The mechanism analysis of exogenous melatonin in limiting pear fruit aroma decrease under low temperature storage

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Exogenous melatonin (MT) is widely used in fruit preservation, and can increase the storage time and delay the quality deterioration. Firstly, it was found that 150 μM MT was the optimal concentration to treat 〔Xinli No.7〕 under storage at 4℃ for 60days. MT could significantly improve oxidase activity and inhibit the reduction of physiological indexes, including pulp hardness, weight loss, titratable acid and soluble solid content. MT could also reduce ethylene release and limit the reduction of fruit aroma. The average content of fruit aroma substance increased by 43.53%. A relevant RNA-Seq database was built to further explore the regulation mechanism of MT. A total of 2761 differentially expressed genes (DEGs) were identified. DEGs were enriched in 64 functional groups and 191 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. DEGs were mainly enriched in alpha-Linolenic acid metabolism, fatty acid metabolism and plant hormone signal transduction pathway. The gene pycom09g05270 belonging to long chain acyl-CoA synthetase family and participating in fatty acid metabolism pathway was identified, and its expression level was consistent with Fragments Per Kilobase per Million mapped reads (FPKM) values, implying that pycom09g05270 might play a vital role in maintaining quality during the storage process.

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17   **Abstract:** Exogenous melatonin (MT) is widely used in fruit preservation, and can increase the

18   storage time and delay the quality deterioration. Firstly, it was found that 150 μM MT was the

19   optimal concentration to treat 'Xinli No.7' under storage at 4℃ for 60days. MT could

20   significantly improve oxidase activity and inhibit the reduction of physiological indexes,

21   including pulp hardness, weight loss, titratable acid and soluble solid content. MT could also

22   reduce ethylene release and limit the reduction of fruit aroma. The average content of fruit aroma

23   substance increased by 43.53%. A relevant RNA-Seq database was built to further explore the

24   regulation mechanism of MT. A total of 2761 differentially expressed genes (DEGs) were

25   identified. DEGs were enriched in 64 functional groups and 191 Kyoto Encyclopedia of Genes

26   and Genomes (KEGG) pathways. DEGs were mainly enriched in alpha-Linolenic acid

27   metabolism, fatty acid metabolism and plant hormone signal transduction pathway. The gene

28   *pycom09g05270* belonging to long chain acyl-CoA synthetase family and participating in fatty

29   acid metabolism pathway was identified, and its expression level was consistent with Fragments

30   Per Kilobase per Million mapped reads (FPKM) values, implying that *pycom09g05270* might

31   play a vital role in maintaining quality during the storage process.

33   **Keywords:** Pear, Melatonin, Aroma

35   **1.   Introduction**

36   Pear (*Pyrus*) is one of the three largest deciduous fruit trees in the world, and fruit aroma is one
37   of the important indexes to evaluate the quality of pear fruit and determine consumers' preference.
38   Occidental pear varieties have richer fruit aromas as compared to Oriental varieties. The fruit
39   aroma and storage are affected by some irreversible physiological and biochemical reactions
40   occurring inside the fruit along with fruit ripening and development (Jia et al., 2018). On the
41   other hand, the pear fruit aroma would become weak under low temperature storage, which leads
42   to a decrease in the edible quality and commercial value.

43   Exogenous MT (N-acetyl-5-methoxytryptamine) can limit the decrease in fruit aroma during
44   storage and prolong the storage time. MT is a kind of indole small molecule substance which is
45   widely spread in animals and plants (Jang et al., 2015; Wang et al., 2016; Jemima et al., 2011),
46   and was discovered in the pineal gland of cattle. Initial research held that the substance only
47   existed in animals and humans. Until 1995, Hattori et al. (1995) found that MT was also present
48   in some plants, such as wheat and corn. Afterwards, MT was discovered in roots, leaves and
49   flowers of plants (Hattori et al., 1995).

50   Studies have shown that MT can scavenge reactive oxide species (ROS) (Lei et al., 2013;
51   Pieri et al., 1995), regulate fruit maturity and senescence (Gao et al., 2016; Xin et al., 2017; Hu
52   et al.,2018) and improve stress resistance (Lei et al., 2010; Jia et al., 2019). In addition, MT has
53   also been proven to have a better preservation effect on fruits and vegetables. In peach, MT
54   treatment could reduce decay rate, delay senescence and maintain total soluble solids and
55   ascorbic acid content during storage (Gao et al., 2016). 0.2 mM MT could delay ripening,
56   maintain and improve quality of mangoes via inhibiting hydrolytic enzymes and enhancing the
57   antioxidant system (Awad et al., 2021). In the apple ripening process, the malondialdehyde
58   (MDA) content was negatively correlated with the MT content. This may be due to the fact that
59   MT can remove the ROS produced by the respiratory jump to maintain the redox balance in the
60   cell (Lei et al., 2013). Additionally, MT could regulate ROS signaling followed by activation of
61   the calcineurin B-like 1-interacting protein kinases 23 (CIPK23) pathway to regulate the
62   expression of the potassium channel protein gene, which then promotes K^+ absorption (Li et al.,
63   2016). MT could inhibit ethylene biosynthesis to alleviate O_3 stress in grape leaves (Liu et al.,
64   2021).

65   The ability of MT to scavenge radicals is higher than that of vitamin E, ascorbic acid and
66   glutathione, and it is an efficient redox balance agent (Pieri et al., 1995). In cucumber, MT could
67   inhibit the respiration rate and reduce ethylene release, MDA content and active oxygen content
68   during storage (Xin et al., 2017). The carrot suspension cell line treated with MT could better
69   maintain the function and integrity of the cell membrane under low temperature stress (Jia et al.,
70   2019). 100 μmol·L^{-1} exogenous MT treatment could alleviate the decrease in chlorophyll,
71   ascorbic acid (Vc), titratable acid and soluble protein contents, inhibit membrane lipid
72   peroxidation and maintain membrane function and integrity of cucumber after harvest (Xin et al.,
73   2017). In cabbage tissues stored at low temperature after harvest, MT could increase the activity
74   of superoxide dismutase (SOD) and peroxidase (POD), reduce MDA accumulation, delay
75   chlorophyll degradation and maintain soluble sugar and soluble protein contents in stem and leaf
76   tissues (Jia et al., 2019). In plum fruits, suitable concentration of MT treatment could keep the
77   contents of fruit soluble solids and titratable acid stable and ensure the fruit flavor and quality
78   (Feng et al., 2020). MT could inhibit phenolic compound metabolism and improve antioxidant
79   capacity to delay the surface discoloration of fresh-cut Chinese water chestnuts and prolong the
80   shelf life (Xu et al., 2022).

81   MT can induce ethylene synthesis and signal transduction, inhibit the accumulation of free
82   radicals and membrane lipid peroxidation and promote fruit maturity and delay fruit senescence.
83   However, research on the effect of MT on pear fruits are few and mainly focus on the quality.
84   MT can participate in antioxidant and antibacterial process in 'Huangguan' pear during storage.
85   Liu (2019) studied the effects of 100 μmol·L^{-1} MT on the softening and senescence of three
86   Western pear cultivars after low temperature storage and further analyzed aroma components
87   change of 'Korla pear' and 'Abate'. However, the suitable concentration of MT treatment in the
88   process of low temperature storage was not explicably stated.

89   Until now, the molecular mechanism of MT in regulating pear fruit aroma is still unclear. In
90   this study, six MT concentrations were used to treat 'Xinli No.7' fruits before storage at low
91   temperature. And then, pulp hardness, weight loss rate, soluble solid content, titratable acid,
92   MDA, POD, SOD, ethylene release rate, fruit aroma content and aroma components were
93   measured at different storage days. Treating the fruits of 'Xinli No.7' with 150 μmol·L^{-1} MT can
94   significantly maintain the physiology quality of pear and delay aroma decrease during low
95   temperature storage. Gene expression types and abundance of MT-treated pear fruits during low
96   temperature storage were further analyzed using digital expression profiling technology. Hence,

97 the key genes involved in regulating aroma production under low temperature were identified

98 and the molecular mechanism of MT in the regulation of fruit aroma was explored.

99

100 **2. Materials and methods**

101 **2.1 Experimental materials**

102 'Xinli No.7' is one of the filial generations of 'Korla pear' × 'ZaoSu', which belongs to *P.*

103 *sinkiangensis* and is a new early-maturing pear variety. Additionally, the scientific name is *P.*

104 *sinkiangensis* Xinli No.7.

105   'Xinli No.7' fruits were collected from Tianping Lake Experimental Demonstration Base of

106 Shandong Institute of Pomology. The pear fruits with uniform maturity, uniform size and

107 without mechanical damage were selected and divided into six groups. Six concentrations (0, 50,

108 100, 150, 200 and 250 μmol·L^{-1}) of MT solution were set to treat six pear groups. The pears

109 were immersed in MT solution for 30 min, and then dried in natural conditions and stored at 4℃.

110 Afterwards, the physiological indicators were determined at 20, 40, 60 and 80 days after storage.

111 Additionally, the MT was purchased from Solarbio (Cas:73-31-4) and dissolved with dimethyl

112 sulfoxide (DMSO).

113

114 **2.2 Analysis of physiological indexes**

115 The fruits treated with different MT concentrations and stored at 4℃ were taken out every 20

116 days to measure physiological data and enzyme activity.

117   Pulp hardness was measured using a GY-4 hardness tester. The skin was peeled off the

118 carcass of the fruit, and the hardness of the sunny side and the dark side of each fruit was

119 measured.

120   The weight loss rate of pear under treatment with MT and storage at 4℃ were calculated

121 using the following formula: weight loss rate (%) = (initial weight - post storage weight)/initial

122 weight×100. Firstly, the weight of MT-treated pear before storage was recorded, and the same

123 pears were taken after storage for 20, 40, 60 and 80 days to weigh and record the weight. Then,

124 the weight loss rate was calculated.

125   The soluble solid contents of pear were measured using ATAGO-PAL-Q type digital

126 refractometer. The detection mirror was cleaned with distilled water, then adjusted to zero for

127 calibration, and the detection mirror was dried with lens wiping paper. Squeezed juice was

128 dropped on the detection mirror of the refraction instrument for measurement, and the value on
129 the digital display device was read. Each treatment was repeated three times.

