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Mechanism of induced soluble sugar accumulation and organic acid reduction in plum fruits by application of melatonin

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Abstract

Melatonin (MT) can improve plant resistance and fruit quality. The mechanism by which MT affects soluble sugar and organic acids accumulation in drupe fruits is not clear. In this study, 100 μmol/L MT was sprayed on the leaves of plum trees at the second stage of rapid fruit expansion (90 and 97 d after flowering), and the effects of MT on plum fruit quality and its effects on the soluble sugar-organic acid metabolism were investigated. At 28 d after MT treatment (at maturity), the longitudinal diameter, fruit weight, and vitamin C content of plum fruits were increased by 5.05%, 12.93%, and 56.09%, respectively, compared to the control. MT caused significant increase in the total soluble solids content and decreased the titratable acid content. MT increased the contents of total soluble sugar, sucrose, sorbitol, and citric acid after 21 and 28 days of treatment, while decreasing the contents of fructose, malic acid, quinic acid, and tartaric acid after 28 days of treatment. Additionally, MT increased the activities of sucrose synthase (catabolism direction), sucrose phosphate synthase, glucokinase, fructokinase, sorbitol oxidase, and $NADP^+$-malic enzyme, and decreased the activities of soluble acid converting enzyme, cell wall insoluble converting enzyme, NAD^+-sorbitol dehydrogenase, and NAD^+-malic dehydrogenase after 21 or 28 days of treatment. Moreover, the differentially expressed genes (DEGs) after 21 and 28 days of treatment were accelerated starch and sucrose metabolism, galactose metabolism, fructose and mannose metabolism, as well as glycolysis, gluconeogenesis, and pentose phosphate metabolism pathways. In conclusion, exogenous MT increases soluble sugar content and decreases organic acid content in plum fruits by regulating various soluble sugar-organic acid metabolic pathways, thereby improving the fruit quality.

Keywords Melatonin, Fruit physiology, Plum fruits, Soluble sugars, Organic acids

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Introduction

Soluble sugars and organic acids are crucial in influencing the sensory characteristics of horticultural fruit products and are essential for developing fruit flavor [1]. The types, concentrations, and ratios of these compounds have a significant impact on the taste and texture of fruits, and soluble sugars typically include glucose, fructose, sucrose, and sorbitol while organic acids commonly found in fruits are malic, citric, oxalic, tartaric, quinic, succinic, and pyruvic acids [2–5]. The composition and levels of soluble sugars and organic acids can vary among different fruit species and varieties, as well as change throughout different stages of fruit development within the same species [6]. However, excessive use of chemical fertilizers in the pursuit of larger fruits and higher yields can result in a decline in fruit quality [7]. Therefore, there is a need to adopt tactics that will improve the quality of fruits.

Melatonin (MT), a safe indole derivative of tryptophan, is widely found in living organisms [8, 9]. Studies have shown that MT treatment at appropriate concentrations can improve plant resistance, maintain the freshness of fruit and vegetable products, and improve fruit quality [10–12]. The reason is the fact that MT exerts its influence on instigating the defense mechanisms within fruits via the generation of polyphenolic substances, thereby playing a crucial role in enhancing the fruits' natural resistance and safeguarding their quality and vitality [13–15]. Depending on the concentration of MT used or the time of application, different fruit species produce different effects [16–18]. For example, application of 100 μmol/L MT on pear trees revealed an increase in the soluble solids content of fruits during the ripening period, decreased the expression level of sucrose converting enzyme gene *Pbinvertase1/2* and its enzyme activity, and increased the expression level of sucrose phosphoribosyl synthase gene *PbSPS1/2/3* and its enzyme activity, resulting in an increase in starch and soluble sugar content in fruits [19]. Exogenous MT increased the yield of sweet cherries under poor climatic conditions, and a concentration of 300 μmol/L increased the quality, color, hardness, titratable acid content, and soluble solids content of fruits at the ripening stage [20]. In pomegranates, exogenous MT increased fruit size, fruit number, and yield, with a concentration of 100 μmol/L MT increasing the contents of total soluble solids and titratable acid [21]. In strawberries, a concentration of 100 μmol/L MT increased the total phenol content, free radical scavenging activity, glucose, and fructose content [22]. Additionally, higher concentrations of MT inhibited the growth of wild-type apple plants, induced the accumulation of fructose, glucose, and sucrose in leaves, down-regulated the expression level of fructose kinase gene *MdFRK2* and fructokinase activity, and increased the activity of sucrose phosphosynthase (SPS) [23]. The ripening of grape berries, SPS activity, and accumulation of soluble sugars were promoted with the application of 100 μmol/L MT [24]. Other studies also showed that 100–150 μmol/L MT improved the quality of fruits, increased their soluble sugar content, and decreased their organic acid content [18, 24, 25]. Therefore, MT can increase the accumulation of soluble sugar and decrease the accumulation of organic acid, though its mechanism is not clear.

Plum (*Prunus salicina*) is a deciduous fruit tree bearing drupe fruits that are rich in nutrients, soluble sugars, organic acids, amino acids, minerals, and antioxidants, and possess a strong flavor [26]. However, due to the misuse of chemical fertilizers and other unscientific cultivation techniques, the quality and taste of plum fruits have deteriorated [27]. Previous studies have indicated that the application of 100 μmol/L MT can enhance the content of soluble sugar and reduce the level of organic acids in drupe fruits [18, 25]. Nevertheless, the exact mechanism behind this effect remains unclear. In this study, we sprayed 100 μmol/L of MT onto the leaves of plum trees during the second phase of rapid fruit expansion to examine the impact of exogenous MT on the quality of plum fruits and the underlying mechanism of soluble sugar-organic acid metabolism. The findings of this study could serve as a cornerstone for enhancing the quality of plum fruits.

Materials and methods

Materials

The plum variety used in this study was 'Qiangcuidali', grafted onto wild peach (rootstock). The four-year-old plum trees were planted in the orchard of Sichuan Huijia Seven Colorful Fields Agricultural Technology Co., located in Chengdu, China (30°38′N, 104°13′E). The planting density was four meters between plants and three meters between rows.

MT was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

Experimental design

In May 2022, the plum fruits reached the second rapid expansion period, which occurred 90 days after flowering, we selected 18 uniform growth plum trees for the experimental treatment. There were two treatments in this experiment: (1) control (CK), where nine plum trees were foliar sprayed with tap water; (2) MT treatment, where nine plum trees were foliar sprayed with 100 μmol/L of MT solution [18, 25]. Each plum tree was sprayed with approximately 3 L of tap water or MT solution until the leaves started dripping. Three replications were conducted for each treatment, with three trees included in each replication. The MT and tap water treatments were reapplied after a 7-day interval. Fruits

were collected at 0, 7, 14, 21, and 28 days following the initial treatment for maturity evaluation. Samples were collected randomly in the east, south, west, and north directions to obtain medium-sized fruits, approximately 10 fruits per direction. Following each sampling, fruits were promptly transferred to an icebox and taken to the laboratory. Upon arrival, basic quality parameters were immediately assessed, after which the pulp was chopped and mixed, frozen in liquid nitrogen, and stored at -80 ℃ for later parameter determination.

Determination of parameters

Determination of fruit quality

An electronic balance was used to measure the single fruit weight. A digital vernier caliper was used to measure both the transverse and longitudinal diameters of the fruits. The total soluble solids content was determined with a PAL-1 handheld refractometer, the titratable acid content was determined by titration with a sodium hydroxide solution, and the vitamin C content was determined using the 2,6-dichloroindophenol method [28].

Determination of soluble sugar components and starch contents

The sulfuric acid-anthrone colorimetric method was utilized to measure the total soluble sugar content [29]. The extraction methods for soluble sugar components (glucose, fructose, sucrose, and sorbitol) followed the protocol established by Li et al. (2014) [30]. The individual contents of soluble sugar components were analyzed using a high-performance liquid chromatography system (Agilent 1260 HPLC, Agilent Technologies) with an Anthena NH2 C18 column (250 mm × 4.6 mm, 5 μm) and a mobile phase of acetonitrile and ultrapure water at a ratio of 80:20. The analysis was conducted with a flow rate of 1 mL/min, a column temperature set to 40 ℃, and an injection volume of 10 μL.

