of 2-chloro-l-phenylalanine (0.3 mg mL⁻¹) dissolved in methanol as internal standard and 1 mL mixture of methanol and water (V: V=7:3) were added to each sample and placed at -20 °C for 2 min. Then ground at 60 HZ for 2 min, and ultrasonicated at ambient temperature for 30 min after vortexed. Afterward, it was placed at -20 °C for 20 min before centrifugation for 10 min (13 000 rpm, 4 °C). 300 μL of the supernatant in the brown and glass vial was dried in a freeze concentration centrifugal dryer and then reconstitute with 400 μL of methanol-water (V: V=1:4), vortexed for 30 s, and ultrasound for 2 min. Samples were centrifuged at 13 000 rpm, 4 °C for 5 min. The supernatants (150 μL) from each tube were collected using crystal syringes,
filtered through a 0.22 μm organic phase pinhole filter and transferred to LC vials, which have been stored at -80 °C until LC-MS analysis.

Sample processing

Samples were analyzed using an LC-MS analysis system composed of an AB ExionLC ultra-high-performance liquid phase series and AB TripleTOF 6600 high-resolution mass spectrometer (AB Sciex, Framingham, MA USA), following the standard operational procedures. An acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm) (Waters Corporation, Milford, MA, USA) was used in both positive and negative modes. The binary gradient elution system consisted of (A) water (containing 0.1 % formic acid, v/v) and (B) acetonitrile (containing 0.1 % formic acid, v/v). The separation was achieved using the following gradient: 0 min, 5 % B; 2 min, 20 % B; 4 min, 25 % B; 9 min, 60 % B; 14 min, 100 % B; 18 min, 100 % B; 18.1 min, 5 % B and 19.5 min, 5 % B. The flow rate was 0.4 mL/min and the column temperature was 45 °C with 5 μL as injection volume. All the samples were kept at 4 °C during the analysis.

Data acquisition was performed in full scan mode (m/z range: 70-1000) using the information-dependent acquisition (IDA) data acquisition mode. The DEMs were selected based on a statistically significant threshold of variable influence on projection (VIP) values obtained from an orthogonal projection to latent structures discriminant analysis (OPLS-DA) model, and on p-values from a two-tailed Student’s t-test performed on the normalized peak areas, where metabolites with VIP values larger than 1.0 and p-values <0.05 were considered as DEMs.

Joint transcriptome and metabolome analysis

The DEGs and differentially expressed metabolites (DEM) in the KEGG pathway enrichment analysis were combined using R 4.1.0 software (https://www.r-project.org/).
Statistical analysis

A physio-biochemical data analysis was performed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA) and SPSS 19.0 (SPSS Inc., Chicago, IL, USA) software. The results are expressed as the mean ± standard deviation of three replicate samples.

FastQC and Trimmomatic software were used to ensure data quality and remove low-quality data, respectively. In addition, Bowtie 2 was used to calculate the expression abundance of each transcript in each sample. Metabolites were identified using Progenesis QI (Waters Corporation) data processing software. The analysis was performed six times and differences between treatments were considered significant at p < 0.05.

Results

Color, visual appearance, and cell ultrastructure

Fig. 1, A, B, and C showed that the a, b and ΔE values gradually increased in both samples during storage. However, at the end of the storage period, 1-MCP-treated Z. latifolia samples had lower a, b and ΔE values. This indicated that the 1-MCP treatment significantly retarded increases in a, b and ΔE values during postharvest storage of Z. latifolia. The appearance of the Z. latifolia control sample was optimal at 0 d, with a bright green shell and white internal color (Fig. 1, D), whereas after 6 d of storage at room temperature the shell turned yellow and its epidermis was browned and blackened, indicating deterioration (Fig. 1, E). In contrast, samples treated with 1-MCP retained an excellent color and visual appearance (Fig. 1, F). Furthermore, as observed in Fig. 1, G, the cell structure of Z. latifolia was intact at 0 d, with a visible cell wall, nucleus, and mitochondria. After 6 d of storage, the cell wall of the control sample was degraded with unclear mitochondria (Fig. 1, H). In contrast, the treated samples retained an intact cell wall and plasma membrane, with visible mitochondria (Fig. 1, I and Fig. S1).
O$_2^-$ production rate and H$_2$O$_2$ content

As seen in Fig. 2, A, there was an increase in the O$_2^-$ production rate on the first day of storage followed by a decrease in both the treated and untreated samples. Fig. 2, B shows the same patterns of H$_2$O$_2$ content under the 1-MCP treatment, while the control sample presented a decreasing trend after 3 d of storage. Interestingly, samples treated with 1-MCP had lower O$_2^-$ and H$_2$O$_2$ contents throughout the storage period compared to the control. These data indicated that the 1-MCP treatment inhibited O$_2^-$ production and the accumulation of H$_2$O$_2$ during the storage of Z. latifolia at 25 °C.

SOD, CAT, and APX activities and AsA content

As indicated by Fig. 3, A, there was a decrease in SOD activity in the control group at the first 1 d of storage, and then increased during the remainder of storage time, with activity reaching 23.99 ± 0.19 U g$^{-1}$ after 6 d of storage. At the same time, under 1-MCP treatment, an upward trend in the first day of storage was followed by a decrease till 24.66 ± 0.21 U g$^{-1}$ at the end of storage, this was higher than that of the untreated samples. In addition, samples treated with 1-MCP had the highest CAT activity throughout the storage period (Fig. 3, B). Furthermore, the APX activity of Z. latifolia decreased during the early storage period and then increased in both the control and treated samples. However, the APX activity of samples treated with 1-MCP was significantly higher than in the control group (Fig. 3, C). As shown in Fig. 3, D, the AsA content decreased in the control and treated samples during the first 3 d of storage, followed by a slight increase. However, the AsA content in the treated samples was still higher than that of the control sample at the end of storage. This indicated that 1-MCP inhibited the decrease of AsA content.