130   The titratable acid content of fruits was determined using the sodium hydroxide titration
131 method. Firstly, 5 g pulp was weighed and grinded into homogenate in a mortar, and transferred
132 into 50 mL conical bottle. The solution was placed in a water bathed for 30min at 80℃, and then
133 cooled to room temperature. Afterwards, the solution was centrifuged at 10000 g for 10 min, and
134 the precipitation was removed. The supernatant was added into a 50 ml volumetric flask and then
135 distilled water was added into it until it reached 50 mL. The supernatant solution was blended,
136 and 10 mL supernatant solution was taken for determination of acid content. ~3-5 drops of
137 phenolphthalein indicator were added into the 10 ml supernatant solution, and 0.10 mol·L^{-1}
138 NaOH standard liquid was added until the solution turned red and did not fade for 30 s. Each
139 treatment was repeated 3 times. The titratable acid content of pear under treatment with MT and
140 storage at 4℃ was calculated using the following formula: titratable acid content (%) =
141 $(C \times V_1 \times k \times V_T)$ /m$\times V_S$. C, V_1, k, V_T, m and Vs represent concentration of NaOH standard
142 solution (mol·L^{-1}), the volume of NaOH standard liquid (mL), organic acid conversion factor,
143 constant volume after extraction (mL), sample quality (g) and volume of sample solution used
144 for titration (mL), respectively.

145

146 **2.3 Analysis of enzyme activity**

147   Firstly, 2 g pulp was weighed and put into a precooled mortar. 5 mL pre-cooled 100 mmol·
148 L^{-1} phosphoric acid buffer with pH 7.0 was added into the mortar, and then, a little of 1%
149 polyvinylpyrrolidone (PVP) and a small amount of quartz sand were added. The homogenate
150 was ground in an ice bath and centrifuged at 10000 rpm and 4 ℃ for 20 min. The supernatant
151 was the crude enzyme extract for determination of malondialdehyde content and antioxidant
152 enzyme activity.

153   MDA content was measured using the thiobarbituric acid (TBA) method. The solution of
154 0.6%TBA (2 mL) was added to the supernatant (2 mL) and the water was used as references,
155 respectively. The solution was mixed and water bathed at 100℃ for15min, and then, they were
156 quickly cooled and centrifuged at 10000 rpm and 4 ℃ for 20 min. The absorbance value of the
157 supernatant was determined at 450, 532 and 600 nm, respectively. MDA content (mmol·g^{-1})
158 =$[6.45(D_{532}-D_{600})-0.56D_{450}] \times$ N/W. N and W represented the volume of extract solution (mL)
159 and the weight of pulp (g), respectively.

160   SOD activity was measured using the azblue tetrazole photochemical reduction process
161   method. Firstly, reaction reagents, including phosphate buffer, Met solution (13 mmol), NBT
162   solution (75 μmol), EDTA-Na$_2$ (10 μmol), riboflavin (2.00 μmol) and distilled water were
163   prepared. Four mixed solutions (2.9 mL) were prepared, and two fractions of mixed solutions
164   were added to the enzyme extract (0.1 ml) and the others were added to phosphate buffer as
165   controls. One of the two controls was covered with foil. They were placed in 4000 lx sunlight
166   lamp for 20 min at 25 ℃. And then, they were quickly transferred into dark condition to stop the
167   reaction. The absorbance value for reaction solutions was determined at 560 nm. SOD
168   enzyme activity $=(A_0-As) \times V_t/C \times 0.5A_0 \times F_w \times V_1$. A_0, As, Vt, V_1, Fw and C represented the
169   absorbance value of the control, the absorbance value of the sample, the total volume of enzyme
170   extract solution (mL), the sample volume used in testing (mL), the weight of sample (g) and
171   protein concentration in crude enzyme solution (mg/g), respectively.

172   POD activity was measured using the guaiacol method. The reaction buffer was prepared,
173   including 0.05 mol/L guaiacol solution, 2% H$_2$O$_2$, 20% TCA, and 0.1% PBS. The enzyme
174   extract solution and PBS (control) were added to the reaction buffer (3ml), respectively. The
175   absorbance value of the reaction solutions was determined at 470 nm and immediately the values
176   were recorded every 10 s, 3 times. POD enzyme activity (U/g min) $=\Delta A_{470} \times Vt/0.01W \times Vs \times t$.
177   ΔA_{470}, W, t, Vt, and Vs represented change of absorbance value in reaction time, the weight of
178   sample (g), reaction time (min), the total volume of enzyme extract solution (mL) and the sample
179   volume used in testing (mL), respectively.

180

181   **2.4 Analysis of ethylene release rate**
182   The pear fruits were placed in a dry container for 2 h after having been sealed.   A 1 mL syringe
183   was used to draw air repeatedly and the air sample was injected into the GC2014C gas
184   chromatograph for determination. The FID detector was used and the test parameters were set as
185   follows. The N$_2$ flow rate was 85 ml/min, H$_2$ pressure was 0.60 kg/cm^2, air pressure was 0.40
186   kg/cm^2, column and injection port temperature were 105 and 125°C, respectively.

187

188   **2.5 Determination of the volatile aroma**
189   The pear fruit were removed from the kernel and cut into 0.20 × 0.20 × 0.20 cm chunks. After
190   sample mixing, 5 g samples were taken and put into a 10 mL sample bottle. 3 μl ance-octanol 2
191   (0.01644 g·L^{-1}) was dropped into the sample bottle and covered with tin foil seal. The methods

192 of fruit extraction and determination of volatile components were as follows. The sample was

193 water bathed for 30 min at 40°C. Meanwhile, the aroma were adsorbed using a SPME extraction

194 head (black). And then, adsorption components were injected into a Gas Chromatography/Mass

195 Spectrometry (GC-MS) for 5 min. The unknown compounds were searched by Internet and

196 matched with mass spectrometry libraries NIST17-1, NIST17-2 and NIST17-3 to confirm all

197 volatile components. The content of each component was calculated according to the following

198 formulae. Each component(ng·g^{-1})= [the peak area of component/(the peak area of internal

199 standard × m)] × C × V ×1000. Among them, m, C and V represented the quality of sample (g),

200 internal standard concentration (g/L) and internal standard volume (μL), respectively.

201

202 **2.6 The transcriptome materials and RNA extraction**

203 The 'Xinli No.7' pear fruits were divided into six groups, and each group contained five pears.

204 Three of these groups were treated with 150 μmol·L^{-1} MT (MT150), and the others were treated

205 with water (CK). The pear fruits were stored at 4°C for 60 days. The pulp of six groups of pear

206 fruits were collected and placed in a freezer tube. They were put into liquid nitrogen and stored

207 at -80°C.

208   The final cDNA libraries were synthesized by Shanghai OE Biotech Co., Ltd.   Total RNA

209 was extracted using the mirVana miRNA Isolation Kit (Ambion) referring the manufacturer's

210 protocol. Agilent 2100 Bioanalyzer was used to tested RNA integrity, and the libraries were

211 constructed using TruSeq Stranded mRNA LTSample Prep Kit following the manufacturer's

212 instructions. Illumina sequencing platform (Illumina HiSeq × Ten) was used to sequenced these

213 libraries, and 125bp/150bp paired-end reads were generated.

214

215 **2.7 Transcriptome data analysis**

216 The published genome database of *Pyrus communis* (taxid:23211) was used as a reference to

217 analyze the sequencing results. Raw data (raw reads) were processed using Trimmomatic. The

218 reads containing ploy-N and the low-quality reads were removed to obtain the clean reads. Then

219 the clean reads were mapped to *Pyrus communis* genome using hisat2. FPKM value of each gene

220 was calculated using cufflinks, and the read counts of each gene were obtained by htseq-count.

221 DESeq was applied to carry out the elimination of biological mutations, and the threshold for

222   significantly differential expression were P value < 0.05 and fold change >2 or < 0.5. The

223   analysis methods of Hierarchical cluster, GO enrichment and KEGG pathway enrichment

224   analysis were collected as previously described (Bolger et al.,2014; Kanehisa et al.,2008; Anders

225   et al.,2013). Additionally, the statistical power of this experimental design was calculated in

226   bioconductor   (version   3.15),   an   open-source   software   for   bioinformatics

227   (https://doi.org/doi:10.18129/B9.bioc.RNASeqPower). We also used the software to analyze

228   RNA-seq data, and 10 × sequencing depth and three technical replicates were used to achieve the

229   claimed statistical power. Additionally, the raw sequence data and reads of RNAseq assembles

230   sequences had been deposited in NCBI, and the SRA and TSA accession numbers are

231   PRJNA861369 (TaxID: 870726) and GKBH00000000, respectively. the actual sequences could

232   be downloaded from Genome Database for Rosaceae (GDR (rosaceae.org)).

233

234   **2.8 qRT-PCR analyses**

235   Based on the analysis of RNA-seq data, 10 genes relating to aroma expression were selected

236   to validate their expression levels. The primers were designed by Shanghai OE Biotech Co., Ltd

237   and synthesized by TsingKe Biotechnology Technology. The primers were shown in **Table S1**.

238   *Actin* was used as an internal control gene. qRT-PCR analysis was carried out using Roche 480

239   SYBR GREEN Master (Roche, Basel, Switzerland). Roche Light-Cycler software was used to

240   analyze the original data, and the gene expression was standardized to the *actin* gene to minimize

241   the difference of cDNA template levels (Chen et al., 2015). The qRT-PCR data were shown in

242   three technical repeats with error bars, representing the mean value ± standard deviation (n = 3).

243   The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak et al., 2001).

244

245   **2.9 Statistics analysis**

246   Microsoft Excel 2010 was used to summarize data and SPSS16.0 software was used to

247   analyze statistical significance. Data were expressed as means ± standard deviation (SD). One-

248   way ANOVA were used to analyze the normality followed by Duncan's multiple range test to obtain

249   the statistical significance between groups. Alphabet indicated that data showed significant

250   difference at P<0.05 level.

