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Effects of different dwarfing interstocks on key enzyme activities and the expression of genes related to malic acid metabolism in Red Fuji apples

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ABSTRACT. In this experiment, the test materials were 'Red Fuji' apple trees grafted onto three interstocks (No. 53, No. 111, and No. 236), which were chosen from SH40 seeding interstocks. The content of malic acid, the enzyme activities, and the expression of genes related to malic acid metabolism were determined during fruit development.The results showed that malic acid content in the ripe fruit on interstock No. 53 was higher than that in the interstock No. 111 fruit. The malate dehydrogenase (NAD-MDH) activity in apples on interstock No. 53 was highest on Day 30, Day 100, and Day 160 after bloom, and the malic enzyme (NADP-ME) activity in apples on interstock No. 111 was higher than in the interstock No. 53 fruit from Day 70 to Day 100 after bloom. The relative expression of NAD-MDH genes in interstock No. 53 fruit was higher than in No. 236 fruit on Day 100 after bloom, but the relative expression of NADP-ME in No. 236 interstock fruit was lower than in No. 53 fruit. The relative expression of NAD-MDH genes in No. 53 interstock fruit was highest on Day 160 after bloom. This might have been the main reason for the difference in the accumulation of malic

acid in the ripe apples.There was a positive correlation between the relative expression of phosphoenolpyruvate carboxylase (PEPC) and the malic acid content of the fruit, and the content of malic acid in the apples was affected by the PEPC activity during the early developmental stage.

Key word: Red Fuji; Dwarfing interstocks; Malic acid; NAD-MDH; Reverse transcription-PCR

INTRODUCTION

The types and content of organic acids influence the inherent quality of fruits. Malic acid is the major organic acid in apples. Apples are malic acid-dominant fruit (Chen et al., 2005) in which acid metabolism is regulated by various enzymes. Under catalysis by phosphoenolpyruvate carboxylase (PEPC) in the fruit cytoplasm, phosphoenolpyruvate (PEP) produced via the glycolytic pathway is converted into oxaloacetic acid (OAA). In the presence of malate dehydrogenase (NAD-MDH), OAA is turned into malic acid, which is decomposed by the catalytic effect of malic enzyme (NADP-ME) into pyruvic acid (Ruffner et al., 1984; Muñoz et al., 2001). PEPC, NADP-ME, and NAD-MDH are the key enzymes regulating malic acid synthesis and decomposition (Martinoia and Rentsch, 1994; Zhao et al., 2007; Wang et al., 2013).

In fact, acid metabolism and regulation in apples are very complex, and the acid content is jointly regulated by the relevant genes and enzymes. Molecular biology techniques have been applied to characterize the key enzymes of acid metabolism. Some researchers have measured the expression levels of PEPC and NADP-ME genes in apple cytoplasm in fruit belonging to high- and low-acid genotypes (Yao et al., 2009). Yao et al. (2011) cloned the full-length NAD-MDA gene into apples and tested its function. Or et al. (2000) found that the transcriptional levels of ME, NADP-MDH, and PEPC genes are crucial for the regulation of malic acid metabolism in grapes during the growth stages.

Environmental conditions and cultivation practice have a large impact on fruit quality (Thakur and Singh, 2012; Xu et al., 2012). Research shows that the organic acid content of apples is also influenced by stocks (Liu et al., 2007). However, the mechanism by which stocks affect the organic acid content of fruit is unknown. In this study, Red Fuji apple trees were grafted onto different dwarfing interstocks and were used as the test materials to determine malic acid content, metabolic enzyme activities, and the expression levels of the relevant genes during fruit growth. We have thereby provided a theoretical basis for ascertaining the mechanism by which interstocks affect the organic acid content of fruit.

MATERIAL AND METHODS

Materials

The test materials were from the Stock Experimental Garden of Baoding Comprehensive Experimental Station, which belongs to the National Modern Apple Industry Technical System. The 6-year-old Red Fuji apple trees were grafted with dwarfing interstocks (numbers 53, 111, and 236 were used). All three types of interstock were the offspring of SH40. Studies have shown that their dwarfing effect and early-fruiting property are superior those of SH40. Using *Malusmicromalus* as the rootstock, Tianhong No. 2 was grafted at a spacing of 0.75 m x 3.0 m. Consistent cultivation and management measures were adopted. Single-tree plot experiments were carried out with five

replicates. Trees showing consistent bloom stage were chosen for artificial pollination. Five fruits were collected from each tree, peeled, and deseeded. The pulp was preserved in liquid nitrogen (-196°C) and placed in an ultra-cold storage freezer (-74°C).