PLD, lipase, and LOX activities, and MDA content

To better understand the effect of 1-MCP during postharvest storage of Z. latifolia, the activities of three enzymes that degraded membrane lipids (PLD, lipase, and LOX) were investigated. As shown
in Fig. 4, A, PLD activity significantly increased from 70.14 ± 0.02 U g⁻¹ at day 0 to 137.57 ± 0.016 and 103.86 ± 0.01 U g⁻¹ at day 3 in the control and treated samples, respectively. After 3 days of storage, the PLD activity of both groups significantly decreased, with a lower value seen in the treated group (93.71 ± 0.03 U g⁻¹) at the end of storage. During the storage period, lipase activity significantly decreased in both the treated and control samples (Fig. 4, B), with the lowest lipase activity observed under the 1-MCP treatment. The LOX activity increased during the first 3 days and then fell back to 52.04 ± 1.46 U g⁻¹ in the control samples and 40.52 ± 2.29 U g⁻¹ in the 1-MCP treated samples (Fig. 4, C). Fig. 4, D shows that there was a decrease in MDA content over the storage period in both the control and treated groups. However, at the end of the storage period, samples treated with 1-MCP had lower MDA content than the control group. These data indicated that the 1-MCP treatment effectively inhibited PLD, lipase, and LOX activities, and also reduced the accumulation of MDA.

**DEGs analysis**

To better understand the mechanism involved in the senescence of *Z. latifolia*, we performed RNA sequencing of untreated and 1-MCP treated samples: 15 libraries (5 samples × 3 biological replicates) were aligned, yielding more than 49 M raw reads with approximately 7.5 raw bases per library. In addition, 2.23-2.41% of the reads were mapped to multiple locations and 87.83-88.88% were mapped to single locations. The low-quality reads were filtered out and 49 M-51 M clean reads were used for further analysis (Table S1).

As shown in Fig. 5, A, 6699 (3254 up-regulated and 3445 down-regulated), and 8248 (3958 up-regulated and 4290 down-regulated) genes were expressed in the control group at 3 d (CK3) and 6 d (CK6) compared to 0 d, respectively, while 3175 and 4143 up-regulated and 3583 and 4778 down-regulated genes were differentially expressed at 3 and 6 d, respectively, under the 1-MCP treatment. When the treated sample was compared to CK3 and CK6, the number of DEGs was 1377 (895 down-regulated and 482 up-regulated) and 499 (301 down-regulated and 198 up-regulated), respectively.
These results indicated that the 1-MCP treatment induced changes in the quantity of DEGs during postharvest storage of *Z. latifolia*.

To recognize the enriched GO terms in our dataset, all expressed genes were divided into three major categories: biological process, cellular component, and metabolism function. Cellular process and metabolic process were the most abundant terms in the biological process category, while cell, cell plant, and organelle were the most abundant terms in the cellular component category. Binding, catalytic activity, and transporter activity were the predominant terms in the metabolic function category (Fig. S2).

The GO enrichment analysis revealed important differences in the gene expression profile under different conditions. Compared to CK0, the 1-MCP treatment had higher enrichment (6239 DEGs; 2705 up- and 3534 down-regulated) after 6 d of storage (Fig. 5, A), while 5757 DEGs (2595 up-regulated and 3162 down-regulated) were enriched in the control group after 6 d of storage. When the 1-MCP treated group was compared to the control at the same sampling times during storage (3 and 6 d), 853 (610 down-regulated and 243 up-regulated) and 269 (171 down-regulated and 98 up-regulated) DEGs were significantly enriched (Fig. 5, B). In addition, a KEGG analysis was performed to determine the biological function of the identified DEGs. The DEGs were classified into 23 categories, among which “signal transduction”, “translation”, “carbohydrate metabolism”, “folding, sorting and degradation”, “global overview maps”, “amino acid metabolism”, “transport and catabolism”, “lipid metabolism”, “energy metabolism”, and “cell growth and death” were the most enriched pathways (Fig. S3).

**DEMs analysis**

To supplement our transcriptome analysis, metabolomics approaches were used to obtain more information about the changes in metabolites during the storage of *Z. latifolia*. The principal component analysis (PCA) model obtained by 7-fold cross-validation showed that the QC samples were closely clustered together, indicating good stability and repeatability of our experiment (Fig. 6, A). In total, 460, 493, 472, and 529 differential metabolites were identified in CK3, CK6, 1-MCP3, and
1-MCP6, respectively, compared to CK0. Compared to the control group at 3 and 6 d, 418 and 568 differential metabolites were quantified in 1-MCP-treated samples, respectively (Fig. 6, B). This indicated that the 1-MCP treatment significantly increased the number of differential metabolites. The identified metabolites were then classified into 11 superclasses of “nucleoside, nucleotide, and analogues”, “organics acids and derivatives”, “lipids and lipid-like molecules”, “alkaloids and derivatives”, “organic oxygen compounds”, “organooxygen compounds”, “organoheterocyclic compounds”, “benzenoids”, “phenylpropanoid and polyketides”, “homogeneous non-metal compounds”, “hydrocarbon derivatives”, and “organic nitrogen compounds”.

Integrative analysis

The DEGs and DEMs were simultaneously mapped to the KEGG pathway database to obtain their common pathway information. Therefore, some pathways were investigated based on the available KEGG pathways, including carbon fixation in photosynthesis, cell cycle-yeast, starch and sucrose metabolism, glycolysis/gluconeogenesis, pentose phosphate pathway (PPP), fructose and mannose metabolism, glyoxylate and dicarboxylate metabolism, amino sugar and nucleotide sugar metabolism, biosynthesis of unsaturated fatty acid, linoleic acid metabolism, glycerophospholipid metabolism, biosynthesis of amino acid, glycine, serine and threonine metabolism, alanine, aspartate, and glutamate metabolism, cysteine and methionine metabolism, glutathione metabolism, pyruvate metabolism, purine metabolism, and plant hormone signal transduction, etc. The most important pathways and their cross network involved in the postharvest senescence of Z. latifolia were summarized as in Fig. 7.