251

3. Results

3.1 Physiological indexes analysis of pear fruit under treatment with MT

In the process of low temperature storage, the fruit became soft and the content of soluble solid decreased, which was one of the important reasons for the decline in fruit quality. To explore the change of physiological indexes of 'Xinli No.7' treated with MT during low temperature storage, 6 concentrations of MT were set to treat 'Xinli No.7' pear fruits, these included: 0, 50, 100, 150, 200 and 250 $\mu mol \cdot L^{-1}$. MT treatment could significantly inhibit the decrease of fruit hardness along with the extension of storage time (**Figure 1A**). MT could inhibit the increase of weight loss during the process of storage. When compared with each group treated with different MT, 150 $\mu mol \cdot L^{-1}$ MT could specifically circumvent fruit weight loss (**Figure 1B**). In the CK group, soluble solid contents increased until 40 days, and then, it slightly came down. When compared with CK, MT could increase soluble solid contents during storage, and 150 $\mu mol \cdot L^{-1}$ MT was beneficial in maintaining and increasing soluble solids in pear fruit (**Figure1C**). Titratable acid content decreased significantly during storage, and MT treatment could slow down the decline of fruit titratable acid content. Compared with other groups treated with MT, 150 $\mu mol \cdot L^{-1}$ MT could significantly maintain titratable acid content (**Figure1D**). Therefore, 150 $\mu mol \cdot L^{-1}$ MT could not only prolong the storage time of fruits but also maintain physiological indexes of pear.

3.2 Biochemical indexes analysis of pear fruit under treatment with MT

Fruit physiological and biochemical indexes are closely related to fruit quality. MT can remove ROS, and regulate fruit ripening and senescence, thus prolonging fruit storage time and maintaining fruit quality. In the CK group, MDA contents increased along with the extension of storage time. MT treatment could significantly delay the rise of MDA content (**Figure2A**). In the CK group, POD activity significantly increased until 40 days storage, and then kept at a similar level. Interestingly, POD activity increased under treatment with MT. In the group treated with 150 $\mu mol \cdot L^{-1}$ MT, POD activity increased significantly higher compared with other concentrations of MT. Additionally, there was no difference in POD enzyme activity between the control group and the high MT concentration (200 and 250$\mu mol \cdot L^{-1}$) groups at the later stage of storage (**Figure2B**). SOD activity increased during the storage period. MT could significantly improve SOD activity, especially the group treated with 150 $\mu mol \cdot L^{-1}$ MT (**Figure2C**).

282   Ethylene participates in the fruit ripening process, and its content is directly related to the

283   formation and release of fruit aroma. The results showed that the ethylene release rate of 'Xinli

284   No.7' without MT treatment continued to increase during low temperature storage. The ethylene

285   release rate of 'Xinli No.7' treated with different MT concentrations was significantly reduced

286   along with the extension of storage time. After 80 days of low temperature storage, the ethylene

287   release rate of pear fruits treated with 50, 100, 150, 200 and 250 μmol·L^{-1} MT decreased by

288   19.02, 33.48, 59.40, 41.62 and 21.90%, respectively. Additionally, after 40 days of storage, the

289   ethylene release rate gradually decreased along with the increase of MT concentration. After 60

290   and 80 days of storage, the ethylene release rate of pear treated with 150 μmol·L^{-1} MT could be

291   significantly inhibited than that treated with 200 and 250 μmol·L^{-1} MT (**Figure 2D**).

292

293   **3.3 Aroma substances variation of 'Xinli No.7' fruits during storage at 4℃**

294   In order to explore the changes of aroma substances under MT treatment, 'Xinli No.7' fruits

295   treated with different exogenous MT and stored for 60 days at 4℃ were selected to detect their

296   variation of aroma substances. The results showed that pear fruits treated with different MT

297   concentrations had a significant increase in total amount of aroma substances. However, there

298   was a slight difference in the types of aroma substances. The treatments with five MT

299   concentrations could increase the types of aroma substances of 'Xinli No.7' except 250 μmol·L^{-1}

300   MT. Thirty-three aroma substances were detected in the control group, and 36, 33, 35, 34 and 26

301   were detected in fruits treated with 50, 100, 150, 200 and 250 μmol·L^{-1} MT, respectively (**Table**

302   **1**). Additionally, we also tested the aroma of 'Xinli No.7' treated with MT and storage for 80

303   days (**Table S2**). The results showed that the aroma contents and quantity decreased compared

304   with CK, except that treated with 200 μmol·L^{-1} MT. This may be due to the fact that after storage

305   for 60 days, the ethylene release rate of pear treated with 200 μmol·L^{-1} was higher than 150

306   μmol·L^{-1}, implying the storage quality of the pear fruit might begin to decline. Hence, we

307   focused on studying the aroma contents of pear treated with different MT and storage for 60 days.

308   The detected aroma substances were mainly esters, alcohols, aldehydes, ketones, and other

309   types of substances. The application of exogenous MT with different concentrations could

310   change types of aroma substances, and the kinds of esters, alcohols and aldehydes reduced along

311   with storage. Exogenous MT treatment can promote the formation of aroma substances,

312   including hexyl acetate, 1-Hexanol, Hexanal, (E)-2-Hexenal, O-xylene and Longicyclen.

313   Meanwhile, it could inhibit the formation of aroma substances such as 1,2,4-

314   benzenetricarboxylic acid, cyclic 1,2-anhydride, nonyl ester, 1-Pentanol, 1-Heptanol, Nonanal,

315   Decanal, 2-Octanone, Ethylbenzene, 13-Methyl tetradecanal and Methyl heptenone (**Table 1**).

316   This also further implicated the complexity of the MT regulatory mechanism.

317

318   **3.4 Aroma substance contents of 'Xinli No.7' during storage at low temperature**

319   The content of aroma substance was further analyzed, and it was found that MT could increase

320   the aroma contents of 'Xinli No.7' fruit during storage. The total aroma contents of treatment

321   with 0, 50, 100, 150, 200 and 250 $\mu mol \cdot L^{-1}$ MT were 108.11, 151.378, 177.661, 176.256,

322   142.774 and 127.819 $ng \cdot g^{-1}$, respectively. Compared with control, the total aroma contents

323   treated with MT significantly increased (**Figure 3F**).

324   In terms of ester, 50 and 100 $\mu mol \cdot L^{-1}$ MT could significantly increase its content, while 100

325    $\mu mol \cdot L^{-1}$ MT could significantly reduce its content, and 200 and 250 $\mu mol \cdot L^{-1}$ MT have no

326   significant effect. The content of ester aroma substances of treatment with 100 $\mu mol \cdot L^{-1}$ MT was

327   higher (54.33%) than that of control, and 150 $\mu mol \cdot L^{-1}$ MT treatment was 25.33% lower than

328   that of CK (**Figure 3A**). In terms of alcohols, different concentrations of MT could reduce

329   alcohol contents excepted 150 $\mu mol \cdot L^{-1}$ MT. In comparison with control, alcohol contents of

330   samples treated with 150 $\mu mol \cdot L^{-1}$ MT increased by 103.33%, and that treated with 100 $\mu mol \cdot L^{-1}$

331   MT decreased by 49.61% (**Figure 3B**). Aldehyde content of samples treated with five MT

332   concentrations increased compared with control. Among them, aldehyde contents treated with

333   100 $\mu mol \cdot L^{-1}$ MT increased by 227.57% (**Figure 3C**). Interestingly, ketone content treated with

334   exogenous MT were reduced (**Figure 3D**). The content of other substances (olefins, acids, etc.)

335   in samples treated with MT significantly increased, and the increase was up to 233.23% when

336   treated with 100 $\mu mol \cdot L^{-1}$ MT (**Figure 3E**). In summary, exogenous MT treatment can

337   significantly increase the content of pear aroma substances. Both 100 and 150 $\mu mol \cdot L^{-1}$

338   exogenous MT could significantly increase aroma substance content of pear fruits (**Figure 3F**),

339   but exogenous MT of 150 $\mu mol \cdot L^{-1}$ can significantly increase alcohol content of the fruit.

340   When compared with the six groups, 150 $\mu mol \cdot L^{-1}$ MT was the optimal concentration, and it

341   could significantly reduce the decrease of fruit aroma during the process of long-term low

342   temperature storage. Additionally, it cannot only maintain the storage quality of fruits, but also

343   improve POD and SOD enzyme activity and inhibit MDA contents and ethylene release, thus

344   prolonging the storage time and improving fruits quality.

345

3.5 Differentially expressed genes (DEGs) analysis

In order to further explore the regulation mechanism of MT on 'Xinli No.7' fruit aroma under low temperature storage, illumina sequencing technology was applied to construct the cDNA library. 'Xinli No.7' pear fruits were treated with 150 μmol·L⁻¹ MT (MT150) and water (CK) and stored at 4°C for 60 days. Hence, more than 48 million high-quality reads were obtained in each sample. The reads were compared with pear genome (taxid:23211)(Gareth et al., 2019; Chagné et al., 2014) and the genome coverage rates were higher than 90.3% (**Table S3**). It showed that total sequencing satisfied the requirement of the downstream analysis. The DEGs were shown by Volcano plot (**Figure 4**). In the Volcano plot, p<0.05 was set as the cut-off criterion of significant difference. 2761 DEGs were identified from the comparison groups. Additionally, we also analysis the function annotations of 2761 DEGs (**Table S4**). Most of DEGs were transcription factors (bHLH, WRKY, MADS) and receptor-like serine/threonine-protein kinase (LRR, wall-associated receptor kinase, MLO-like protein), suggesting that DEGs could be involved in multiple process of biology. Among them, 734 genes were up-regulated (orange dots), and 2027 genes were down-regulated (blue dots).

3.6 GO annotation analysis of DEGs

GO is an international standard classification system for gene functions. In this study, GO annotations were performed on DEGs of different comparison groups, and DEGs were enriched in 64 functional groups. Among them, 23, 20 and 21 functional groups belonged to three functional family groups, including biological process, cellular component and molecular function. Additionally, the top 10 functional groups of   the three functional families were further explored.

   544 DEGs were enriched in biological process. 213, 161, 48, 33 and 32 DEGs were enriched in protein phosphorylation, regulation of transcription, signal transduction, lipid metabolic process and protein ubiquitination functional groups, respectively. 174 DEGs were enriched in cellular component. 117, 14, 11 and 10 DEGs were enriched in membrane, extracellular region, apoplast and cell wall functional groups, respectively. 959 DEGs were enriched in molecular function. 390, 213, 119, 73, 59 and 33 DEGs were enriched in ATP binding, protein kinase activity, DNA-binding transcription factor activity, ADP binding, sequence-specific DNA binding and flavin adenine dinucleotide binding functional groups, respectively (**Figure 5; Table**

377 **S5**). Most of the DEGs were distributed in biological process, implying that these genes might

378 play an essential role in the aroma synthesis and pear metabolism under treatment with MT.