The starch content was determined using a starch kit (Suzhou Grace Biotechnology Co., Ltd., Suzhou, China), according to the manufacturer's instructions.

Determination of soluble sugar and starch metabolism enzymes activities

The activities of sucrose synthetase (synthetic direction, SS-s), sucrose synthetase (catabolism direction, SS-c), SPS, soluble acid invertase (S-AI), cell wall invertase (CWINV), neutral invertase (NI), NAD^+-sorbitol dehydrogenase (NAD^+-SDH), sorbitol oxidase (SOX), hexokinase (HK), glucokinase (GK), fructokinase (FK), ATP-phosphate fructose kinase (PFK), α-amylase, and β-amylase were determined using the correspond kit (Suzhou Grace Biotechnology Co., Ltd., Suzhou, China), according to the manufacturer's instructions.

Determination of organic acid components contents

The organic acid components (malic acid, quinic acid, citric acid, oxalic acid, tartaric acid, and α-ketoglutaric acid) were extracted using the method described by Bao (2022) [31]. The contents of organic acid components were analyzed using high-performance liquid chromatography (Thermo Scientific UltiMate 3000, Thermo Fisher Scientific Inc.). The chromatographic column used was Inertsil AQ-C18 (250 mm × 4.6 mm, 5 μm), with a mobile phase of potassium phosphate buffer (0.04 mol/L): methanol=99:1 (pH 2.6). The analysis was conducted with a flow rate of 0.8 mL/min, the column temperature set at 30 ℃, and an injection volume of 10 μL.

Determination of organic acid metabolism enzymes activities

The activities of $NADP^+$-malate dehydrogenase (NAD^+-MDH), $NADP^+$-malate synthase ($NADP^+$-ME), and phosphoenolpyruvic acid carboxylase (PEPC) were determined using the correspond kit (Suzhou Grace Biotechnology Co., Ltd., Suzhou, China), according to the manufacturer's instructions.

Transcriptome sequencing analysis

The transcriptome data of plum pulps at 21 and 28 d after treatment [32] were utilized in this study. Transcriptome sequencing yielded a total of 79.83 Gb of clean data, with a Q30% exceeding 94.20% and a GC content greater than 45.94%. The clean reads were aligned with the reference gene (*Prunus_salicina.v1.0.genome.fa*), and the alignment of clean reads from each sample with the reference genome ranged from 93.29 to 94.61%. A total of 3,194 new genes were identified, 2,018 of which were functionally annotated. In this study, a fold change of ≥1.5 and a *P* value of <0.01 were used as criteria for screening differentially expressed genes (DEGs). At 21 d after treatment, the DEGs annotated to the GO, KEGG, and NR databases were 350, 302, and 436, respectively. At 28 d after treatment, the DEGs annotated to the GO, KEGG, and NR databases were 306, 260, and 367, respectively.

Candidate DEGs expression analysis by quantitative RT-PCR (qRT-PCR)

The M5 Super Plus qPCR RT kit with gDNA remover from Mei5 Biotechnology Co., Ltd, Beijing, China was used to perform the first strand cDNA synthesis of the chosen DEGs, following the manufacturer's guidelines. Primers were designed with Primer premier 5.0 software using the reference gene sequences in plum (Table 1), with the lattice protein articulator complex medium subunit family protein gene (*CAC*) utilized as an internal reference gene [33]. qRT-PCR of the DEGs was carried out using the 2X M5 HiPer SYBR Premix EsTaq (with Tli RNaseH) kit from Mei5 Biotechnology Co., Ltd., following the manufacturer's instructions.

Table 1 qRT-PCR primer sequences

Gene ID	Gene name	Sequence (5′-3′)
At1g60780	*CAC*	F: GGGATACGCTACAAGAA GAATGAG R: CTTACACTCTGGCATAC CACTCAA
gene.evm.model.LG02.2078	*SWEET17*	F: CTGAGGCGTAGATCAA CTGAGG R: GGCCTTCATTCTTGGT GGTG
gene.evm.model.LG02.647	*GK*	F: GCTGTTGTGATTATGGG TGTCAG R: GTGTTTCAAGCCAAGG CATCC
gene.evm.model.LG04.694	*SWEET2a*	F: GCCTTCGTGTTGTTTGT GTCAC R: CCAAATGAATTGACTGT AGCCACC
gene.evm.model.LG08.1659	*MDH*	F: GACCCAAAGCGACTTC TAGGAG R: AGGCTTGACCTGTGAC AGAAG
gene.evm.model.LG04.87	*TDT*	F: TCCTCTGCTACTTCCTT R: TGCTTGTGGGTGTGACTA
gene.evm.model.LG04.2538	*HK3*	F: CGGAAGTGGAGAAGAG TGGTTG R: ACCATCAGAGGCCAA ACCTG
gene.evm.model.LG01.2283	*PFK3*	F: CTCATCTCTGCGACTAC TTGTCC R: CTTGGACTATCCGTGTG GACAAC

Statistical analysis

The data were analyzed in triplicate by Student's t-test ($0.01 \leq p < 0.05$ or $p < 0.01$) using the SPSS 20.0.0 software (IBM, Chicago, IL, USA).

Results

Fruit quality of plum

Longer period after treatment, there was a rise in the single fruit weight, transverse and longitudinal diameter, total soluble solids contents, and vitamin C content of plum fruits, while the titratable acid content declined (Fig. 1). Compared with CK, MT treatment increased the single fruit weight by 18.56% and 12.93%, longitudinal diameter by 5.37% and 5.05%, soluble solids contents by 1.36 and 1.13% points, and vitamin C content by 115.66% and 56.09%, respectively, at 21 and 28 d after treatment, while did not affect these parameters at other treatment times. Further, MT treatment did not affect the transverse diameter at different treatment times. In comparison to CK, MT decreased the titratable acid content by 0.18 and 0.10% points, respectively, at 7 and 28 d after treatment, while did not affect this parameter at other treatment times.

Soluble sugar and starch metabolisms of plum fruits

Contents of total soluble sugar and its components

Longer period after treatment, the contents of total soluble sugar, sucrose and sorbitol in plum fruits exhibited a gradual increase, whereas the levels of glucose and fructose showed a pattern of initially increasing and then decreasing (Fig. 2). Compared with CK, MT increased the content of soluble sugar by 19.96%, 14.52%, 7.78%, and 9.74%, respectively, at 7, 14, 21, and 28 d after treatment. It only increased the content of glucose at 7 d after treatment and decreased the content of fructose at 28 d after treatment. The sucrose content in MT-treated fruits increased by 45.66%%, 50.72%, 47.81%, and 20.07%, respectively, at 7, 14, 21, and 28 d after treatment, compared with CK. The sorbitol content in MT-treated fruits was higher than in CK at 14, 21, and 28 d after treatment, with increases of 31.80%, 19.95%, and 8.67% over CK, respectively. There was no significant difference for sorbitol content in MT-treated fruits at 7 d after treatment.

Activities of soluble sugar metabolism enzymes

The treatment of MT did not have a significant effect on the activities of SS-s, NI, and HK in plum fruits at different treatment times (Fig. 3). In comparison to CK, MT treatment led to a decrease in the activities of SS-c and FK at 21 d after treatment, an increase at 28 d after treatment, and no significant impact at other treatment times. For the activities of SPS and GK, MT treatment increased their activities at 7, 14, and 28 d after treatment, with the increases of 16.55%, 33.84%, and 23.89% for SPS activity, and the increases of 8.55%, 7.91%, and 12.33% for GK activity, respectively, compared with CK. There were no significant differences for the activities of SPS and GK between MT treatment and CK at 14 d after treatment. Additionally, the activities of S-AI and CWINV were enhanced by MT treatment at 7 and 21 d post-treatment but reduced at 14 and 28 d post-treatment. Furthermore, MT treatment boosted the activity of NAD^+-SDH at 14 days post-treatment but decreased it at 21 and 28 d post-treatment. It also increased the activity of SOX at 7 and 28 d post-treatment but lowered it at 14 days post-treatment. The activity of PFK decreased at 7 days post-treatment and increased at 14 and 28 d post-treatment with MT treatment, while remaining unaffected at other time points.