Discussion

Plant senescence is a genetically and environmentally regulated process associated with physiological activities, especially under adverse environmental conditions. It results in a degradation of the visual appearance as well as the degradation and cells death throughout a sequence of irreversible processes (Ali, 2020; Gundewadi et al., 2018). It has also been reported that
changes in metabolism due to preharvest and postharvest stresses, even of short duration, cause rapid loss of quality in leafy products, such as salads (Cavaiuolo et al., 2017). Genes related to the control of plant senescence encode enzymes responsible for protein processing, transport, and carbohydrate metabolism (Ostrowska et al., 2019). In addition, it has been demonstrated that cells exert control over the metabolic state by modulating the expression of genes encoding enzymes (Zeleznjak et al., 2014). Moreover, plants can respond to environmental stresses by developing signaling mechanism based on endogenous hormones, i.e. abscisic acid, brassinolide, cytokinin, gibberellin, jasmonic acid, and salicylic acid (Okazaki and Saito, 2012). In wheat and Arabidopsis, leaf senescence and water deficit were regulated via the interaction between endogenous hormones (Luo et al., 2019). Nevertheless, endogenous hormone such as ethylene, was reported to be involved in plant senescence and fruit ripening (Ahlawat and Liu, 2021). Henceforth, the reduction of ethylene synthesis could be of utmost importance in delaying senescence. In our investigation, we found that 1-MCP treatment significantly down-regulated the activities of genes encoded S-adenosylmethionine (SAM) synthetase, 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO), which in return are involved in ethylene biosynthesis reactions. SAM synthetase is a useful enzyme for the conversion of methionine to SAM, which is converted to 1-aminocyclopropane-1-carboxylate (ACC) by ACC synthetase. At the end of a reaction, ACC was transformed to ethylene by ACO. Our finding was consistent with those of Yang et al. (2012) who found that 1-MCP treatment delayed the activities of ACO1, ACO2 and ACS1 related genes during ripening of apple fruit. In addition, 1-MCP treatment enhanced the expression of ERS2 and reduced that of ETR3 comparing to the control group where only ETR3 expression was induced. ERS2 is generally implicated in the perception of ethylene; the increase level of its mRNA may decrease the ethylene sensitivity and delayed petal and sepal wilding (Tanase and Ichimura, 2006). Xue et al. (2008) and Xu et al. (2010) reported an inhibitory effect of 1-MCP during the increase activity of Rh-ETR3 in cut rose and rose floral. They also found that the expression of Rh-ETR1 and Rh-ETR3 were correlated with the production of ethylene in Flora tissue and Rose Samantha. The
increase of ethylene receptor levels could induce different combinations among them. Thus, leading to the formation of protein complexes which could confer low ethylene-sensitivity (Agarwal et al., 2012) and consistently Zhu et al. (2019) demonstrate that 1-MCP exerts inhibitory effects to regulate the level of ethylene by interacting with ethylene receptors. Recent evidence also indicated that 1-MCP treatment preserved the shelf-life of Pak choi by decreasing leaf ethylene production (Song et al., 2020). Therefore, we suggest that the inhibition of Z. latifolia senescence by 1-MCP may be due to its effectiveness to decrease the activities of ethylene biosynthesis related genes (SAM1, SAM2, ACC1 and ACO1), and the up-regulation activity of ERS2.

In this study, 1-MCP treatment also had a large effect on the up-regulation of genes related to transcription factor A (TFA), jasmonic acid-amino synthetase, coronatine-insensitive protein1, which serves as a receptor for jasmonates (Yan et al., 2009), and brassinosteroid insensitive 1 (BRI1) kinase inhibitor 1, in return inhibited the decrease of jasmonic acid. It has been reported that jasmonic acid-amino synthetase is important for activating jasmonate, which protects plants against abiotic stress by inducing plant defense mechanism involving antioxidant enzymes and other defensive compounds (Ali, 2020). These results were consistent with the physiobiochemical analysis, which revealed the increase of antioxidant enzymes activities such as SOD, CAT, and APX under 1-MCP treatment than the control group. In addition, the integrative analysis revealed a positive effect of the 1-MCP treatment on the activity of genes related to CAT than the control group. It has been reported that SOD can convert superoxide ion into H$_2$O$_2$, that is eliminated by CAT (Luo et al., 2012; Song et al., 2020). Our results also corroborated recent studies in broccoli (Yuan et al., 2010) and green bell pepper (Cao et al., 2012), respectively, found that the 1-MCP treatment increased the activity of antioxidant enzymes (SOD, CAT, and APX), which indirectly lowered the content of ROS (these are among the toxic intermediate products of abiotic stress).

Previous studies have reported that polyamine oxidase and ornithine decarboxylase are associated with the catabolism of polyamine, which in return, is involved in the regulation of
oxidative stress caused by environmental stresses and ultimately leads to the inhibition of membrane lipid peroxidation (Chen et al., 2015). It has also been reported that the cellular defense against oxidative damage may involve ferredoxin nicotinamide adenine dinucleotide phosphate (NADP⁺) reductase and a reduced glutathione (Krapp et al., 1997). Ziosi et al. (2009) demonstrated that jasmonate induced a delay in the ripening of peaches, accompanied by up-regulation of polyamine. In our study, the 1-MCP treatment had a positive effect on the activity of ornithine decarboxylase, polyamine oxidase, ferredoxin NADP⁺ reductase, and glutathione reductase-related genes. Glutathione reductase is an enzyme that can restore the glutathione content (important for oxidative damage prevention) in the case of glutathione oxidation (Bata et al., 2020). A previous study reported that the overexpression of glutathione reductase increases the amount of AsA in poplar (Blauer et al., 2013). Furthermore, Song et al. (2020) reported that AsA and glutathione are important for scavenging ROS. Our physiobiochemical investigation showed that the 1-MCP treatment inhibited the decrease of AsA, thus maintaining a higher AsA content than the control group. Which correlates with the up-regulation activity of glutathione related gene. Therefore, we suggest that the 1-MCP treatment retarded the senescence of Z. latifolia by enhancing the activity of ornithine decarboxylase-, polyamine oxidase-, ferredoxin NADP⁺ reductase-, and glutathione reductase-related genes, as well as maintaining a high AsA content.