379

380 **3.7 KEGG pathway analysis of DEGs**

381 In organisms, different genes coordinate with each other to perform their biological functions.

382 Pathway enrichment analysis helps to further understand the biological functions of genes. The

383 DEGs were enriched in 191 KEGG pathways based on metabolic pathways (**Table S6**).

384 The top 20 KEGG enrichment pathways of total DEGs, up-regulation DEGs and down-

385 regulation DEGs, respectively, were also further explored. 39, 26, 22, 17, 14 and 12 DEGs were

386 involved in plant hormone signal transduction (ko04075), starch and sucrose metabolism

387 (ko00500), endocytosis (ko04144), flavonoid biosynthesis (ko00941), pentose and glucuronate

388 interconversions (ko00040) and alpha-Linolenic acid metabolism (ko00592), respectively

389 (**Figure 6**). On the other hand, it was found that 13, 12, 12, 12 and 10 up-regulation DEGs were

390 involved in starch and sucrose metabolism (ko00500), flavonoid biosynthesis (ko00941),

391 endocytosis (ko04144), plant hormone signal transduction (ko04075) and protein processing in

392 endoplasmic reticulum (ko04141), respectively (**Figure S1**). Among them, the KEGG pathways

393 of up-regulation DEGs were nearly consistent with the total DEGs.

394 27, 21, 12, 9, 9, 9, 9 and 8 up-regulation DEGs were involved in plant hormone signal

395 transduction (ko04075), biosynthesis of amino acids (ko01230), pentose and glucuronate

396 interconversions (ko00040), sesquiterpenoid and triterpenoid biosynthesis (ko00909), steroid

397 biosynthesis (ko00100), alpha-Linolenic acid metabolism (ko00592), glycine, serine and

398 threonine metabolism (ko00260), fructose and mannose metabolism (ko00051), and

399 phenylalanine, tyrosine and tryptophan biosynthesis (ko00400), respectively (**Figure S2**).

400 Interestingly, the enrichment KEGG pathways of down-regulation DEGs were significantly

401 different from those of up-regulation DEGs and total DEGs. Additionally, down-regulation

402 DEGs were involved in unique pathways in the top 20 KEGG pathways, including SNARE

403 interactions in vesicular transport (ko04130) and biosynthesis of amino acids (ko01230) (**Figure**

404 **S1, S2**).

405 The gene expression levels involved in amino acid metabolism and biosynthesis pathways

406 were suppressed, implying that the metabolic processes of pear during low temperature storage

407 might be reduced. On the other hand, the genes involved in fatty acid metabolism, containing

408 alpha-linolenic acid metabolism, linoleic acid metabolism and fatty acid elongation, were up-

409   regulated, implying that fatty acid metabolism was the main pathway of aroma production during

410   low temperature storage. Interestingly, *pycom11g01870* (ko00592) and *pycom09g05270*

411   (ko02024) belonging to the long chain Acyl-CoA synthetase family were identified, and they

412   were reported to participate in fatty acids β-oxidation process. Although, quorum sensing

413   (ko02024) pathway was not divided into fatty acid metabolism, it might be localized in

414   peroxisome, and participate in transporting fatty acids into peroxisome for the β-oxidation

415   process. Additionally, the FPKM values of *pycom09g05270* were significantly higher than those

416   of *pycom11g01870*. Therefore, it was assumed in the present study that *pycom09g05270* might

417   be the key gene in limiting the aroma reduction under MT treatment.

418

419   **3.8 DEGs expression level analysis**

420   To further explore the regulatory mechanism of DEGs, 10 candidate genes related to multiple

421   metabolic pathways from the database based on GO and KEGG analysis were selected to verify

422   their expression trends by qRT-PCR. The 10 genes were involved in fatty acid synthesis pathway,

423   amino acid pathway, transcription factor regulation and calcium signal pathway.

424   The results showed that the 10 candidate genes shared a similar trend with the FPKM values

425   (**Figure 7**). Interestingly, the expression levels of both *pycom09g05270* and *pycom06g10250*

426   significantly increased under treatment of MT and storage at 4°C for 60 days, and the rest of the

427   genes showed a downward trend. Based on the above results, it was assumed in the present study

428   that pycom09g05270 might be involved in transporting aroma substrate, and pycom06g10250

429   might participate in the process of fruit aroma synthesis as a signal.

430

431   **4. Discussion**

432   MT treatment could contribute to prolonging the storage time and improving pear aroma, which

433   may be due to a decreased release of ethylene and the delay of respiration jump of pear fruits. In

434   the respiratory jump fruit, aroma synthesis was related to ethylene release. The synthesis of fruit

435   aroma was positively correlated with the release rate of ethylene, and the fruit aroma also began

436   to synthesize in a large amount during the peak period of ethylene release. In pear, the expression

437   peak of *PuACS1* and *PuACO4* were consistent with the peak of ester aroma release (Li et al.,

438   2014). In mango, the biosynthesis of monoterpenes, esters and aldehydes were strongly

439   dependent on the production of ethylene (Lalel et al., 2003). In melon, the aroma release has also

440   been confirmed to be strongly associated with ethylene (Lucchetta et al., 2007). Ester

441   biosynthetic pathway was dependent on ethylene because ethylene suppressed fruit had reduced

442   fatty acids and aldehydes (Flores et al., 2002). These reports indicated that ethylene has a

443   significant role in regulating aroma biosynthesis. In Jujube, 25 $\mu mol \cdot L^{-1}$ exogenous MT could

444   inhibit the fruit respiration and ethylene release, and delay fruit softening (Tang, 2019). However,

445   50 $\mu mol \cdot L^{-1}$ exogenous MT could promote ethylene production and reach the peak of respiratory

446   jump earlier than the control and improve its quality in tomato (Gross, 1985; Sun, 2016). In this

447   study, 150 $\mu mol \cdot L^{-1}$ exogenous MT was selected as the optimal concentration to treat 'Xinli No.7'

448   fruits, which greatly reduced ethylene release. On the other hand, combined with the results of

449   previous studies, the reduction of ethylene release may also lead to a decrease in fruit aroma, but

450   compared with the control group, the components of fruit aroma increased. This may be due to

451   the fact that MT treatment can significantly increase alcohol contents and reduce ketone contents,

452   increase the concentration of substrates in the process of fatty acid metabolism, thereby limiting

453   the decrease in fruit aroma.

454   MT could reduce the occurrence of post-harvest fruit diseases, such as pear fruit ring blight,

455   banana anthracnose, jujube penicillium and tomato gray mold (Liu, 2019; Tang, 2019; Li et al.,

456   2019a; Li et al., 2019b). MT enhances fruit disease resistance by enhancing mitogen-activated

457   protein kinase (MAPK) signaling, inducing ROS accumulation and improving the activities of

458   defense enzymes, including chitinase, β-1,3-glucanase, POD, SOD, ascorbic peroxidase (APX)

459   and catalase (CAT). However, MT treatment effectively eliminated defense-related ROS in

460   citrus fruit tissues and accelerated the spread of postharvest green mildew (Wang et al., 2019).

461   On the other hand, MT treatment can improve the resistance of apple (*Malus domestica Mill.*)

462   leaves to brown spot (Yin et al. 2013). After the tomato bacteria *Pseudomonas syringae* (*Pst*

463   *DC3000*) infection in *Arabidopsis thaliana*, it could lead to a significant decrease in endogenous

464   MT content. We also screened relevant genes from transcriptome data (Shi et al., 2014). We

465   found that 3 and 26 DEGs were enriched in regulation of defense response to fungus and defense

466   response function groups, respectively. Among them, the FPKM values of 5 DEGs were up-

467   regulated, and the others were down-regulated. The expression levels of *pycom16g02200*,

468   *pycom16g13650* and *pycom16g00860* were significantly up-regulated under treatment with MT.

469   Interestingly, the expression level of *pycom01g16720* was significantly inhibited, implying that

470   the gene might play a key role in defense response **(Figure5)**. Additionally, 8 DEGs were

471   enriched in MAPK signaling pathway (ko04010), and 7 DEGs expression levels were up-

472   regulated (**Figure 6**). Therefore, MT may play an important role in plant resistance to fungal
473   diseases.

474   Through the analysis of transcriptome data, it was found that the expression levels of genes
475   involved in the regulation of fatty acid metabolism have undergone significant changes. The
476   expression level of long chain Acyl-CoA synthetase 8 (*pycom09g05270*) was up-regulated. The
477   expression levels of linoleate 13S-lipoxygenase 3-1 (*pycom09g09820* and *pycom09g09830*) and
478   allene oxide synthase (*pycom08g03050*) were down-regulated. It was suggested that fatty acid
479   metabolism may play an essential role in pear fruit aroma metabolism in response to MT
480   treatment during storage. In addition, *LACS* played an important role in the process of fatty acid
481   transport and activation. Nine *LACS* genes were identified in *Arabidopsis* (Shockey et al., 2002),
482   and fatty acids degradation mainly occurred in peroxidase through the β-oxidation cycle in plants.
483   *LACS6* and *LACS7* were localized in peroxisomes and expressed in the germination and early
484   seedlings, indicating that they may be involved in the oxidation process of phthalyl-CoA (Fulda
485   et al., 2002, 2004; Zhao et al., 2019). *LACS9* was localized in the plasma membrane, and it was
486   inferred that it may be involved in the synthesis of lipids. Additionally, the activity of long-chain
487   acyl-CoA in *lacs9-1* mutant was decreased by 90% than wide type (Schnurr et al., 2002). LACS4
488   and LACS2 also provided substrates for the extension of wax fatty acid precursors (Schnurr et al.,
489   2004). L*ACS1* and *LACS4* participated in the formation of *Arabidopsis* pollen coat (Dirk et al.,
490   2011). In the *lacs1lacs9* and *lacs4lacs9*, the fatty acid content decreased by 11% and 27%,
491   respectively (Zhao et al., 2010; Jessen et al., 2015). MdLACS2 could also regulate the
492   accumulation of cuticular wax to enhance plant resistance to abiotic stress and MdLACS4
493   induced early flowering and increased the adaptability to stress (Zhang et al., 2020a, b). However,
494   *LACS* involved in β-oxidation process of fruit fatty acids still remained unclear. It was assumed
495   in the present study that during low temperature storage, MT treatment could improve expression
496   level of *LACS*, which increased the β-oxidation process of fatty acids and the synthesis of
497   substrate, and therefore increased fruit aroma. The mechanism of how fatty acids enter the
498   peroxisome and are activated after entering the peroxisome is still unclear. In conclusion, we
499   could transfer pycom09g05270 into yeast mutants (*Δpxa1pxa2faa*) to test its critical role in fatty
500   acid transport and analysis β-oxidation capacity of yeast mutant.