Content of starch and its activities of metabolic enzymes

In comparison to CK, MT treatment resulted in a 14.73% increase in starch content in plum fruits at 7 d after treatment, while no significant effect was observed at other time points (Fig. 4). Furthermore, at 7 d after treatment, MT treatment led to a 22.95% increase in α-amylase activity and a 20.93% increase in β-amylase activity compared to CK. Conversely, at 14 d after treatment, MT

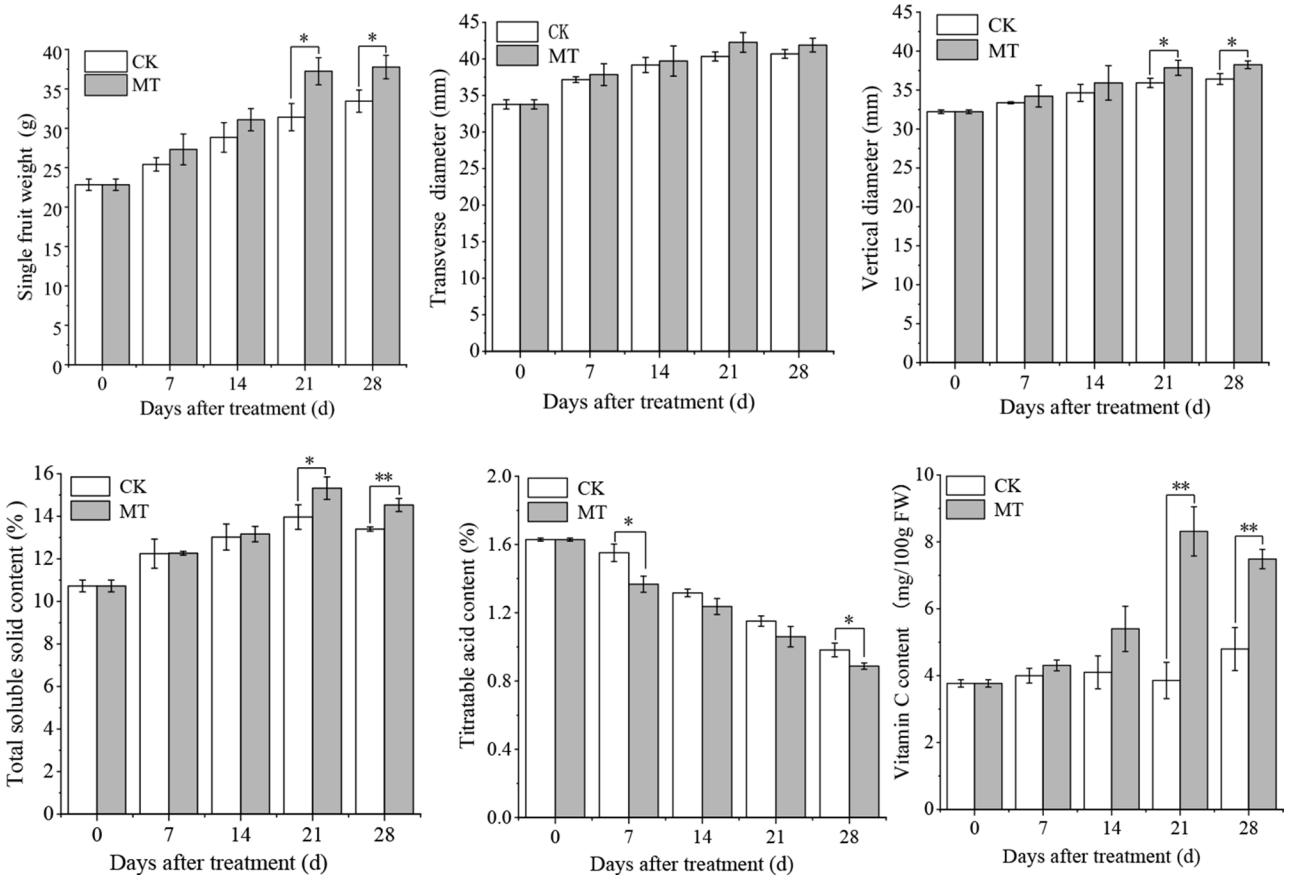

Fig. 1 Effects of melatonin on the fruit quality of plum. Values represent the mean ± SE ($n = 3$). Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

treatment caused a decrease of 13.22% and 11.65% in α-amylase and β-amylase activities, respectively, compared to CK, with no impact on these parameters at other time points.

Organic acid metabolism of plum fruits

Contents of organic acid components

In comparison to CK, MT treatment decreased the content of malic acid in plum fruits by 6.45% at 28 d after treatment, while did not affect this parameter at other treatment times (Fig. 5). Additionally, the application of MT resulted in higher levels of quinic acid after 7 and 21 d, lower levels after 28 d, and no significant change after 14 d. Moreover, when compared to the CK treatment, MT treatment led to a 59.76%, 25.79%, 25.60%, and 14.71% increase in citric acid content after 7, 14, 21, and 28 d of treatment. MT treatment did not affect the contents of oxalic acid and α-ketoglutaric acid. Furthermore, at 21 and 28 d after treatment, the content of tartaric acid increased by 36.14% and 24.42% with MT treatment compared to CK, respectively, but there was no effect on this parameter at 7 and 14 days after treatment.

Activities of organic acid metabolism enzymes

In comparison to CK, MT treatment resulted in a 58.50% increase in the activity of NAD^+-MDH at 7 d after treatment, a 7.42% decrease at 28 d after treatment, and no significant effect at 14 and 21 d after treatment (Fig. 6). Additionally, MT treatment led to a 72.28% and 35.43% increase in the activity of $NADP^+$-ME at 7 and 28 d after treatment, a 21.98% decrease at 14 d after treatment, and no change at 21 d after treatment compared to CK. In terms of the activity of PEPC, MT treatment only showed a decrease at 7 d after treatment and had no impact at 14, 21, and 28 d after treatment.

Transcriptome sequencing analysis

Analysis of soluble sugar and organic acid metabolisms genes

The metabolism of soluble sugars and organic acids in plum fruits showed more noticeable differences at 21 and 28 d after treatment. These specific time points were chosen for transcriptome sequencing. The report by Li et al. (2023) [32] provides the details regarding the number of DEGs in MT vs. CK at 21 and 28 d after treatment.