Extraction of organic acids

Gao's method (Gao et al., 2007) was adopted with modifications. Frozen pulp (5 g) was ground in anhydrous alcohol, transferred to a 50-mL conical bottle, and ultrasonically extracted for 10 min. Suction filtration was performed to extract the solution, and the solution was evaporated on a rotary evaporator. Next, 15 mL 12% sulfuric acid in methanol was added to dissolve the product, followed by transfer to a 50-mL volumetric flask. This was carried out three times. After heating in a water bath at 80°C for 1 h, the solution was titrated with ultrapure water and centrifuged at 4000 *g* for 5 min. The supernatant was collected and placed in a triangular bottle with a stopper. A pipette was used to transfer the solution described above (30 mL), and extraction was carried out three times with dichloromethane (10 mL each time). The extraction liquor was added with an appropriate amount of anhydrous sodium sulfate and passed through a 0.22-μm milipore filter.

Quantification of organic acids

Gas chromatography-mass spectrometry (GC-MS) was performed for quantification of the organic acids in the apples. The GC-MS conditions were as follows.

The GC was conducted using a 6890GC/5973MSD instrument (Agilent, CA, USA). An HP-5MS column (30 m x 250 μm x 0.25 μm) was used with an inlet temperature of 280°C. The programmed temperature regimen comprised: a column temperature of 70°C (0.5 min); a temperature increase rate of 10°C/min to 280°C (maintained for 1 min); then an increase at 5°C/min to 290°C (maintained for 15 min). We used helium as a carrier; the flow rate was 1 mL/min; the loading amount was 1 μL; and the system was operated in non-split mode.

The MS conditions were: electron ionization (EI); electron energy, 70 eV; ion source temperature, 230°C; quadrupole temperature, 150°C; electron multiplier voltage, 1.89 kV; scanning range, 35-500 U; and full-scan mode.

Quantification of malic acid

Malic acid content was determined by GC. The GC was conducted using an Agilent 7890A Gas Chromatograph (USA). Derivatives detection was by GC. We used FID apparatus, a G4513A automatic liquid sampler (Agilent, USA), an Hp-5 capillary column (5%-phenyl-methyl polysiloxane, 30 m x 25 μm x 0.1 μm) with an inlet temperature of 230°C and a monitor temperature of 250°C. We used high purity N_2 as carrier, a flow rate of 45 mL/min, an N_2 flow rate of 40 mL/min, a column pressure of 12.00 psi, a loading amount of 1 μL, and a split ratio of 30:1. The programmed temperature regimen comprised: an initial temperature of 60°C maintained for 2 min; an increase to 120°C at a rate of 8°C/min; an increase to 160°C at 20°C/min (maintained for 4 min); and finally an increase to 250°C at 40°C/min (maintained for 10 min).

Plotting of the standard curve: 0.9983 g malic acid was accurately weighed and placed in a 5-mL volumetric flask; methanol was added to the graduation mark. Aliquots of the above solution (10, 100, 250, 500, and 1000 μL) were transferred to a 50-mL volumetric flask, and 15 mL 12% sulfuric acid in methanol was added for derivation and extraction. Linear regression analysis was

carried out according to peak area of malic acid (Y) and corresponding mass (X). The standard curve was plotted as y = 18.648x - 9.347, with coefficient of correlation R = 0.99900.

Determination of enzyme activities

Preparation of enzyme solutions

Hirai's method (Hirai and Ueno, 1977) was adopted with slightly modifications. Pulp (3 g) was ground with 3 mL grinding fluid in an ice bath (4°C) and centrifuged at 4000 *g* for 20 min. The supernatant liquor was collected and diluted to 5 mL, and 2.5 mL was taken and centrifuged at 4°C and 15,000 *g* for 15 min. The precipitate was dissolved with extraction solution to 2.5 mL to obtain NAD-IDH enzyme solution. Then, 2.5 mL extraction solution was added to obtain NAD-MDH and NAD-ME enzyme solution. All procedures were completed at 0-4°C. The grinding buffer comprised 0.2 M Tris-HCl, pH 8.2; 0.6 M sucrose; and 10 mM arabo-ascorbic acid. The extraction buffer comprised: 0.2 M Tris-HCl, pH 8.2; 10 mM arabo-ascorbic acid; and 0.1% Triton X-100.