501   Calcium is one of the essential nutrient elements in plants. It is closely related to plant
502   growth and development, and can be used as a signal molecule to regulate the development
503   process. Studies have shown that calcium is one of the inhibitors of ethylene synthesis.

504   Exogenous calcium could reduce ethylene production and delay ethylene peak of respiratory

505   jump fruits. In 'Nanguoli', calcium treatment could improve its aroma by increasing the contents

506   of metabolic substrates (linoleic acid and linolenic acid) and the activities of alcohol

507   dehydrogenase (ADH), pyruvate decarboxylase (PDC) and lipoxygenase (LOX) (Wei et al.,

508   2017). Combined with RNA-Seq and qRT-PCR results, pycom06g10250 encoding calcium ions

509   was identified and the expression level was up-regulated under MT treatment. Virus induced

510   gene silencing (VIGS) could be used to further verify the function of pycom06g10250 in

511   promoting pear aroma. This result indirectly showed that in the process of low temperature

512   storage, exogenous MT treatment can activate the calcium ions pathway which could limit the

513   decrease of aroma.

514

515   **5. Conclusion**

516   In this article, 'Xinli No.7' pear fruits were treated with six concentrations of MT, and MT could

517   contribute to the improvement of oxidase activity, and the inhibition of the reduction of

518   physiological indexes, such as MDA, SOD, POD, etc. 150 µmol·L^{-1} was also identified as the

519   optimal concentration for treating pear fruits, and it could significantly reduce ethylene release

520   and limit the reduction of fruit aroma during storage. RNA-Seq library was constructed to

521   explore the potential molecular mechanism of MT in slowing down the decrease of fruit aroma.

522   2761 DEGs were identified. Among them, 734 DEGs were up-regulated and 2027 DEGs showed

523   tendency to be downregulated. DEGs were enriched in 64 functional groups and 191 KEGG

524   pathways. The DEGs were involved in plant hormone signal transduction, alpha-linolenic acid

525   metabolism and linoleic acid metabolism pathway. qRT-PCR method was also used to verify the

526   expression trends of DEGs, and it was found that the gene expression patterns were consistent

527   with the FPKM values. The two DEGs, *pycom09g05270* and *pycom06g10250*, belonging to long

528   chain acyl-CoA synthetase 8 and calcineurin B-like protein 7 family, were identified. Their

529   expression levels were up-regulated when treated with MT at low temperature storage, which

530   indirectly indicated their role in the storage process.

531

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539 **Conflicts of interests**

540 The authors declare that they have no competing interests.

541

542 **Authors' contributions**

543 Wei Shuwei contributed to the experimental design and data analysis.

544 Jiao Huijun performed experiments and wrote the manuscript.

545 Wang Hongwei and Ran Kun participated in analysis of RNA-Seq data.

546 Dong Ran and Dong Xiaochang contributed to data analysis and manuscript modification.

547 Yan Wenjing prepared the plant materials.

548 Wang Shaomin designed and managed the experiments.

549

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685   Zhang, C. L., Zhang Y. L., Hu, X., Xu.X., Wang,G.L., You, C.X., Li,Y.Y., Hao,Y.J., An apple
686   long-chain acyl-CoA synthetase, MdLACS4, induces early flowering and enhances abiotic
687   stress resistance in Arabidopsis[J]. *Plant Science,* 2020, 297:110529.

688   Zhang, C. L., Hu, X., Zhang, Y. L., Liu, Y.,Wang, G.L., You, C.X., Li,Y.Y., Hao,Y.J., An apple
689   long-chain acyl-CoA synthetase 2 gene enhances plant resistance to abiotic stress by
690   regulating the accumulation of cuticular wax[J]. *Tree Physiology*, 2020, 40(10),1450-1465.

691

Figure 1

Figure 1: Physiological indexes analysis of 'Xinli No.7' treated with MT. including pulp hardness(A), weight loss rate(B), soluble solid contents(C) and titratable acid contents(D).

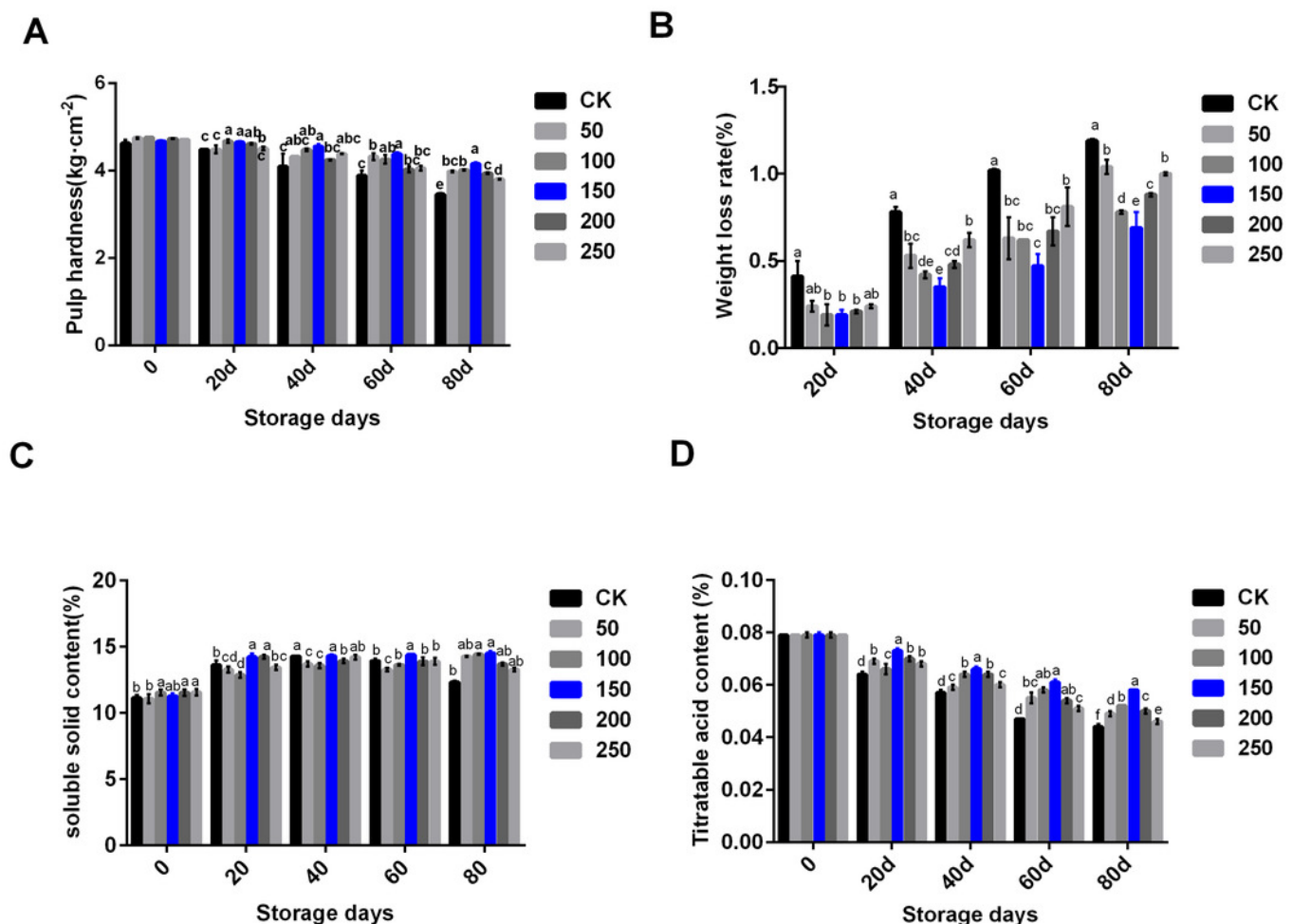



Figure 2

Figure 2: Biochemical indexes analysis of 'Xinli No.7' treated with MT. including MDA contents(A), POD activity(B), SOD activity(C) and Ethylene release rate (D).