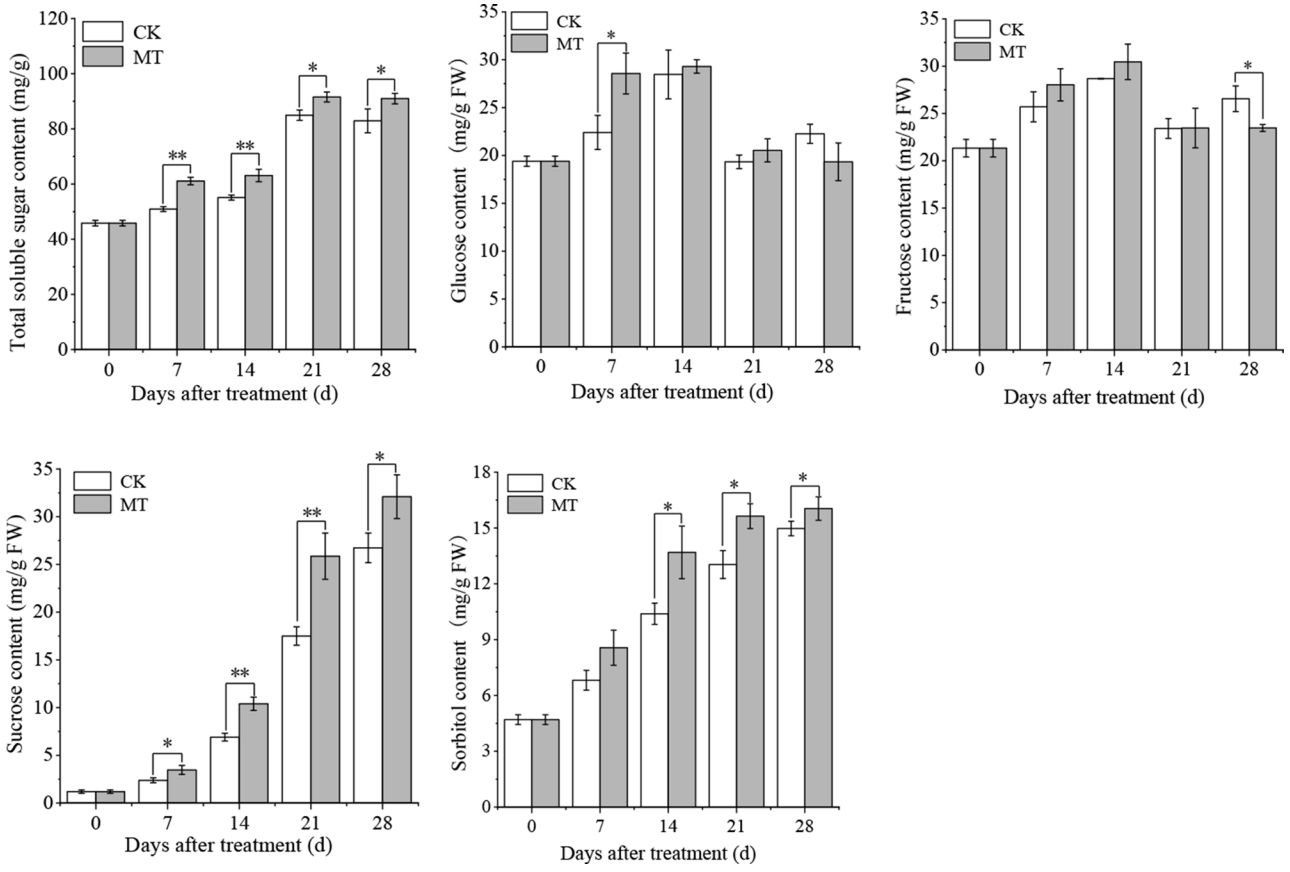

Fig. 2 Effects of melatonin on the contents of total soluble sugar and its components in plum fruits. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

At 21 d after treatment, there were 13 DEGs in MT vs. CK associated with soluble sugar metabolism. These genes were enriched for starch and sucrose metabolism, galactose metabolism, fructose and mannose metabolism, and glycolysis, gluconeogenesis, and pentose phosphate metabolism (Table 2; Fig. 7). In the starch and sucrose metabolic pathway, the expression levels of three *β-1,3-glucan endonuclease* genes were up-regulated, along with the expression level of *hexokinase 3* (*HK3*). Within the glycolysis and gluconeogenesis pathway, the expression levels of *6-phosphofructokinase 3* (*PFK3*) and *glucokinase* (*GK*) were up-regulated. Notably, *HK3* and *PFK3* were also involved in the fructose and mannose metabolism pathway. Additionally, the expression level of *glycosyltransferase* (*UGT73C2*) was up-regulated, while the expression levels of two *glycosyltransferase* (*UGT86A1* and *UGT74G1*) genes were down-regulated. Furthermore, three DEGs related to soluble sugar and organic acid transports were identified. The expression level of *soluble sugar transporter protein* (*SWEET17*) was down-regulated and the expression level of *aluminum-activated malate transporter protein 2* (*ALMT2*) was up-regulated, while the expression level of *polyol transporter protein* (*PLT5*) was down-regulated. In the ascorbate and acetaldehyde metabolic pathway, the expression level of *ascorbic acid oxidase* (*AAO*) was up-regulated.

At 28 d after treatment, 20 DGEs in MT vs. CK were associated with soluble sugar metabolism. These DGEs were enriched for pathways including starch and sucrose metabolism, galactose metabolism, fructose and mannose metabolism, as well as glycolysis, gluconeogenesis, and pentose phosphate metabolism (Table 3; Fig. 7). Specifically, in the starch and sucrose metabolic pathway, the expression levels of four *β-glucosidase* genes and one *β-furanofructosidase* gene were up-regulated. In the fructose and mannose metabolism, as well as glycolysis and gluconeogenesis pathways, the expression level of *fructose bisphosphate aldolase* (*FBA*) was up-regulated, and the expression level of *pyruvate decarboxylase* (*PDC*) was down-regulated. In the pentose phosphate pathway, the expression level of *GK* was down-regulated, while the expression level of *SWEET2a* was up-regulated. The expression levels of *sucrose phosphate synthase* (*SPS*) and *phosphofructokinase* (*PFK*) treated with MT were higher than CK. In addition, there were four DEGs related to malate metabolism in MT vs. CK. The expression levels of two *malate dehydrogenase* (*MDH*) genes and *ALMT2* were down-regulated, while the expression level of