Determination of enzyme activity

The methods used by Hirai (Hirai and Ueno, 1977) and Luo (Luo et al., 2003) were referred to. The 3-mL reaction system for the detection of each enzyme was as follows. For NADP-ME, the system comprised 300 µL 0.8 M Tris-HCl, pH 7.4; 150 µL 4 mM NADP; 150 µL 4 mM $MnSO_4$; 300 µL ultrapure water; 500 µL enzyme solution; 1600 µL 4 mM malic acid; and a detection wavelength of 340 nm. For NAD-MDH, the system comprised: 300 µL 0.8 M Tris-HCl, pH 8.2; 150 µL 0.01 M glutathione (GSH); 150 µL 3 mM NADH; 150 µL 0.2 M $KHCO_3$; 150 µL 0.04 M $MgCl_2$; 500 µL enzyme solution; 1600 µL 4 mM OAA; and a detection wavelength of 340 nm. For PEPC, the system comprised: 300 µL 0.8 M Tris-HCl, pH 8.5; 150 µL 0.01 M GSH; 150 µL 3 mM NADH; 150 µL 0.04 M$MgCl_2$; 150 µL 0.2 M$KHCO_3$; 500 µL enzyme solution; 1600 µL 4 mM PEP; and a detection wavelength of 340 nm. A UV-2450 ultraviolet spectrophotometer was used to measure the absorbance. The scan was performed for 3 min at a unit of 5 s to record the changes of absorbance, with three replicates. One enzyme unit was defined as 0.01 absorbance change per min and expressed as $U \cdot g^{-1} \cdot FW \cdot min^{-1}$.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) detection of enzymes related to malic acid metabolism

Total RNA extraction and reverse transcription

Total RNA extraction and RT-qPCR detection were performed for fruit samples at Day 30, Day 70, and Day 100 after bloom, using two replicates for each sample. Total RNA was extracted using an Easy-spin Plus Plant RNA Kit (Nanjing Zhongding Biotechnology Co., Ltd.) in accordance with the manufacturer instructions. The extracted total RNA was then detected by agarose gel electrophoresis and ultraviolet spectrophotometry. Genomic DNA (gDNA) was removed before reverse transcription through the following procedures: 4X gDNA wiper Mix (2 µL), template RNA (500 ng), and RNase-free double-distilled H_2O were added to an RNase-free centrifuge tube to 10 µL, with gentle blowing using a pipette. The reaction proceeded at 42°C for 2 min. Then, 5X qRT Super Mix II (2 µL) and the reaction solution (8 µL) from the first step were added. RNA was reverse

transcribed into complementary DNA (cDNA) under the following conditions: 25°C for 10 min, 42°C for 30 min, and 85°C for 5 min.

RT-qPCR

A quantitative fluorescence PCR was carried out according to the manufacturer instructions using 2X SYBR Green qPCR Mix. The reaction system (20 µL) comprised 2X qPCR Master Mix (10 µL); 10 µM forward primer (0.4 µL); 10 µM reverse primer (0.4 µL); template (cDNA) (2 µL); and double-distilled H_2O. The conditions for the quantitative fluorescence PCR were as follows. For the amplification curve: 94°C for 30 s; 94°C for 10 s; 60°C for 12 s; 72°C for 30 s, for a total of 45 cycles; and a final extension at 72°C. For the dissolution curve: 95°C for 0 s; 65°C for 15 s; 95°C for 0 s; and continuous detection. The primers for the quantitative fluorescence PCR were designed according to the gene sequences with β-actin as internal control (Apple Research Team of the School of Horticulture and Agricultural at the University of Hebei Province). The primer sequences are listed in Table 1. The expression levels of three genes, NAD-MDH, NADP-ME, and PEPC, were detected.

Table 1. Primer sequences for reverse transcription polymerase chain reaction (RT-PCR).

Gene	Sequence (5' to 3')	GenBank accession No.
NAD-MDH-F	GTCAAGATGGAGTTGGTGGAG	DQ221207
NAD-MDH-R	TGGTGTTTGCGGGATTAG	
NADP-ME-F	GGATTCGGTCTGGGTTT	DQ280492
NADP-ME-R	GTAGTTTCGGTAGATGGGAC	
PEPC-F	CCTCCAAATGAACCCTACC	EU315246
PEPC-R	CACTGGCTAACAACTGACGA	
NF	GGATTTGCTGGTGATGATGCT	
NR	AGTTGCTCACTATGCCGTGCT	

Statistical analysis

Data sorting and plotting were conducted using Excel 2003. Significance tests and correlation analyses were carried out using DPS and SPSS 13.0 softwares, respectively.