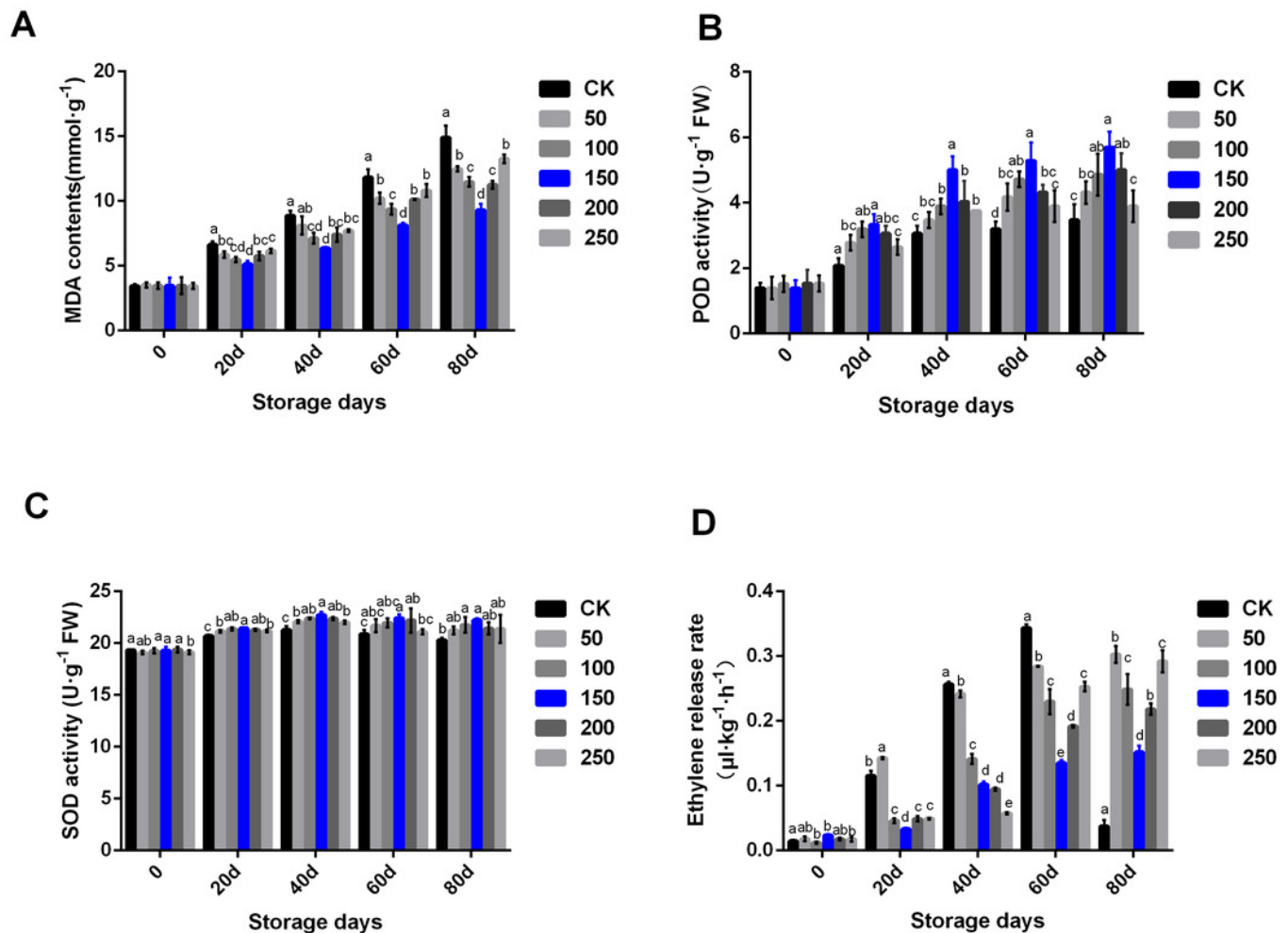
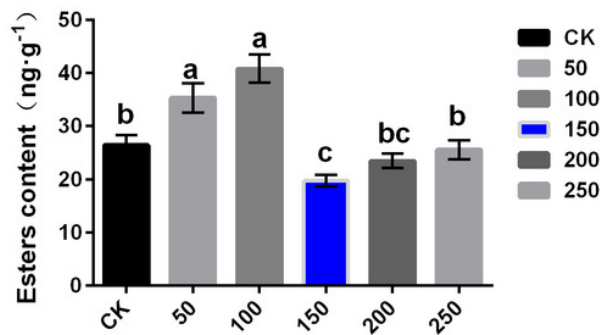
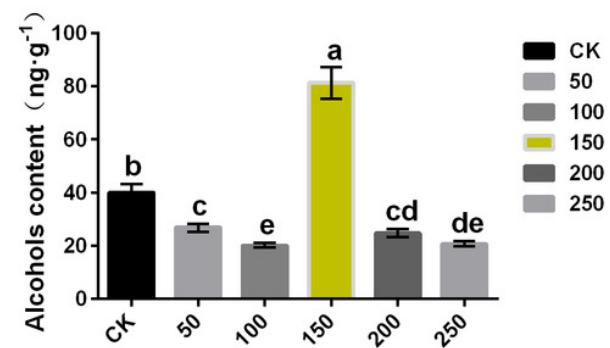
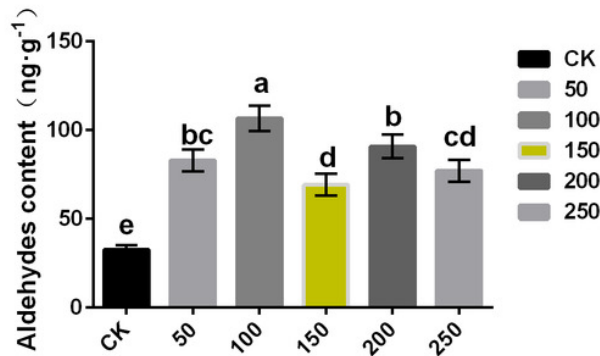
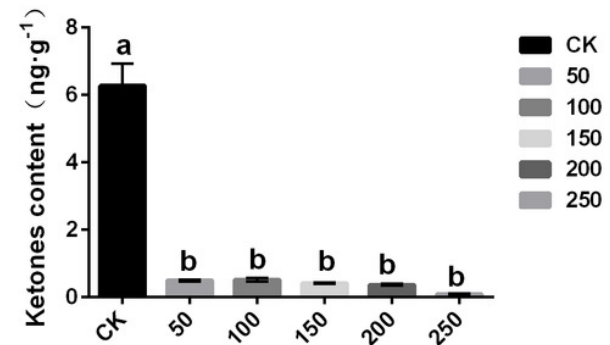
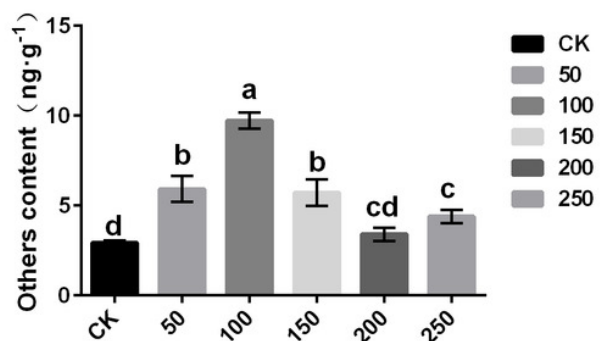



Figure 3

Figure 3: Comparison of aroma substance contents of 'XinliNo.7' treated with different MTs and storage 4℃ for 60days. (A-F) Esters, Alcohols, Aldehydes, Ketones , Other and Aroma total contents of 'Xinli No.7' treated with MT.

A

B

C

D

E

F

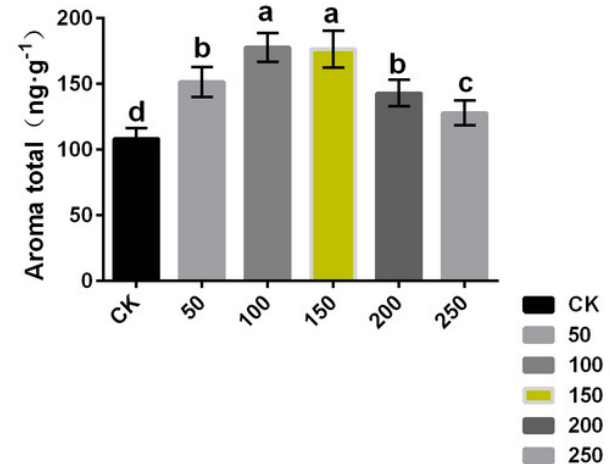



Figure 4

Figure 4: Volcanic maps analysis of DEGs. Orange dots and blue dots represented significantly up-regulated genes and down-regulated genes, respectively.

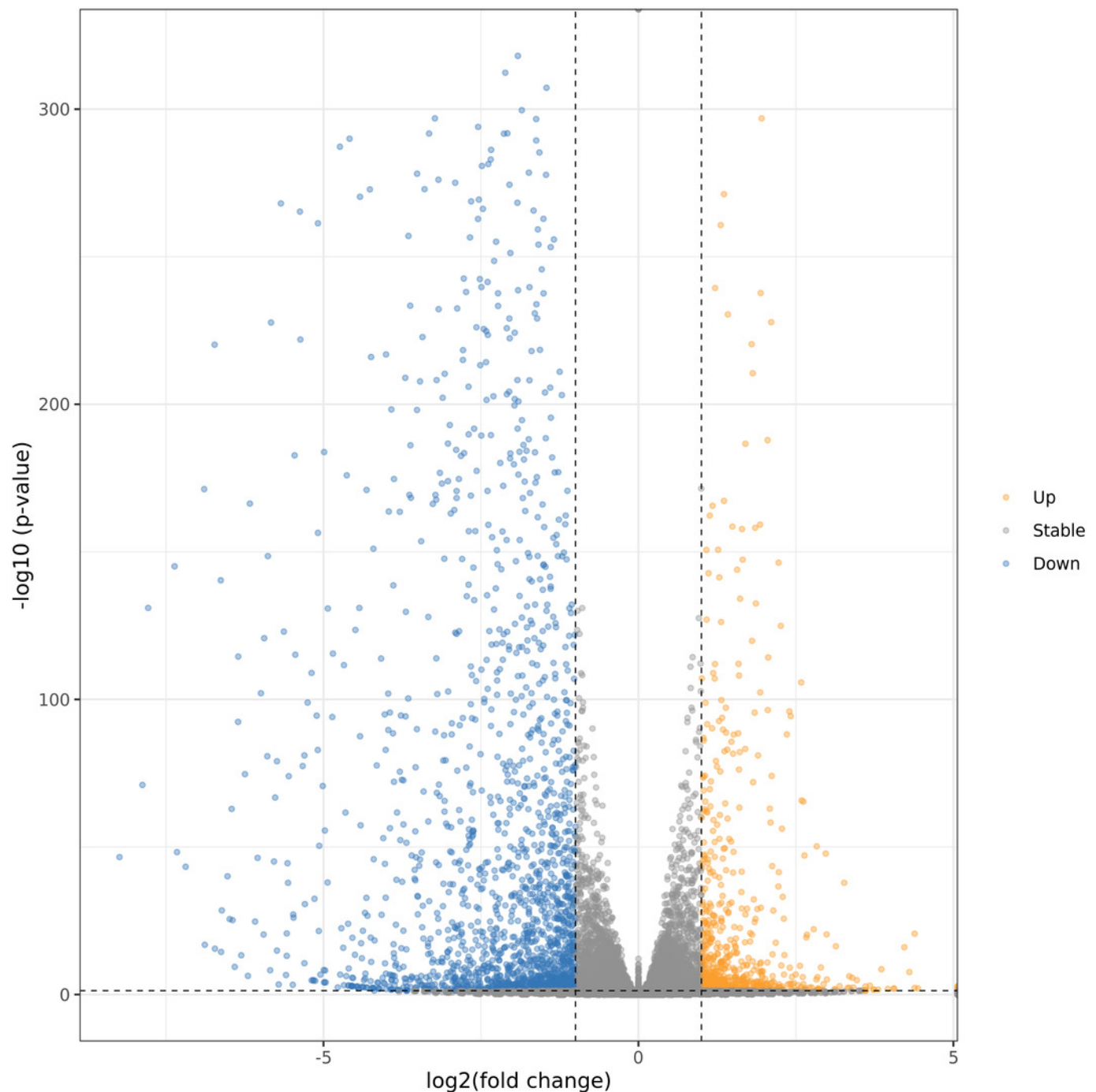



Figure 5

Figure 5: GO functional classification of DEGs under treated with MT.