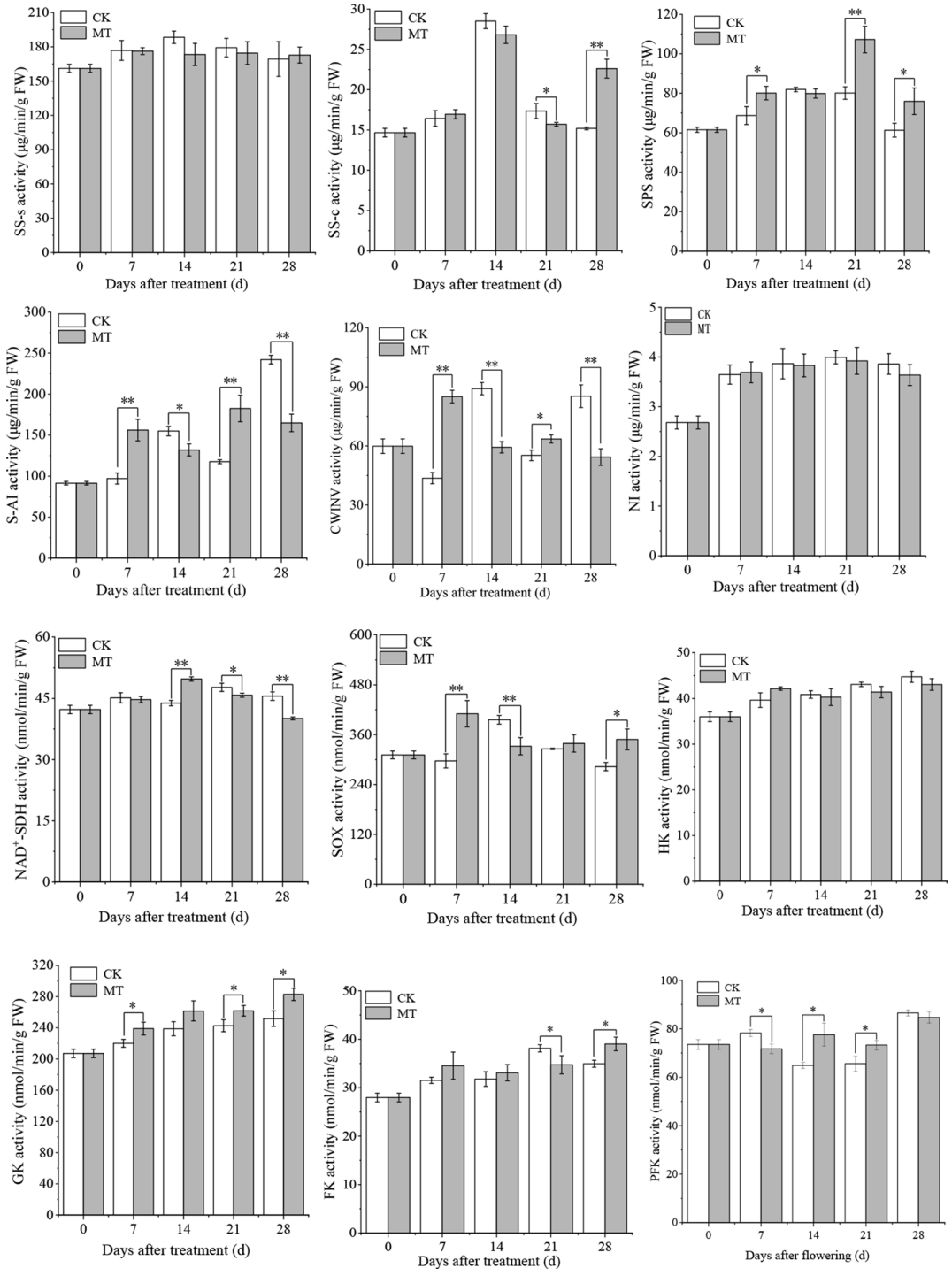

Fig. 3 Effects of melatonin on the activities of soluble sugar metabolism enzymes in plum fruits. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \leq p < 0.05$; **: $p < 0.01$)

vesicular membrane dicarboxylic acid transporter protein (*TDT*) was up-regulated. In the ascorbate and acetaldehyde metabolic pathway, the expression levels of three *AAO* genes were down-regulated.

DEGs expression levels of qRT-PCR and RNA-Seq

Four DEGs in MT vs. CK were screened for qRT-PCR validation at 21 and 28 d after treatment, respectively (Fig. 8). The expression levels of these DEGs in qRT-PCR were consistent with the RNA-Seq results in MT vs. CK. At 21 d after treatment, the expression levels of *HK3*, *GK*,

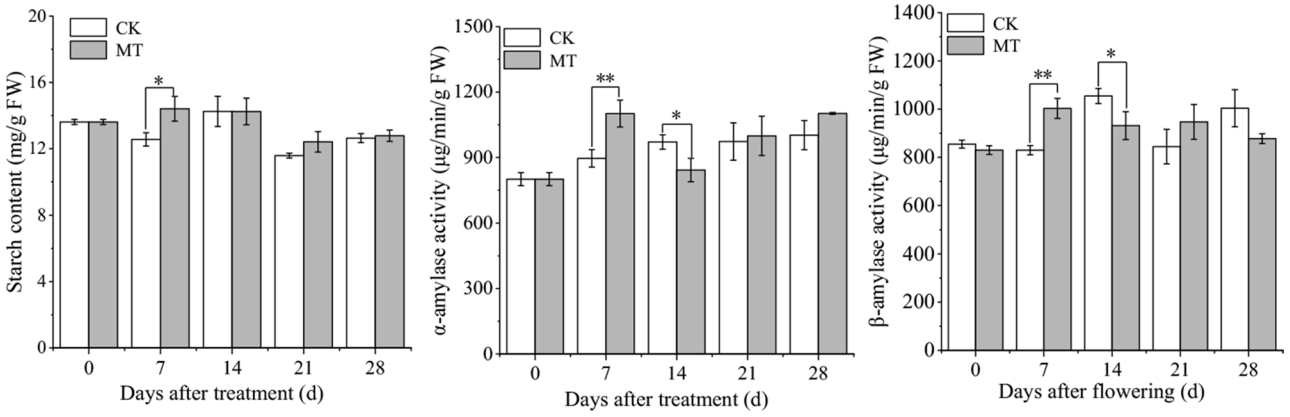

Fig. 4 Effects of melatonin on the content of starch and its activities of metabolism enzymes in plum fruits. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

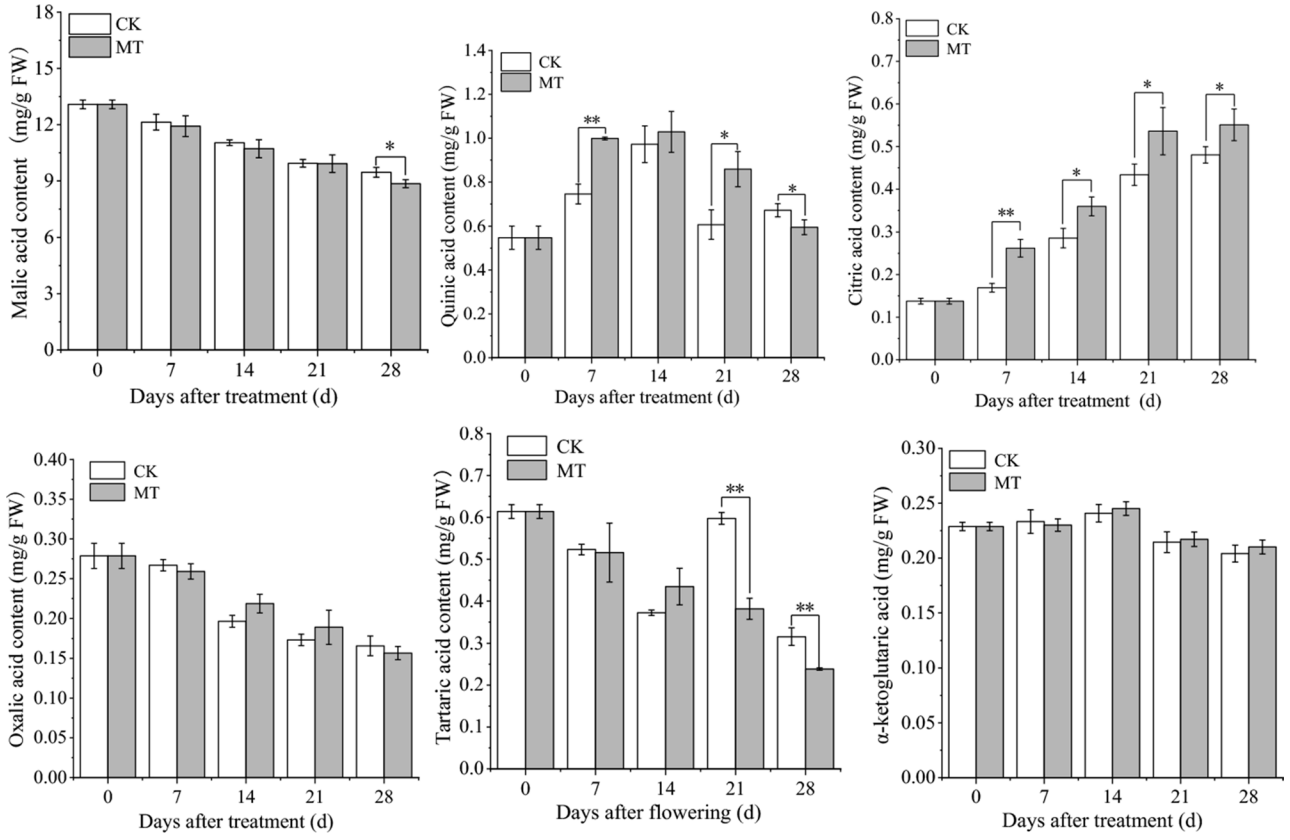

Fig. 5 Effects of melatonin on the contents of organic acid components in plum fruits. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

and *PFK3* were up-regulated, and the expression level of *SWEET17* was down-regulated. At 28 d after treatment, the expression levels of *SWEET2a* and *TDT* were up-regulated, and the expression levels of *GK* and *MDH* were down-regulated.