It has been reported that plants also respond to biotic and abiotic stress via MAPK, which can be activated by ROS (Bata et al., 2020). In animals, plants, and fungi, MAPK cascade genes are essential components of the signal transduction pathways that contribute in the conversion of extracellular signals to intracellular responses (Neupane et al., 2019). In our study, the up-regulated activity of 4 genes encoding MAPK were found in CK3, 1-MCP3, and 1-MCP6 compared to CK0 and also to CK3. This reveals that 1-MCP treatment could enhance the activity of those MAPK related genes to protect Z. latifolia from stress, thereby prolonging its shelf-life. However, two genes encoding MAPK showed a down-regulated activity. Thus, further investigation of the roles of these MAPK genes is needed. Protein kinase activity can be regulated by serine/threonine phosphatase 2A.
regulator subunit A, which is an important component of the stress signal transduction pathway and jasmonic acid signal transmission (Pais et al., 2009). While, cyclic adenosine monophosphate (cAMP), responsible for many signaling cascades in cells, stimulates protein kinase A, leading to the phosphorylation of mitochondrial proteins (Luo et al., 2019). In our study, 1-MCP treatment up-regulated the activities of cAMP and genes related to serine/threonine phosphatase 2A regulator subunit A. These results are consistent with those of Yu et al. (2005) who demonstrated that type 2A protein phosphatases were up-regulated in rice (Oryza sativa) in response to high salinity and with our previous investigation on the effect of 1-MCP on the mitochondria proteome (Luo et al., 2019).

Taken together, we hypothesize that the down-regulated activity of those two MAPK related genes may be due to the regulatory effect of serine/threonine phosphatase 2A regulator subunit A and is crucial for the shelf-life of Z. latifolia. However, research is needed to confirm this hypothesis.

Furthermore, down-and-up regulated patterns of 1-phosphatidyl inositol-4- phosphate-5-kinase activity were observed in the treated sample compared to CK3 and CK6, respectively. It has been reported that 1-phosphatidyl inositol-4-phosphate-5-kinase is a key enzyme in the reversible reaction of phosphatidylinositol-4-phosphate and phosphatidylinositol-5-phosphate, which is hydrolyzed by phospholipase C (PLC) into various secondary signaling messengers, such as diacylglycerol and their corresponding phosphoinositide phosphate. Inositol 1,3,4-trisphosphate can interact with specific receptors to allow Ca\(^{2+}\) to flow out and bind with calmodulin (CaM) (Yang et al., 2020; Jia et al., 2019). A previous study reported that CaM is an important calcium sensor protein that can perform different functions by binding to a range of targets located in different cell compartments. CaM1 gene was found to be implicated in Arabidopsis leaf senescence regulation. However, its accumulation can lead to the overproduction of ROS (Kurubaş et al., 2021). And the resulting abundance could induce oxidative reactions with lipids, thereby inducing the production of harmful substances, including MDA. In this study, up-regulation of CaM-related genes was observed only at 3 d (1-MCP3vsCK3), while the physiobiochemical analysis indicated lower ROS (O\(_{2}^{-}\), H\(_{2}O_{2}\)) contents under the 1-MCP treatment at the end of storage, leading to a decrease in the MDA.
content. This could be due to the non-excess accumulation of CaM and the increase activities of the antioxidant enzymes mentioned above. A similar effect of 1-MCP on the inhibition of ROS was also observed in baby squash (Kurubaş et al., 2021). These results suggest that 1-MCP treatment may delay the postharvest senescence of *Z. latifolia* by regulating oxidative phosphorylation, ROS metabolism, cell growth, and death.

Changes in membrane lipids influence the properties of the membrane and could contribute to senescence (Bata et al., 2021). Membrane-related lipolytic enzymes, including PLD, lipase, and LOX, have been reported to play a crucial role in membrane deterioration and senescence. PLD produces phosphatidic acid (PA), which is converted to diacylglycerol by PA phosphatase. At the end of the reaction, the free fatty acids obtained from diacylglycerol are peroxidized by lipoxygenase (Wang et al., 2020; Yuan et al., 2010). Therefore, any change in PLD gene expression could alter cellular processes. In our study, phosphatidylserine content significantly decreased. This was followed by an accumulation of phosphatidylethanolamine, significant down-regulation of the activity of PLD-related genes, and a consequent decrease in PA and lecithin contents under the 1-MCP treatment.