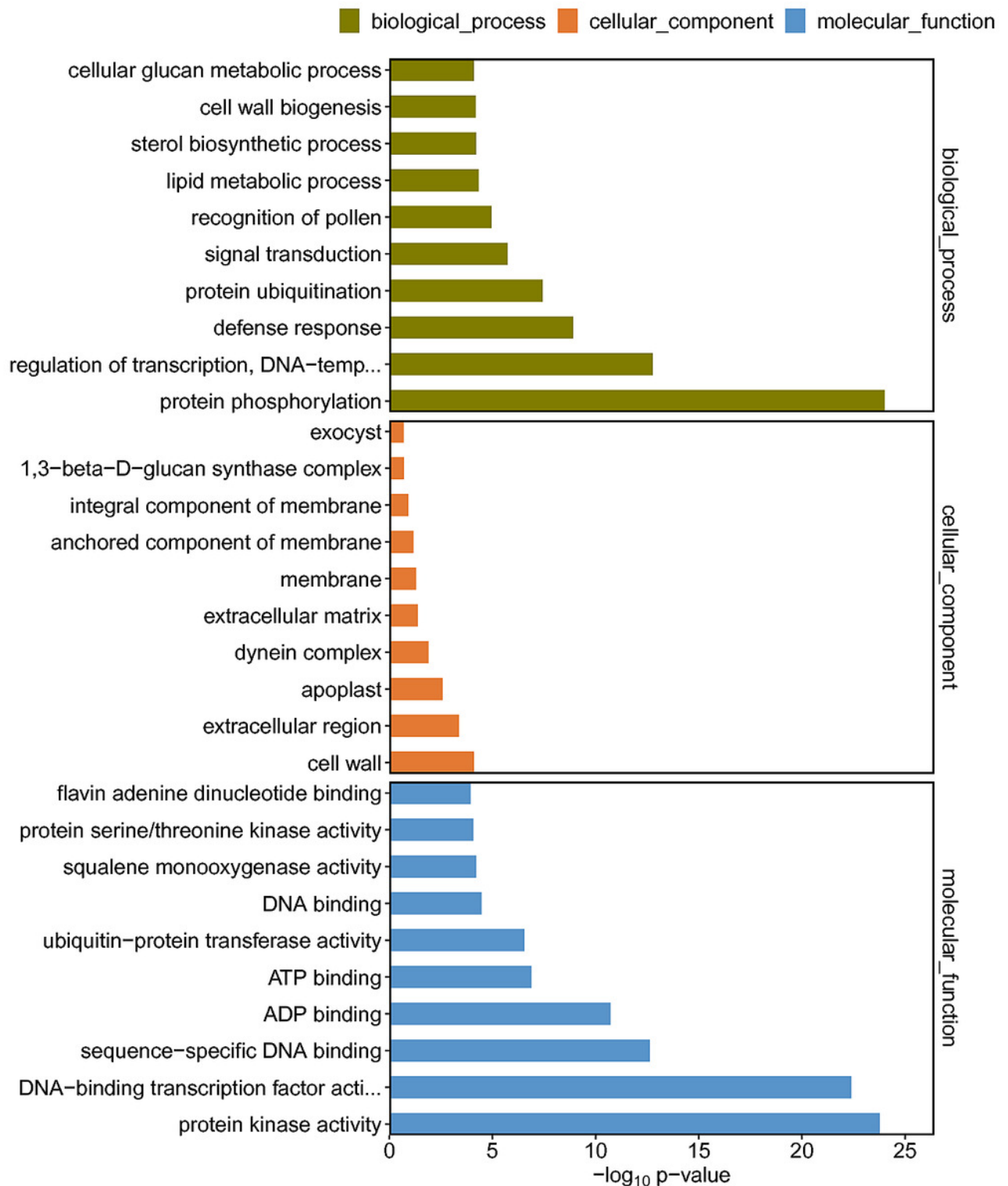



Figure 6

Figure 6: KEGG functional classification analysis of DEGs under treated with MT.

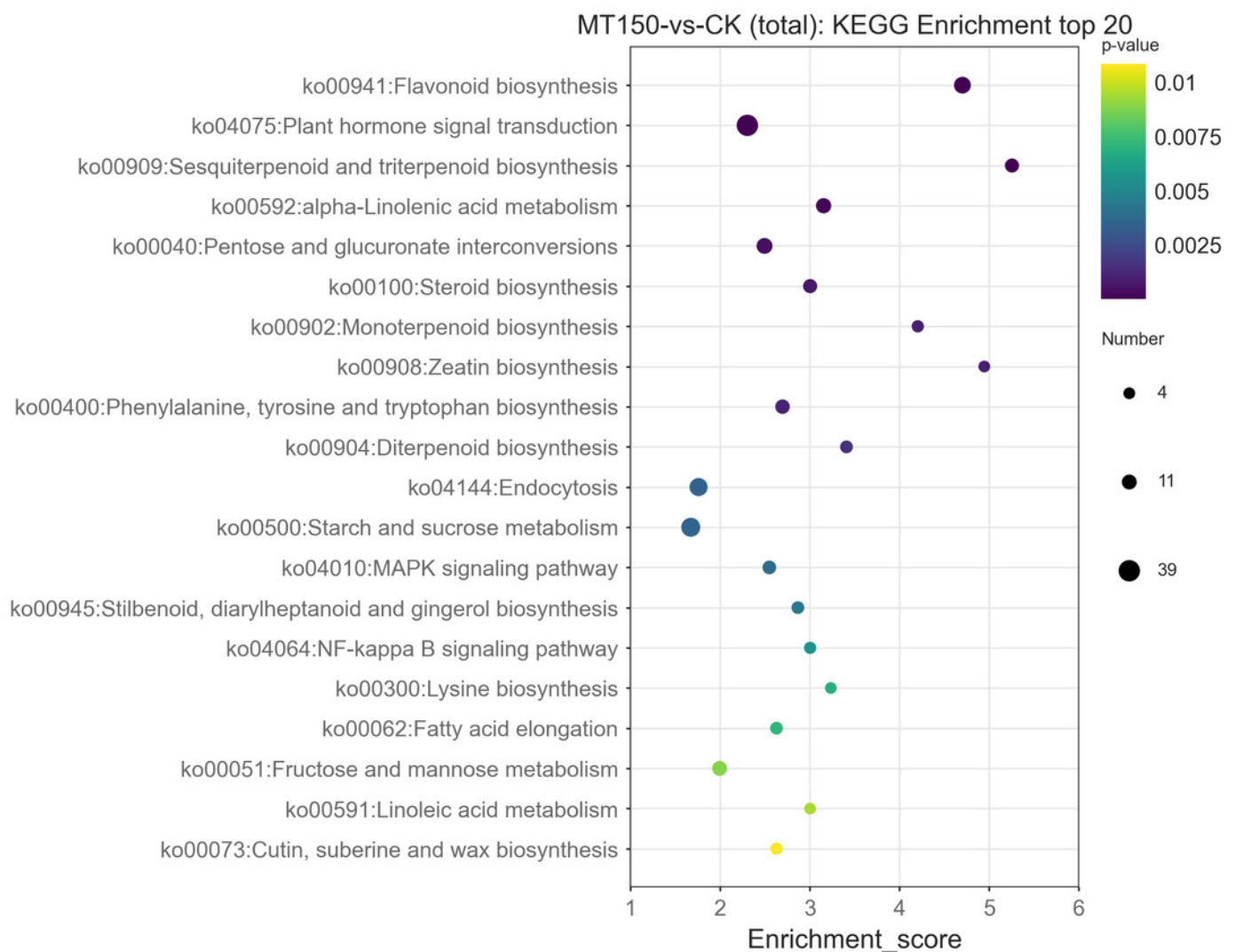



Figure 7

Figure 7:qRT-PCR analysis of ten DEGs expression level combined with RNA-Seq. CK and MT indicate that 'Xinli No.7' were treated with water and MT, and stored at 4℃ for 60 days.