Discussion

Effects of MT on fruit quality

MT can enhance the transcription of genes involved in chlorophyll synthesis, PSI and PSII, and antioxidant enzymes, while reducing the expression of genes associated with chlorophyll breakdown, resulting in improved photosynthesis in plants [34]. The application of MT has been demonstrated to elevate the net photosynthetic rate, transpiration rate, and stomatal conductance in

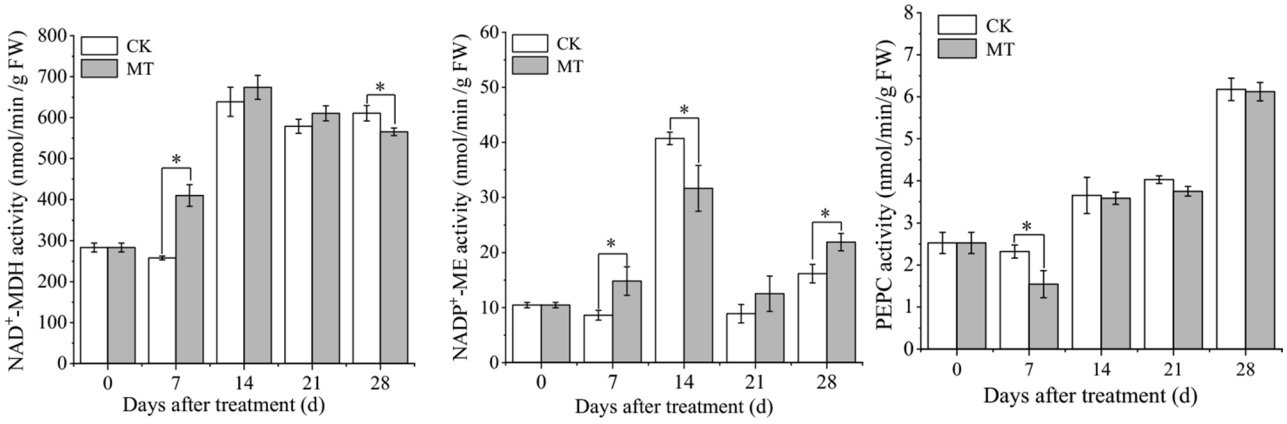

Fig. 6 Effects of melatonin on the activities of organic acid metabolism enzymes in plum fruits. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

Table 2 DEGs in MT vs. CK at 21 d after treatment

Gene ID	Gene name	log_2FC	Expression
gene.evm.model.LG03.2136	*6-UDP glucose dehydrogenase*	0.6233	Up-regulated
gene.evm.model.LG01.2283	*6-Phosphofructokinase 3*	0.6445	Up-regulated
gene.evm.model.LG08.1514	*L-ascorbate oxidase*	2.5948	Up-regulated
gene.evm.model.LG05.215	*UDP-glycosyltransferase*	1.0010	Up-regulated
gene.evm.model.LG06.672	*UDP-glycosyltransferase*	-0.9753	Down-regulated
gene.evm.model.LG08.1387	*UDP-glycosyltransferase*	-0.6671	Down-regulated
gene.evm.model.LG02.949	*β-1,3-Glucan endonuclease*	0.8844	Up-regulated
gene.evm.model.LG04.2376	*β-1,3-Glucan endonuclease*	1.1088	Up-regulated
gene.evm.model.LG04.2529	*β-1,3-Glucan endonuclease*	1.4818	Up-regulated
gene.evm.model.LG01.4557	*Alginate phosphate synthase*	-1.0349	Down-regulated
gene.evm.model.LG04.2538	*Hexokinase 3*	0.5946	Up-regulated
gene.evm.model.LG08.2198	*Polyol transporter protein*	-0.8572	Down-regulated
gene.evm.model.LG06.672	*Phosphoglycerate kinase*	-0.9753	Down-regulated
gene.evm.model.LG06.3381	*Aluminum-activated malate transporter protein 2*	1.6243	Up-regulated
gene.evm.model.LG02.647	*Glucose kinase*	0.9792	Up-regulated
gene.evm.model.LG02.2078	*Sugar efflux transporter 17*	-0.7279	Down-regulated
gene.evm.model.LG03.2521	*Isocitrate lyase*	1.6679	Up-regulated

strawberry leaves, leading to enhanced photosynthesis and increased individual fruit weight in strawberries [35]. In this experiment, MT treatment increased the transverse diameter and single fruit weight of plum fruits. These results align with the results of previous studies [17, 18], indicating that MT treatment may improve photosynthesis in the leaves of plum trees and promote the accumulation of nutrients to be transferred to fruits. Total soluble solids, soluble sugars, and titratable acid are important indicators for fruit quality [1]. Previous studies have shown that MT treatment can increase the contents of total soluble solids and soluble sugar and decrease the content of organic acid in fruits [19–22]. In this study, MT treatment increased the contents of total soluble solids and soluble sugars and decreased the content of titratable acid in plum fruits at the mature stage. This outcome was in line with prior research [25], indicating that MT treatment can enhance soluble sugar accumulation and reduce organic acid accumulation in plum fruits, thus improving the quality of fruits. Additionally, at later treatment stages, The levels of sucrose and sorbitol in plum fruits were found to increase with MT treatment, mirroring the findings in a study on pear fruits [19], but not consistent with the study of pomegranate fruits [21]. This discrepancy may be due to the different major soluble sugar components in fruits of various species. Furthermore, malic acid was found to be the main organic acid component in plum fruits, followed by quinic acid, citric acid, and tartaric acid. At later treatment stages, MT treatment decreased the contents of malic acid, quinic acid, and tartaric acid, and increased the contents of citric acid and vitamin C in plum fruits, resulting in a decrease in the titratable acid content, similar to the results observed in grapes and tomatoes [36, 37]. Therefore, MT treatment can improve the quality of plum fruits.