RESULTS

Quantification of organic acids in apples

After methylation, the pulp was analyzed by GC-MS and the total ion chromatograms of the methylated organic acids were obtained. By searching the NIST98 database, 12 methylated organic acids were identified. Thus, the samples detected contained 2-butenedioic acid, 4-oxo-pentanoic acid, malic acid, 2-butynedioic acid, 3-acetoxy-3-hydroxypropionic acid, carbamic acid, methoxy-butanedioic acid, 2-methyl-pentanoic acid, citric acid, 4-hydroxy-3-methoxy-benzeneacetic acid, 2,3-dihydro-3-benzofurancarboxylic acid, and hexadecanoic acid. The content of each methylated organic acid was determined by using the area normalization method (Table 2). It was found that the dimethyl ester of malic acid had the largest relative content, which was 78.17%, indicating that malic acid was the predominant organic acid in Red Fuji apples.

Table 2. Constituents and contents of the methylated organic acids from Red Fuji apples.

Peak No.	Retention time	Compounds	Relative contents (%)
1	6.027	2-Butenedioic acid-, dimethyl ester	0.53
2	6.597	Pentanoic acid, 4-oxo-, methyl ester	0.63
3	7.587	Malic acid, dimethyl ester	78.17
4	8.015	2-Butynedioic acid, dimethyl ester	13.64
5	8.56	3-Acetoxy-3-hydroxypropionic acid, methyl ester	0.61
6	9.55	Carbamic acid, methyl ester	1.01
7	10.431	Butanedioic acid, methoxy-, dimethyl ester	0.69
8	11.571	Pentanoic acid, 2-methyl-, methyl ester	0.17
9	12.251	Citric acid, trimethyl ester	1.22
10	13.132	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	0.24
11	16.94	3-Benzofurancarboxylic acid, 2,3-dihydro-, methyl ester	2.89
12	17.544	Hexadecanoic acid, methyl ester	0.20

Changes of malic acid content in Red Fuji apples on different dwarfing interstocks

The overall change trend of malic acid content in apples on the three different interstocks was similar to that of titratable acid content (Figure 1). On Day 20 after bloom, the malic acid content began to rise, reaching a peak on Day 30. After that, the malic acid content declined until maturity. On Day 20 after bloom, the malic acid content in the apples on the No. 53 interstock was significantly higher than in apples on the No. 111 interstock. On Day 40 after bloom, the malic acid content in the apples on the No. 236 interstock was much lower than in apples on the other two interstocks. On Day 160 after bloom, the malic acid content in the apples on the No. 53 interstock was considerably higher than that in the apples on the No. 111 interstock ($P < 0.05$); the malic acid content of the apples in the two interstocks was 5.08 and 4.47 mg/g, respectively.

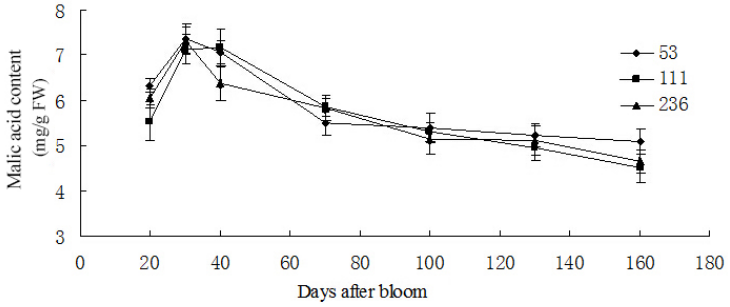

Figure 1. Changes of malic acid content in Red Fuji apples on the different interstocks.

Changes in NAD-MDH activity and gene expression

NAD-MDH activities in the apples on the three interstocks all reached the lowest level on Day 20 after bloom (Figure 2). The NAD-MDH activities showed an increasing trend on Day 20 after bloom and reached a peak on Day 30. At this time, the NAD-MDH activity in the apples on the No. 53 interstock was significantly higher than in the apples on the No. 111 interstock, and the two NAD-MDH activities were 163.50 $U \cdot g^{-1}$ $FW \cdot min^{-1}$ and 134.43 $U \cdot g^{-1}$ $FW \cdot min^{-1}$, respectively. On Day 100 and Day 160 after bloom, the NAD-MDH activities in all apples declined; the highest NAD-MDH activity was found in apples on the No. 53 interstock, and the lowest in apples on the No. 111 interstock; the difference was statistically significant.

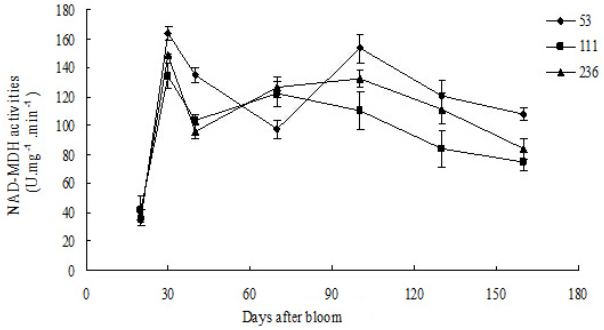

Figure 2. Changes of NAD-MDH activity in Red Fuji apples on the different interstocks.