Phosphatidylethanolamine is an important component of the cell membrane. It hydrolysis leads to the formation of lysophosphatidylethanolamine (LPE) involved in the attenuation of ethylene production consequently the delay of PLD activity which is activated during the ethylene induced senescence process and thereby inhibition of postharvest senescence (Cowan, 2009). Study found that LPE treatment could maintain the firmness, enhance the color and prolong the shelf-life of fruit (Ahmed et al., 2016). These results corroborate those of our physiobiochemical analysis, which revealed that the 1-MCP treatment inhibited PLD, lipase, and LOX activities, preserved the cell wall of *Z. latifolia*, and maintained the visual appearance by ensuring low a, b, and ΔE values. Moreover, Huang et al. (2012) found that 1-MCP treatment significantly delayed the loss of membrane integrity in Okra (*Hibiscus esculentus*) during storage at 7 °C for 18 d. Therefore, we suggest that the senescence of *Z. latifolia* can be retarded due to a decrease in PLD activity and its related genes, as well as an accumulation of phosphatidylethanolamine.
Adenosine triphosphate (ATP), which is mainly distributed in chloroplasts, the cytoplasmic matrix, and mitochondria, is the principal source of energy for metabolism regulation. To a certain extent, the physiological status of plants can be measured by the amount of ATP that they contain. A reduction in the energy deficit caused by ATP synthesis, is one of the main causes of membrane damage and browning of postharvest horticultural products (Lin et al., 2017). Thus, a sufficient intracellular ATP supply and friendly extracellular ATP signaling are crucial for attenuating stresses, retarding senescence, and maintaining the quality of horticultural crops (Aghdam et al., 2018). In the present study, the 1-MCP treatment enhanced the activities of ATP biosynthesis-related genes namely, acetyl-CoA carboxylase/biotin carboxylase 1, malate dehydrogenase, phosphoglucomutase, phosphoglycerate kinase, 6-phosphogluconolactonase, citrate synthase, and ATP citrate (pro-S)-lyase, while the activities of genes related to 6-phosphofructokinase 1 and α-amylase were low compared to the control. There were high amounts of ATP biosynthesis-related metabolites such as citrate, isocitrate, fumarate, gluconate, α-D-glucose-1P, and glycerate-2P due to the decrease in sucrose content. Studies have also reported that fumarate, which can be converted to malate, is needed as a respiratory substrate to avoid dark-induced senescence and serves as an alternative carbon sink for photosynthate (Araújo et al., 2011). These outcomes support our previous proteomic investigation, where we found that 1-MCP treatment inhibited the down-regulation of proteins involved in the electron transport chain and oxidative phosphorylation while the down-regulation was promoted under ethylene treatment, meaning that 1-MCP treatment has a positive effect on the accumulation of energy needed to delay the senescence (Luo et al., 2019). Taken together, we suggest that 1-MCP treatment delayed Z. latifolia senescence by promoting the production and accumulation of ATP.

Conclusions

In summary, an integrated transcriptomics and metabolomics approach was used to gain insight into the mechanism of Z. latifolia senescence during storage at 25 °C. Z. latifolia senescence was
closely related to the accumulation of ROS, biosynthesis of ethylene, membrane degradation, and depletion of energy metabolism due to abiotic stress. These findings were also confirmed by the integrated analysis, which revealed that *Z. latifolia* undergoes senescence due to the up-regulation activities of several genes encoding enzymes and an accumulation of metabolites, such as ethylene biosynthesis-related genes, PLD, omega-6 fatty acid desaturase, hexokinase, phosphofructokinase, and α-amylase-related genes, as well as PA, oxidized glutathione, 9-cis,11-trans-octadecadienoate, metabolites. Senescence may also be associated with the down-regulated activity of ornithine decarboxylase, polyamine oxidase, TFA, jasmonic acid-amino synthetase, coronatine-insensitive protein1, BRI1, MAPK, CaM, and CAT genes, and lower organic acid, L-alanine, and γ-linolenate contents. Although, changes in the activities of these genes, metabolites, and enzymes were noticed under 1-MCP treatment, which subsequently delayed the postharvest senescence of *Z. latifolia*. The results presented here support our previous research on the differential expressed proteins (DEPs) and improve our understanding of the mechanism underlying the senescence of *Z. latifolia* during storage at 25 °C. However, further investigations are needed to determine the specific roles of MAPK- and CaM-related genes.
Author Contributions
MHBG, SP, and RY: Methodology, Investigation. MHBG and HL: Writing - original draft. HS: Writing - review & editing. SP and YC: Software, Data curation. JL and GZ: Methodology, Data curation. HS and HL: Project administration, Supervision, Resources.

Acknowledgements
This work was supported by the Qingchuang Science and Technology Support Program of Shandong Provincial College (Lujiaorenzi[2019]No.6), the Research Start-up Funding from Nanjing Normal University (184080H202B117) and the Doctoral Fund (XY19BS18) of Heze University (provided to Huibo Song).

Conflict of Interest
No declared.

Supplementary data
Supplementary data may be found in the online version of this article, at http://....
References

Agarwal, G., Choudhari, D., Singh, V., et al. (2012). Role of ethylene receptors during senescence and ripening horticultural crops. *Plant Signaling and Behavior, 7*(7): 827–846.

Aghdam, M. S., Jannatizadeh, A., Luo, Z., et al. (2018). Ensuring sufficient intracellular ATP supplying and friendly extracellular ATP signaling attenuates stresses, delays senescence and maintains quality in horticultural crops during postharvest life. *Trends in Food Science & Technology, 76*: 67–81.

Ahlawat, Y., Liu, T. (2021). Varied expression of senescence-associated and ethylene-related genes during postharvest of *Brassica* vegetables. *International Journal of Molecular Sciences, 22*(2): 839.

Ahmed, Z. F. R., Palta, J. P. (2016). Postharvest dip treatment with a natural lysophospholipid plus soy lecithin extended the shelf life of banana fruit. *Postharvest Biology and Technology, 113*: 58–65.

Ali, M., Raza, M. A., Li, S., et al. (2020). 1-MCP regulates ethanol fermentation and GABA shunt pathway involved in kiwifruit quality during postharvest storage. *Horticultural Plant Journal, 7*(1): 23–30.

Ali, S., Baek, K. H. (2020). Jasmonic acid signaling pathway in response to abiotic stresses in plants. *International Journal of Molecular Sciences, 21*(2): 621.

Araújo, W. L., Nunes-Nesi, A., Fernie, A. R. (2011). Fumarate: Multiple functions of a simple metabolite. *Journal of Phytochemistry, 72*(9): 838–843.

Bata, G. M. H., Zhang, C., Peng, S., et al. (2021). Combination of sodium alginate-based coating with L-cysteine and citric acid extends the shelf-life of fresh-cut lotus root slices by inhibiting browning and microbial growth. *Postharvest Biology and Technology, 175*: 111502.
Bata, G. M. H., Zhang, C., Wang, J., et al. (2020). ROS and MAPK cascades in the post-harvest senescence of horticultural products. *Journal of Proteomics & Bioinformatics*, 13(1): 1–7.