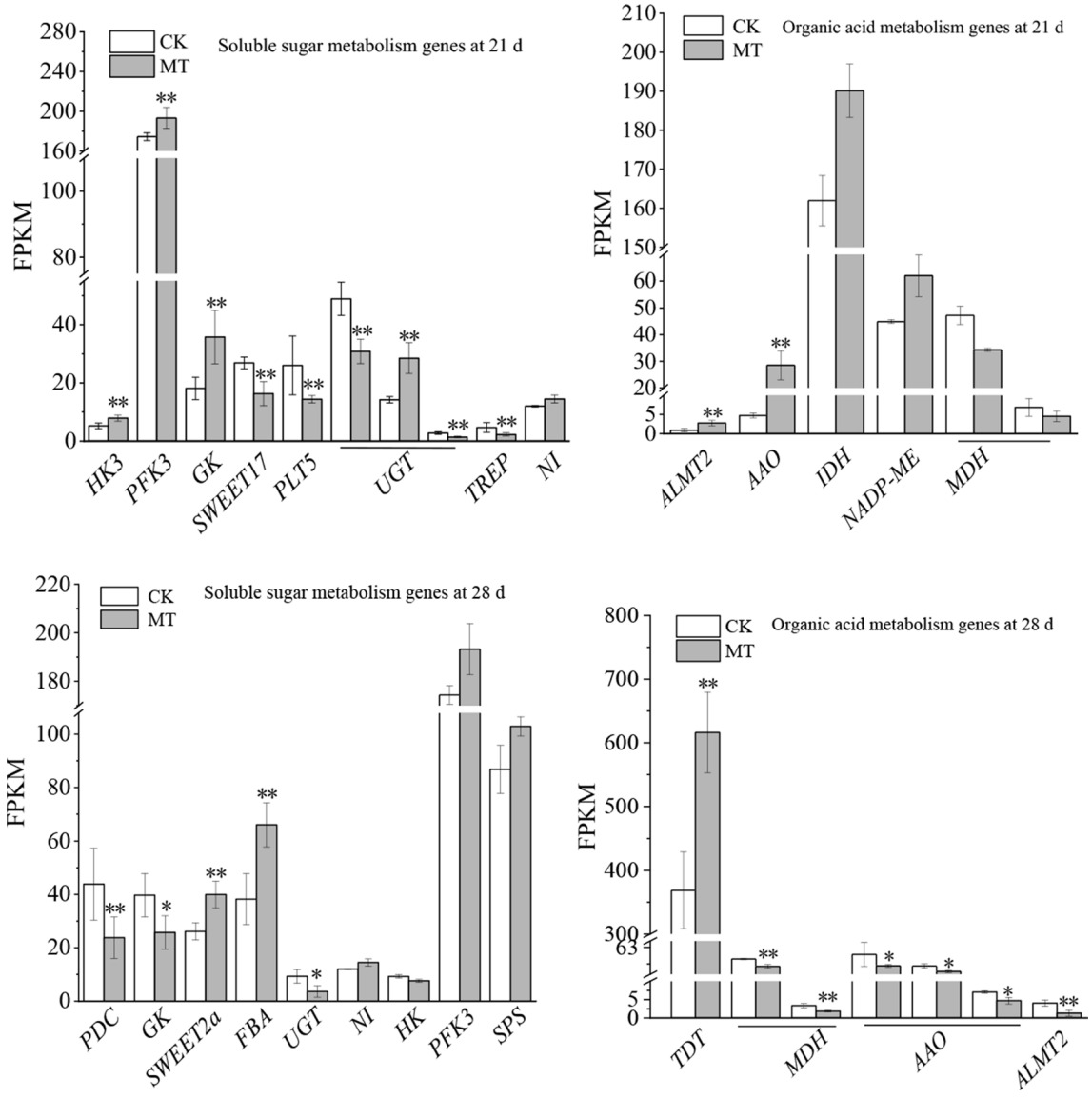

Fig. 7 Expression levels of to soluble sugar and organic acid metabolisms genes at 21 and 28 d after treatment. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

Effects of MT on soluble sugar metabolism and related genes in fruits

Soluble sugar accumulation in fruits is primarily affected by 'bank' strength, with the key indicator being the sugar metabolism enzymes activities [38]. MT treatment resulted in an increase in SPS activity in plum fruits at later stages in this study, with no noticeable impact on NI activity, similar to the results seen in tomatoes [39]. Additionally, MT treatment increased the SS-c enzyme activity of plum fruits at later stages after treatment, with no significant effect on the SS-s activity. However, the SS-s activity was notably higher than SS-c, indicating that the synthetic direction enzyme activity of SS played a significant role. Further, MT treatment decreased the activities of S-AI and CWINV, resulting in a lower rate of sucrose conversion, while increasing the SPS activity led to a higher rate of sucrose synthesis. The combined effects of SPS, S-AI, and CWINV led to a decrease in fruit sucrose content consumption and an increase in accumulation. Transcriptome analysis revealed an up-regulation of the sucrose synthase gene *SPS1* at a later stage after treatment, aligning with the trend of increased SPS activity. This finding is consistent with results seen in pear fruits [19]. In contrast to findings in grapes [40], MT treatment increased the activities of AI, NI, and SS-c in plums, promoting the conversion of sucrose to glucose and fructose. This difference may be attributed to plums and pears being sucrose-accumulating fruits, while grapes are hexose-accumulating fruits [41].

Table 3 DEGs in MT vs. CK at 28 d after treatment

Gene ID	Gene name	log_2FC	Expression
gene.evm.model.LG07.1505	*Fructose 1,6-bisphosphate aldolase*	0.7675	Up-regulated
gene.evm.model.LG02.939	*Phosphogalacturonan isomerase*	-0.8314	Down-regulated
gene.evm.model.LG06.1193	*Pyruvate decarboxylase*	-0.9062	Down-regulated
gene.evm.model.LG08.1659	*Malate dehydrogenase*	-0.8723	Down-regulated
gene.evm.model.LG02.2322	*Malate dehydrogenase*	-0.6224	Down-regulated
gene.evm.model.LG06.3381	*Aluminum-activated malate transporter protein 2*	-1.6499	Down-regulated
gene.evm.model.LG04.87	*Vesicular membrane dicarboxylic acid malate transporter protein*	0.7246	Up-regulated
gene.evm.model.LG02.647	*Glucokinase*	-0.9062	Down-regulated
gene.evm.model.LG04.694	*Sugar efflux transporter protein 2a*	0.5889	Up-regulated
Prunus salicina newGene 280	*Glucose pyrophosphorylase*	-0.8872	Down-regulated
gene.evm.model.Contig1.995	*Uridine diphosphate-glucose dehydrogenase*	-1.0379	Down-regulated
gene.evm.model.LG05.707	*β-Fructofuranosidase*	1.0369	Up-regulated
gene.evm.model.LG01.794	*UDP-glycosyltransferase*	-1.3694	Down-regulated
gene.evm.model.LG06.2277	*Cotton seed sugar synthase*	0.8167	Up-regulated
gene.evm.model.LG03.1037	*β-galactosidase*	-0.9069	Down-regulated
gene.evm.model.LG06.2940	*β-galactosidase*	-1.0282	Down-regulated
gene.evm.model.LG08.1514	*L-ascorbic acid oxidase*	-0.8455	Down-regulated
gene.evm.model.LG05.708	*L-ascorbic acid oxidase*	-0.6973	Down-regulated
gene.evm.model.LG07.1943	*L-ascorbic acid oxidase*	-0.6058	Down-regulated
gene.evm.model.LG02.2581	*β-Glucosidase*	0.5999	Up-regulated
gene.evm.model.LG04.1860	*β-Glucosidase*	0.6500	Up-regulated
gene.evm.model.LG06.3584	*β-Glucosidase*	0.9171	Up-regulated
gene.evm.model.LG07.1915	*Fructose 1,6-bisphosphate aldolase*	0.6579	Up-regulated

Research has indicated that fructose phosphate kinase is the enzyme that limits the rate of the glycolytic pathway [42]. In lychee pulp, phosphofructokinase activity was reduced and PFK expression was down-regulated, leading to inhibition of the glycolytic pathway and the accumulation of fructose and glucose in the pulp [43]. In this experiment, at 21 d after treatment, MT treatment increased PFK activity and up-regulated the expression level of *PFK3* in plum fruit. This promoted the entry of hexose into the glycolysis and pentose phosphate pathway. MT treatment also increased GK activity in plum fruit, facilitating the decomposition of glucose and fructose. At 28 d after treatment, MT treatment resulted in an increase of FK activity and decrease of NAD^+-SDH, S-AI, and CWINV activities in plum fruits. This promoted the catabolic conversion of fructose, reduced the rate of fructose production, and decreased the accumulation of fructose in fruits, consistent with previous studies [44]. In addition, MT treatment resulted in an increase of GK and SOX activities in plum fruits at 28 d after treatment, promoting both glucose catabolism and gluconeogenesis and keeping glucose levels relatively stable in the fruits. This may explain the non-significant difference in glucose content between MT-treated fruits and the control.

The *SWEET* family is a group of protein genes primarily responsible for sugar transport within cells, which can transport sucrose or hexose down concentration gradients, and these sugar transport proteins play a crucial role in fruit sugar accumulation [45]. Previous studies have shown that *SWEET17* acts as a fructose transporter in citrus fruits [46], while *SlSWEET17*, found in the vesicle membrane of tomato fruits, functions as a bidirectional fructose transporter [45]. In this experiment, the expression level of *SWEET17* was down-regulated in plum fruits 21 d after treatment, with no significant difference in fructose content between the treatment and control groups. At 28 d after treatment, MT treatment increased the activity of FK in plum fruits, facilitating the conversion of fructose to fructose 6-phosphate, resulting in a significant decrease in fructose content. Meanwhile, MT treatment up-regulated the expression of *SWEET2a* and increased the sucrose content in plum fruits, in line with previous studies in grapes [47]. Therefore, it is hypothesized that *SWEET2a* and *SWEET17* play a role in the transmembrane transport process of fructose and sucrose within plum fruit cells, ultimately impacting sugar content within the fruit vesicles.