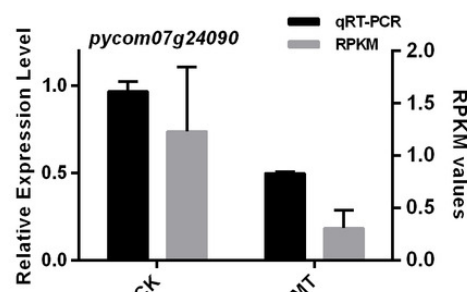
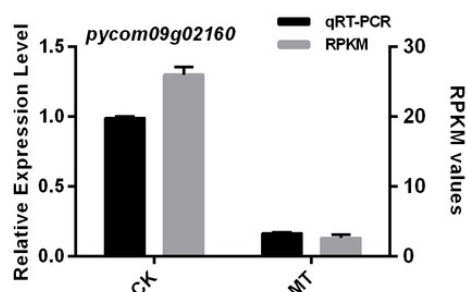
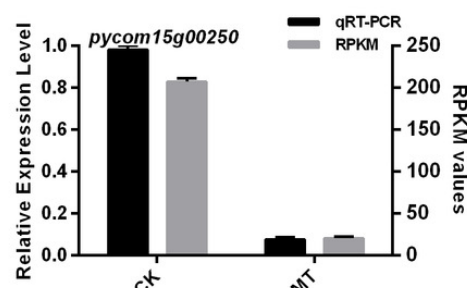
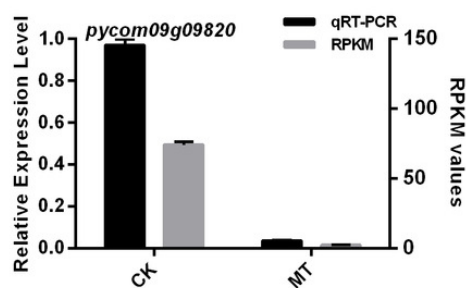
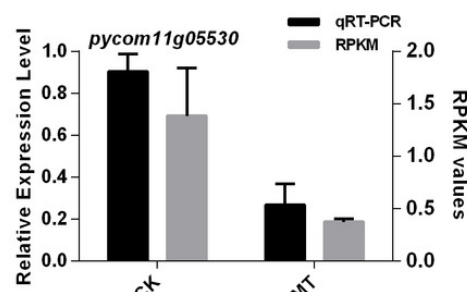
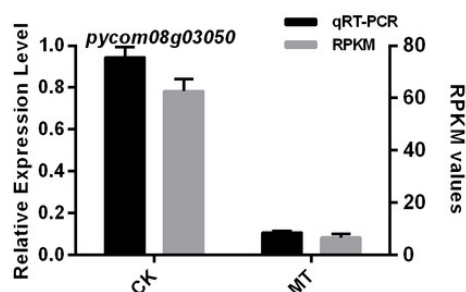
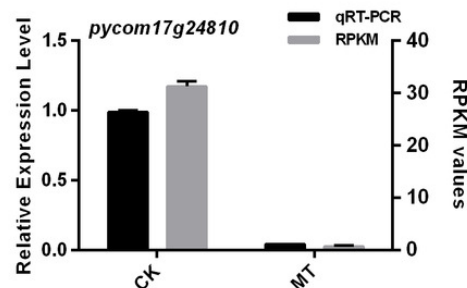
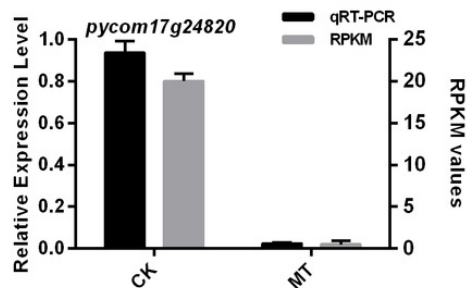
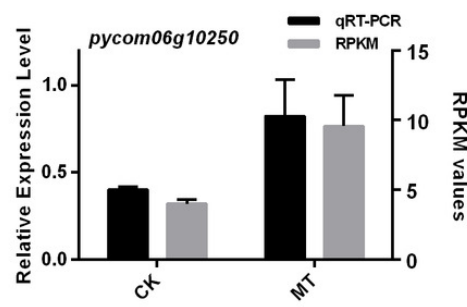
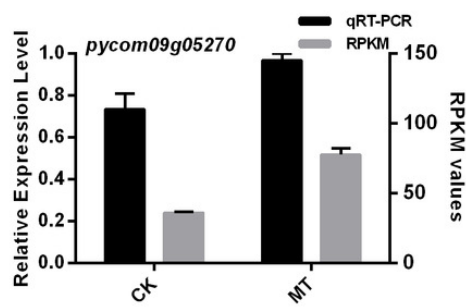



Table 1 Aroma substance of 'Xinli No.7' treated with MT and stored at 4℃ for 60days

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Volatiles Name	Aroma content(ng·g^{-1})					
	CK	50 µmol·L^{-1}	100 µmol·L^{-1}	150 µmol·L^{-1}	200 µmol·L^{-1}	250 µmol·L^{-1}
Esters						
Ethyl acetate	1.005±0.155	1.197±0.106	4.213±0.318	0.592±0.042	ND	ND
Butyl acetate	24.607±1.734	24.612±1.827	22.112±1.283	11.128±0.738	14.828±0.772	20.335±1.428
Acetic acid, pentyl ester	0.051±0.007	1.116±0.102	1.236±0.123	0.892±0.092	1.243±0.113	ND
Hexyl acetate	ND	5.349±0.427	6.198±0.416	4.321±0.066	4.962±0.284	4.255±0.248
n-Propyl acetate	0.264±0.014	0.916±0.042	0.725±0.041	0.709±0.06	0.607±0.046	ND
Isobutyl acetate	0.091±0.011	0.395±0.016	0.303±0.022	0.27±0.011	0.19±0.062	ND
Isoamyl acetate	0.365±0.02	1.451±0.172	5.732±0.424	ND	1.373±0.104	0.971±0.094
Allyl decyl carbonate	ND	0.153±0.022	ND	ND	ND	ND
1,2,4-Benzenetricarboxylic acid, cyclic 1,2-anhydride, nonyl ester	0.035±0.005	0.014±0.002	ND	ND	ND	ND
2,2,4-Trimethyl-1,4-pentanediol diisobutyrate	ND	0.093±0.011	0.258±0.016	0.145±0.014	ND	ND
Hexanedioic acid, 3-methyl-, dimethyl ester	0.003±0.001	ND	ND	ND	0.003±0.001	ND
(Z)-3,7-Dimethyl-2,7-octadien-1-ol, propanoate(ester)	ND	ND	ND	1.672±0.072	ND	ND
(all-Z)-5,8,11,14-Eicosatetraenoic acid, methyl ester	ND	ND	ND	ND	0.286±0.02	ND
Alcohols						
Ethanol	26.283±1.937	ND	ND	58.502±4.827	ND	ND
Dipentaerythritol	ND	ND	ND	0.005	0.042±0.006	ND

(Z)-2-hexenol	ND	ND	ND	ND	ND	0.246±0.023
1-Butanol	ND	ND	0.595±0.024	0.517±0.081	ND	ND
1-Butanol, 2-methyl-, acetate	0.716±0.036	3.773±0.124	ND	ND	3.697±0.283	1.423±0.133
1-Pentanol	0.182±0.009	ND	ND	ND	ND	ND
1-Hexanol	2.208±0.204	10.133±0.631	6.417±0.381	12.442±0.882	8.431±0.502	6.852±0.253
1-Heptanol	0.453±0.026	0.191±0.033	ND	ND	ND	ND
2-Methyl-1-butanol	0.044±0.006	0.063±0.023	ND	ND	ND	ND
2-Ethyl-1hexanol	0.221±0.013	ND	3.251±0.081	ND	ND	1.566±0.163
2-Octanol	9.864±0.849	9.864±0.473	9.864±0.371	9.864±0.183	9.864±0.542	9.864±0.327
2,5-Monomethylene-l-rhamnitol	ND	0.092±0.029	0.03±0.003	ND	0.039±0.004	0.033±0.006
2-Buten-1-ol, 3-methyl-, acetate	ND	0.384±0.041	ND	ND	0.405±0.033	ND
1-Octyn-3-ol, 4-ethyl-	ND	2.339±0.213	ND	ND	2.334±0.224	ND
6-Methyl-2-heptanol	0.029±0.004	ND	ND	ND	ND	0.789±0.052
Aldehydes						
Acetaldehyde	0.85±0.034	ND	ND	2.952±0.182	ND	ND
Hexanal	27.102±2.011	63.446±4.826	89.382±5.927	55.053±5.001	75.662±6.264	71.032±5.927
Heptanal	0.257±0.022	0.835±0.036	0.94±0.052	0.662±0.089	0.947±0.093	0.273±0.031
Nonanal	0.892±0.042	0.259±0.026	0.463±0.032	0.22±0.021	0.338±0.022	0.122±0.014
Decanal	0.355±0.027	0.11±0.017	0.061±0.004	ND	ND	ND
(E)-2-Hexenal	2.942±0.304	18.168±1.273	15.651±0.992	10.162±0.826	13.415±0.273	5.579±0.123
2,4,6-Trihydroxybenzaldehyde	ND	ND	ND	ND	0.111±0.012	ND
3,5-Dimethyl-4-hydroxybenzaldehyde	ND	ND	ND	ND	0.213±0.035	ND
3-Methyl butanal	0.011±0.002	0.016±0.002	ND	0.016±0.001	0.021±0.003	ND
13-Methyl tetradecanal	0.102±0.012	ND	ND	ND	ND	ND
Ketones						
Methyl heptenone	0.167±0.021	ND	ND	ND	ND	0.091±0.015

Cyclopentanone	ND	0.048±0.007	ND	0.008±0.001	0.011±0.001	ND
2-Octanone	6.094±0.635	0.441±0.023	0.513±0.052	0.405±0.019	0.349±0.03	ND
Others						
Toluene	0.375±0.028	0.816±0.041	0.525±0.031	0.782±0.057	0.397±0.036	ND
Ethylbenzene	1.27±0.052	ND	ND	ND	ND	ND
O-xylene	0.974±0.034	2.152±0.22	2.517±0.073	1.685±0.163	2.065±0.163	1.09±0.082
Biphenylene	ND	0.253±0.021	0.515±0.038	0.302±0.068	ND	0.078±0.015
1,3-Dimethyl benzene	ND	ND	ND	ND	ND	0.854±0.061
2-Allyl-4-methylphenol	ND	ND	ND	0.391±0.072	ND	0.158±0.016
Butylated hydroxytoluene	ND	ND	0.505±0.033	0.099±0.011	ND	ND
1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	ND	ND	ND	0.138±0.042	ND	0.016±0.002
n-Hexane	ND	0.122±0.017	0.29±0.013	0.15±0.045	0.231±0.032	ND
Dodecane	0.025±0.003	0.047±0.008	ND	0.032±0.006	0.049±0.007	ND
Tetradecane	ND	ND	0.27±0.011	ND	ND	ND
Dodecane, 2,6,11-trimethyl-	ND	ND	0.24±0.018	ND	ND	0.262±0.02
Nonane, 5-(1-methylpropyl)-	ND	ND	0.017±0.002	ND	ND	0.03±0.001
Longicyclen	ND	0.025±0.004	0.134±0.011	0.02±0.003	0.026±0.003	0.105±0.022
Estragole	0.232±0.024	2.011±0.371	0.95±0.056	ND	ND	0.398±0.028
Chamigren	ND	ND	2.045±0.052	ND	ND	ND
Valencene	ND	ND	ND	ND	0.207±0.073	ND
Limonene	ND	ND	ND	ND	ND	1.241±0.094
acenaphthene	ND	0.184±0.013	0.382±0.021	0.305±0.072	ND	ND
Fluorene	ND	0.287±0.025	0	0.31±0.067	ND	ND
Retinoic acid	ND	0.023±0.006	0	ND	0.003	ND
1,3-Dioxolane, 2,4,5-trimethyl-	ND	ND	0.034±0.008	0.089±0.01	0.059±0.005	ND
Methyleugenol	0.035±0.005	ND	1.293±0.082	0.818±0.062	0.32±0.036	0.156±0.01
beta-arone	ND	ND	ND	ND	0.046±0.008	ND
1,6-dimethylnaphthalene	0.004	ND	ND	0.598±0.061	ND	ND

Aroma numbers total	34	36	33	35	34	26

1 ND means not detected.