Blauer, J.M., Kumar, G.N.M., Knowles, L.O., et al. (2012). Changes in ascorbate and associated genes expression during development and storage of potato tubers (*Solanum tuberosum* L.). *Postharvest Biology and Technology*, 78: 76–91.

Cao, S., Yang, Z., Zheng, Y. (2012). Effect of 1-methylcyclopene on senescence and quality maintenance of green bell pepper fruit during storage at 20 °C. *Postharvest Biology and Technology*, 70: 1–6.

Cavaiuolo, M., Cocetta, G., Spadafora, N. D., et al. (2017). Gene expression analysis of rocket salad under pre-harvest and postharvest stresses: A transcriptomic resource for diplotaxis tenuifolia. *Plos One*, 12(5): 1–27.

Chen, Y., Lin, H., Shi, J., et al. (2015). Effects of a feasible 1-methylcyclopropene postharvest treatment on senescence and quality maintenance of harvested huanghua pears during storage at ambient temperature. *LWT-Food Science and Technology*, 64(1): 6–13.

Cowan, A. K., (2009). Plant growth promotion by 18:0-lyso-phoshatidylethanolamine involves senescence delay. *Plant Signaling and Behavior*, 4(4): 324–327.

da Silva, T. L., Aguiar-Oliveira, E., Mazalli, M. R., et al. (2017). Comparison between titrimetric and spectrophotometric methods for quantification of vitamin C. *Food Chemistry*, 224: 92–96.

Dai, X., Lu, Y., Yang, Y., et al. (2021). 1-methylcyclopropene preserves the quality of Chive (*Allium schoenoprasum* L.) by enhancing its antioxidant capacities and organosulfur profile during storage. *Foods*, 10(8): 1792.

Ding, Y., Chang, J., Ma, Q., et al. (2015). Network analysis of postharvest senescence process in citrus fruits revealed by transcriptomic and metabolomic profiling. *Plant Physiology*, 168(5): 357–376.
Fu, F., Peng, Y., Wang, G., et al. (2021). Integrative analysis of the metabolome and transcriptome reveals seed germination mechanism in Punica granatum L. *Journal of Integrative Agriculture*, 20(1): 132–146.

Gundewadi, G., Rakesh, V., Bb, R. (2018). Physiological and biochemical basis of fruit development and ripening. *Journal of Hill Agriculture*, 9(1): 7–21.

Huang, S., Li, T., Jiang, G., et al. (2012). 1-Methylcyclopropene reduces chilling injury of harvested okra (Hibiscus esculentus L.) pods. *Scientia Horticulturae*, 141: 42–46.

Jia, Q., Kong, D., Li, Q., Sun, et al. (2019). The function of inositol phosphatases in plant tolerance to abiotic stress. *International Journal of Molecular Sciences*, 20(16): 1–15.

Kanehisa, M., Araki, M., Goto, S., et al. (2008). KEGG for linking genomes to life and the environment. *Nucleic Acids Research*, 36(1): 480–484.

Krapp, A. R., Tognetti, V. B., Carrillo, N., et al. (1997). The role of ferredoxin-NADP+ reductase in the concerted cell defense against oxidative damage. Studies using Escherichia coli mutants and cloned plant genes. *European Journal of Biochemistry*, 249(2): 556–563.

Kuruş, M. S., Sabotic, J., Erkan, M. (2021). The effects of 1-methylcyclopropene (1-MCP) treatment on antioxidant enzymes and fruit quality parameters of cold-stored baby squashes. *Turkish Journal of Agriculture and Forestry*, 45: 33–45.

Li, D., Cheng, Y., Guan, J. (2019). Effects of 1-methylcyclopropene on surface wax and related gene expression in cold-stored ‘Hongxiangsu’ pears. *Journal of the Science of Food and Agriculture*, 99(5): 2438–2446.

Li, X., Zhao, C., Li, H., et al. (2009). Bacterial impact on H₂O₂ accumulation during the interaction between Xanthomonas and rice. *Plant Production Science*, 12(2): 133–138.
Lin, Y., Lin, Y., Lin, H., et al. (2017). Hydrogen peroxide-induced pericarp browning of harvested longan fruit in association with energy metabolism. *Food Chemistry*, 225: 31–36.

Liu, L., Wang, B., Liu, D., et al. (2020). Transcriptomic and metabolomic analyses reveal mechanisms of adaptation to salinity in which carbon and nitrogen metabolism is altered in sugar beet roots. *BMC Plant Biology*, 20(1): 1–21.

Luo, H., Jiang, L., Zhang, L., et al. (2012). Quality changes of whole and fresh-cut *Zizania latifolia* during refrigerated (1 °C) storage. *Food and Bioprocess Technology*, 5, 1411–1415.

Luo, H., Zhou, T., Kong, X., et al. (2019). iTRAQ-based mitochondrial proteome analysis of the molecular mechanisms underlying postharvest senescence of *Zizania latifolia*. *Journal of Food Biochemistry*, 43(12): e13053.

Lv, J., Zhang, M., Bai, L., et al. (2020). Effects of 1-methylcyclopropene (1-MCP) on the expression of genes involved in the chlorophyll degradation pathway of apple fruit during storage. *Food Chemistry*, 308: 125707.

Massolo, J. F., Roberto, A., Concepción, A., et al. (2019). Effects of ethylene and 1-MCP on quality maintenance of fresh cut celery. *Postharvest Biology and Technology*, 148(1): 176–183.

Min, D., Li, F., Zhang, X., et al. (2018). Effect of methyl salicylate in combination with 1-methylcyclopropene on postharvest quality and decay caused by *Botrytis cinerea* in tomato fruit. *Journal of the Science of Food and Agriculture*, 9(10): 3815–3822.

Neupane, S., Schweitzer, S.E., Neupane, A., et al. (2019). Identification and characterization of mitogen-activated protein kinase (MAPK) genes in sunflower (*Helianthus annuus* L.). *Plants*, 8(2): 1–25.