Effects of MT on organic acid metabolism and related genes in fruits

In this experiment, the NAD^+-MDH activity was found to be higher than the PEPC activity in plum fruits at different treatment times, indicating that NAD^+-MDH played a dominant role in the synthesis of malic acid. During the early stages of treatment, the NAD^+-MDH activity gradually increased, contributing to the synthesis of malic acid to support the tricarboxylic acid cycle

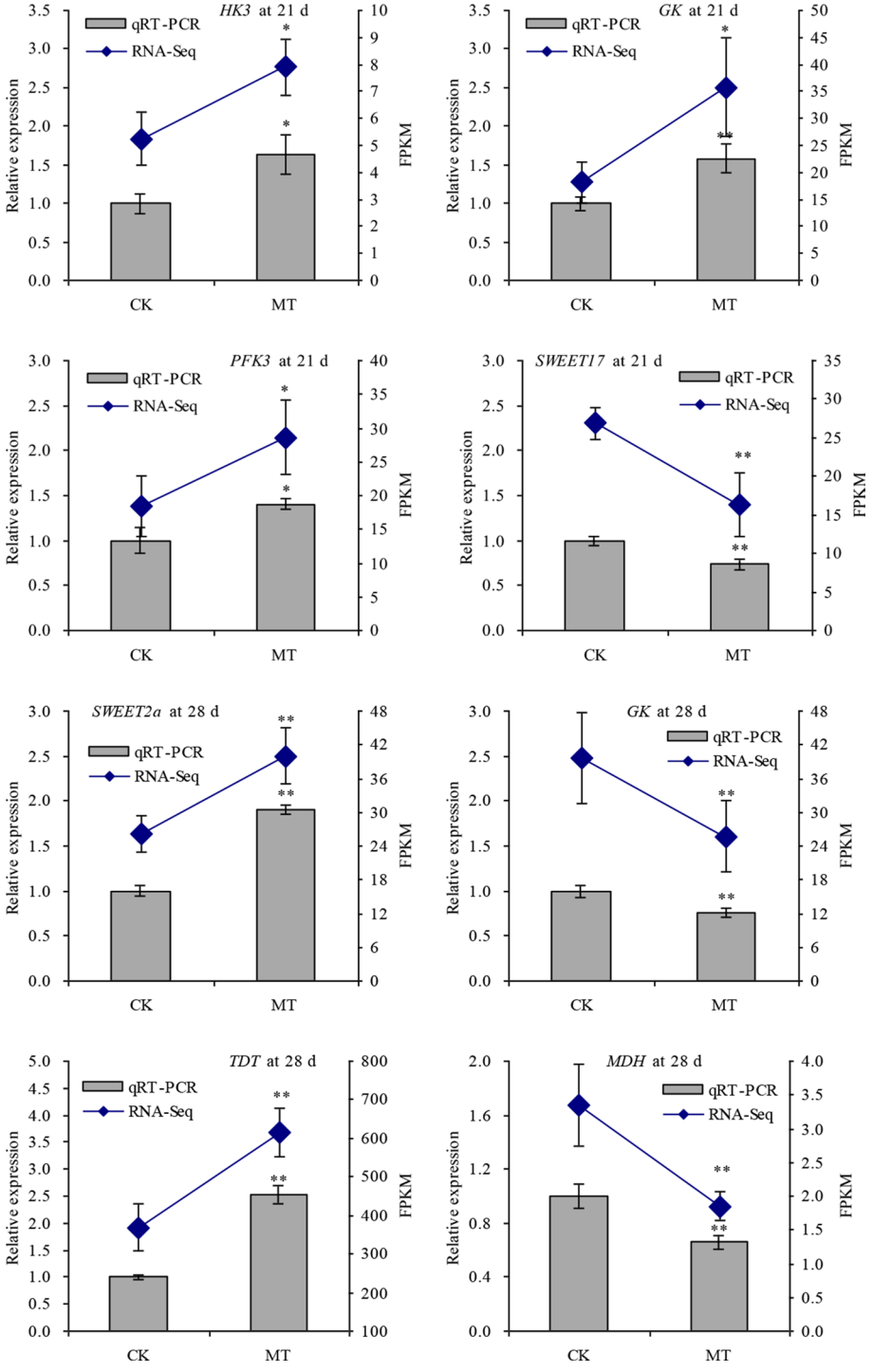

Fig. 8 DEGs expression levels of qRT-PCR and RNA-Seq in MT vs. CK at 21 and 28 d after treatment. Asterisks indicate significant differences between the treatments using the Student's t-test (*: $0.01 \le p < 0.05$; **: $p < 0.01$)

and provide energy for fruit development [48]. However, at 28 d after treatment, the MT treatment resulted in a decrease in NAD^+-MDH activity and an increase in $NADP^+$-ME activity. This shift led to the decomposition of malic acid in the fruits, consequently reducing the malic acid content [49]. Transcriptome analysis revealed that the expression level of *MDH* was down-regulated at 28 d after treatment, aligning with the decrease in malate dehydrogenase activity. This discovery aligns with earlier studies conducted on apples [49], suggesting that *MDH* plays a regulatory role in malate synthesis in plum fruits.

ALMT2 belongs to the *ALMT* family of homologous genes, and the *ALMT* family is involved in the transport of various organic acids [50]. In this experiment, MT treatment down-regulated the expression levels of *ALMT2* at 28 d after treatment, was consistent with the trend of the decrease in malic acid content, indicating that *ALMT2* may be involved in the transport of malic acid in fruit cells. Meanwhile, MT treatment up-regulated the expression levels of malate transport related gene *TDT*. The previous study showed the high expression level of *TDT1* in pear fruits promoted its accumulation of malic acid content [51], and the expression levels of *tDT-like* (a *TDT* homologous gene) had a positive association with malic acid content in plum fruits [52]. The expression level of *PstDT* had a positive correlation with the content of citric acid in plum fruits [53]. In this experiment, MT treatment up-regulated the expression level of *TDT* and increased the citric acid content, while decreased the malic acid content at 28 d after treatment. This may be due to the fact that the amount of malic acid accumulated in plum fruits depended on the effects of the integrated regulation of several genes such as malic acid synthesis and degradation related genes, as well as malic acid transport related transporter proteins, ion channel genes, etc., and the changes in the malic acid content could not be explained by the changes in one or two genes only [52].

Conclusion

MT treatment improves fruit quality, increases the soluble sugar content, and decreases the organic acid content of plum fruits. At 21 d after treatment, a total of 13 DEGs are related to sugar metabolism, along with one DEG related to malic acid transport. Among these DEGs, the expression levels of *HK3*, *PFK3*, *GK*, and *ALMT2* are up-regulated. At 28 d after treatment, total of 20 DEGs are associated with sugar metabolism, and four DEGs are linked to malate metabolism and transport. In MT treated plum fruits, the expression levels of *SWEET2a*, *FBA*, and *TDT* are up-regulated, while the expression levels of *GK*, *ALMT2*, and *MDH* are down-regulated. Future study should focus on verifying the functions of these genes and elucidating their molecular mechanisms and regulatory networks.

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Author contributions
Y.X., J.W. and L.L. conceived and designed the research. Y.X. wrote the main manuscript text. J.W. and L.L. checked and revised the manuscript. Y.W., Z.H., M.G., L.Z., X.L. and H.X. performed the experiments. X.Z., D.L. and X.L. analyzed the data. All authors contributed to this article and approved the submitted version.

Data availability
The raw sequence data reported in this paper have been deposited in the National Center for Biotechnology Information (NCBI) and are publicly accessible at http://www.ncbi.nlm.nih.gov/bioproject/1178090.

Declarations

Ethics approval and consent to participate
We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. Plant samples were collected from university research area. Study protocol must comply with relevant institutional, national, and international guidelines and legislation. Our experiment follows with the relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

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The authors declare no competing interests.

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