Okazaki, Y., Saito, K. (2012). Recent advances of metabolomics in plant biotechnology. *Plant Biotechnology Reports*, 6: 1–15.
Ostrowska, A., Tyrka, M., Dziurka, M., et al. (2019). Participation of wheat and rye genome in drought induced senescence in winter triticale (X Triticosecale Wittm.). *Agronomy*, 9(4): 195.

Pais, S. M., Téllez-Iñón, M. T., Capiati, D. A. (2009). Serine/threonine protein phosphatases type 2A and their roles in stress signaling. *Plant Signaling & Behavior*, 4(11): 1013–1015.

Qian C., Ji Z., Zhu Q., et al. (2021). Effects of 1-MCP on proline, polyamine, and nitric oxide metabolism in postharvest peach fruit under chilling stress. *Horticultural Plant Journal*, 7(3): 188–196.

Singh, S.P., Pal, R.K. (2008). Reponse of climacteric-type guava (*Psidium guajava* L.) to postharvest treatment with 1-MCP. *Postharvest Biology and Technology*, 47(3): 307–314.

Song, L., Luo, H., Jiang, L., et al. (2020). Integrative analysis of transcriptome and metabolome reveals the possible mechanism of leaf yellowing in pak choi (*Brassica rapa* subsp. chinensis) with 1-methylcyclopropene treatment during storage at 20 °C. *Postharvest Biology and Technology*, 169: 111300.

Tanase, K., Ichimura, K. (2006). Expression of ethylene receptors DI-ERS1-3 and DI-ERS2, and ethylene response during flower senescence in *Delphinium*. *Journal of Plant Physiology*, 163(11): 1159–1166.

Trapnell, C., Williams, B.A., Pertea, G., et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology*, 28(5): 511–515.

Wang, H., Chen, G., Shi, L., et al. (2020). Influences of 1-methylcyclopropene-containing papers on the metabolisms of membrane lipids in Anxi persimmons during storage. *Food Quality and Safety*, 4(3): 143–150.
Witzel, K., Neugart, S., Ruppel, S., et al. (2015). Recent progress in the use of omics technologies in brassicaceous vegetables. *Frontiers Plant Science*, 6(4): 1–14.

Xoca-Orozco, L.Á., Cuellar-Torres, E.A., González-Morales, S., et al. (2017). Transcriptomic analysis of avocado hass (*Persea americana* Mill) in the interaction system Fruit-Chitosan-Colletotrichum. *Frontiers in Plant Science*, 8: 1–13.

Xu, Y., Wang, J., Wang, H., et al. (2010). Relationship between Rh-RTH1 and ethylene receptor gene expression in response to ethylene in cut rose. *Plant Cell Report*, 29(8): 895–904.

Xue, J., Li, Y., Tan, H., et al. (2008). Expression of ethylene biosynthesis and receptor genes in rose floral tissues during ethylene-enhanced flower opening. *Journal of Experimental botany*, 59(8): 2161–2169.

Yan, J., Zhang, C., Gu, M., et al. (2009). The arabidopsis coronatine insensitive1 protein is a jasmonate receptor. *Plant Cell*, 21(8): 2220–2236.

Yan, N., Du, Y., Liu, X., et al. (2018). Morphological characteristics, nutrients, and bioactive compounds of *Zizania latifolia*, and health benefits of its seeds. *Molecules*, 23(1561): 1–16.

Yang, S., Fang, G., Zhang, A., et al. (2020). Rice early senescence 2, encoding an inositol polyphosphate kinase, is involved in leaf senescence. *BMC Plant Biology*, 20(1): 1–15.

Yang, X.T., Song, J., Campbell-Palmer, L., Fillmore, S., et al. (2012). Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during during ripening of apple fruit. *Postharvest Biology and Technology*, 78: 55–66.

Ying, H., Shi, J., Zhang, S., et al. (2019). Transcriptomic and metabolomic profiling provide novel insights into fruit development and flesh coloration in Prunus mira Koehne, a special wild peach species. *BMC Plant Biology*, 19(1): 1–16.
Yu, R.M.K., Wong, M.M.L., Jack, R.W., et al. (2005). Structure, evolution and expression of a second subfamily of protein phosphatase 2A catalytic subunit genes in the rice plant (Oryza sativa L.). Planta, 222(5): 757–768.

Yuan, G., Sun, B., Yuan, J., et al. (2010). Effect of 1-methylcyclopropene on shelf life, visual quality, antioxidant enzymes and health-promoting compounds in broccoli florets. Food Chemistry, 118(3): 774–781.

Zelezniak, A., Sheridan, S., Patil, K.R. (2014). Contribution of network connectivity in determining the relationship between gene expression and metabolite concentration changes. Plos Computational Biology, 10(4): e1003512.

Zhang, C., Hao, Y. (2020). Advances in genomic, transcriptomic, and metabolomic analyses of fruit quality in fruit crops. Horticultural Plant Journal, 6(6): 361–371.

Zhang, Z., Xu, S., Kong, M., et al. (2021). Isolation, identification and artificial inoculation of Ustilago esculenta on Zizania latifolia. Horticultural Plant Journal, 7(4): 347–358.

Zhu, X., Ye, L., Ding, X., et al. (2019). Transcriptomic analysis reveals key factors in fruit ripening and rubbery texture caused by 1-MCP in papaya. BMC Plant Biology, 19(1): 309.

Ziosi, V., Bregoli, A.M., Fregola, F., et al. (2009). Jasmonate-induced ripening delay is associated with up-regulation of polyamine levels in peach fruit. Journal of Plant Physiology, 166(9): 938–946.
Figure 1. The color (A, B, C), appearance (D, E, F) and ultrastructure visualization (G, H, I) of \textit{Z. latifolia} during storage at 25 °C. Vertical bars represent the standard error of three replicates. Means with different letters for the same storage time are significantly different ($p < 0.05$).
Figure 2. $O_2^\cdot$ production rate (A) and $H_2O_2$ content (B) of *Z. latifolia* during storage at 25 °C. Vertical bars represent the standard error of three replicates. Means with different letters for the same storage time are significantly different ($p < 0.05$).
Figure 3. SOD (A), CAT (B), APX (C) activities and AsA content (D) of *Z. latifolia* during storage at 25 °C. Vertical bars represent the standard error of three replicates. Means with different letters for the same storage time are significantly different (*p* < 0.05).
Figure 4. PLD (A), Lipase (B), LOX (C) activities and MDA content (D) of *Z. latifolia* during storage at 25 °C. Vertical bars represent the standard error of three replicates. Means with different letters for the same storage time are significantly different (*p* <0.05).
Figure 5. Global view of transcriptome analysis. Differentially expressed genes (A) and GO enrichment (B).
Figure 6. PCA score chart of all samples (A) and number of differential metabolites (B).
Figure 7. Overview of the main metabolic pathway and possible roles in up- or down-regulated expressed genes encoding enzyme and metabolites in the control and 1-MCP treated groups. In the figure, red grid represents up-regulation, green grid represents down-regulation, and yellow grid represents both up regulation and down regulation and white grid means no expression of gene or metabolite. 1, CK3vsCK0; 2, CK6vsCK0; 3, 1-MCP3vsCK0; 4, 1-MCP6vsCK0; 5, 1-MCP3vsCK3; 6, 1-MCP6vsCK6.
ACACA, acetyl-CoA carboxylase/biotin carboxylase 1; ACAT, acetyl-CoA C-acetyltransferase; ACE B, malate synthase; ACE E, pyruvate dehydrogenase E1 component; ACLY, ATP citrate (pro-S)-lyase; ACOX, acyl-CoA oxidase; ACO, aminocyclopropane carboxylate oxidase; ACS, 1-aminocyclopropane-1-carboxylate synthase; ADHL, aldehyde dehydrogenase (NAD+); AGPAT1-2, lysophosphatidic acid acyltransferase; AGXT, alanine-glyoxylate transaminase/serine-glyoxylate transaminase/serine-pyruvate transaminase; ALDO, fructose-bisphosphate aldolase, class I; AMY, α-amylase; AOC, allene oxide cyclase; AOS, hydroperoxide dehydratase; BRI1, brassinosteroid insensitive kinase inhibitor1; CAT, catalase; COI, coronatine-insensitive protein1; CS, citrate synthase; DHOME, dihydroxyoctadec-12-enonic acid; DLAT, pyruvate dehydrogenase E2 component (dihydrolipoamide acyltransferase); DLD, dihydrolipoamide dehydrogenase; E2.4.1.14, sucrose-phosphate synthase; E3.2.1.21, β-glucosidase; E3.2.1.4, endoglucanase; ENO, enolase; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase family member 1/3; EpOME, epoxy-12-octadecenoic acid; ETKN, ethanolamine kinase; ERS, ethylene responsive sensor; ETR, ethylene receptor; FAD A, acetyl-CoA acyltransferase; FDH, formate dehydrogenase; FPGD, 6-phosphogluconate dehydrogenase; FUM, fumarate hydratase class I; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GGT, γ-glutamyl transpeptidase/glutathione hydrolase; GLY A, glycine hydroxymethyltransferase; GLG A, starch synthase; GLG B, 1,4-alpha-glucan branching enzyme; GLG C, glucose-1-phosphate adenyl transferase; Gln A, glutamine synthetase; GPAT, glycerol-3-phosphate acyltransferase; GPAT1-2, glycerol-3-phosphate O-acyltransferase ½; GPI, glucose-6-phosphate isomerase; GPMI, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase; G6PD, glucose-6-phosphate 1-dehydrogenase; GPX, glutathione peroxidase; GRHPR, glyoxylate/hydroxy pyruvate reductase; GSR, glutathione reductase (NADPH); GSS, glutathione synthase; GST, glutathione S-transferase; HAO, (S)-2-hydroxy-acid oxidase; HPR A, glycerate dehydrogenase; HK, hexokinase; ICD, isocitrate dehydrogenase; IDH, isocitrate dehydrogenase; INV, β-fructofuranosidase; JAR, jasmonic acid-aminotransferase; LOX2S, lipoxigenase; LTA E, threonine aldolase; MDH1, malate dehydrogenase; MFP2, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; NMT, phosphoethanolamine N-methyltransferase; OPR, 12-oxophytodienoic acid reductase; OTS A, trehalose 6-phosphate synthase; OTS B, trehalose 6-phosphate phosphatase; PEP, phosphoenolpyruvate; PFK, 6-phosphofructokinase 1; PGAM, 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase; PGK, phosphoglycerate kinase; PGLS, 6-phosphogluconolactonase; PGM, phosphoglucomutase; PHOSPHO1, phosphoethanolamine/phosphocholine phosphatase; PISD, phosphatidylserine decarboxylase; PK, pyruvate kinase; PLA2G, secretory phospholipase A2; PLD1-2, phospholipase D1/2; PRPS, ribose-phosphate pyrophosphokinase; PRPP, 5-phospho-alpha-D-ribose 1-diphosphate; PTDS52, phosphatidylserine synthase 2; RPI A, ribose 5-phosphate isomerase A; SAM, S-adenosylmethionine synthetase; SCRK, fructokinase; SDHA, succinate dehydrogenase (ubiquinone) flavoprotein subunit; SDS, L-serine/L-threonine ammonia-lyase; SER B, phosphoserine phosphatase; SER C, phosphoserine aminotransferase; SUS, sucrose synthase; TAL, transaldolase; TGA, transcription factor A; TKT, transketolase; TPS, trehalose 6-phosphate synthase/phosphatase; TREH, α-treholase; UGP2, UTP-glucose-1-phosphate uridylyl transferase.