The results of real-time fluorescence quantitative PCR detection for the NAD-MDH gene in the apples are shown in Figure 3. The relative expression of the NAD-MDH gene in apples on the No. 111 interstock on Day 30 after bloom was considerably higher than in the apples on the other interstocks. On Day 100 after bloom, the relative expression levels of the NAD-MDH gene in apples on the No. 53 interstock and the No. 236 interstock were significantly higher than in the apples on the No. 111 interstock, reaching 4.77 and 4.07 times the latter, respectively. On Day 160 after bloom, the relative expression of the NAD-MDH gene in the apples on the No. 53 interstock was significantly higher than in apples on the other interstocks. The relative expression of the NAD-MDH gene in apples on the No. 53 interstock was 1.84 and 4.45 times that for the No. 111 interstock and the No. 236 interstock, respectively.

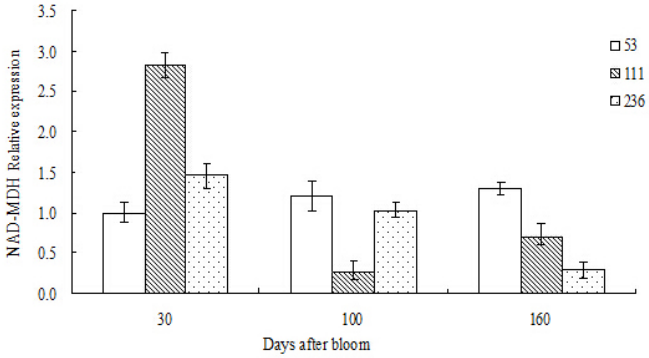

Figure 3. Relative expression of NAD-NDH in Red Fuji apples on the different interstocks.

Changes in PEPC activity and gene expression

On all three interstocks, PEPC activities initially increased and then decreased along with fruit growth (Figure 4). From Day 20 after bloom, PEPC activity levels in the fruits began to rise. PEPC activity levels in apples on the No. 111 interstock and the No. 236 interstock reached a peak on Day 40 after bloom. At this time, the PEPC activity in the apples on the No. 236 interstock was significantly higher than in the other interstocks. PEPC activity in apples on the No. 53 interstock reached a peak on Day 30, which was much higher than in the No. 111 interstock apples, and then declined. From Day 100 to maturity, PEPC activity in apples on the No. 53 interstock decreased

slowly; on Day 130, PEPC activity in apples on the No. 53 interstock was significantly higher than in the other interstocks. However, no significant differences were observed among other treatments.

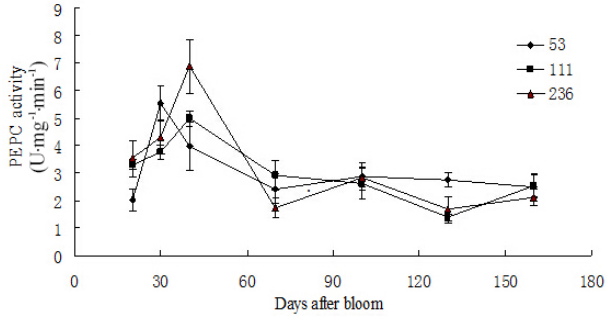

Figure 4. Change of PEPC activity in Red Fuji apples on the different interstocks.

The results of real-time fluorescence quantitative PCR for the PEPC gene are shown in Figure 5. On Day 30 after bloom, the relative expression of the PEPC gene in the apples on the No. 236 interstock was significantly higher than in the other interstocks. On Day 100 after bloom, the relative expression of the PEPC gene in the apples on the No. 236 interstock was much higher than in the No. 111 interstock apples; the relative expression of the PEPC gene in the apples on the No. 53 interstock was not significantly different from that in the No. 111 and No. 236 interstock apples. On Day 160, the apples on the No. 53 interstock showed an obvious increase in relative expression of the PEPC gene compared with the other interstocks. The relative expression of the PEPC gene in apples on the No. 53 interstock was 4.49 times and 1.22 times that in the No. 111 and No. 236 interstock apples, respectively; the relative expression of the PEPC gene in apples on the No. 236 interstock was 3.67 times that in the No. 111 interstock apples; the difference was statistically significant.

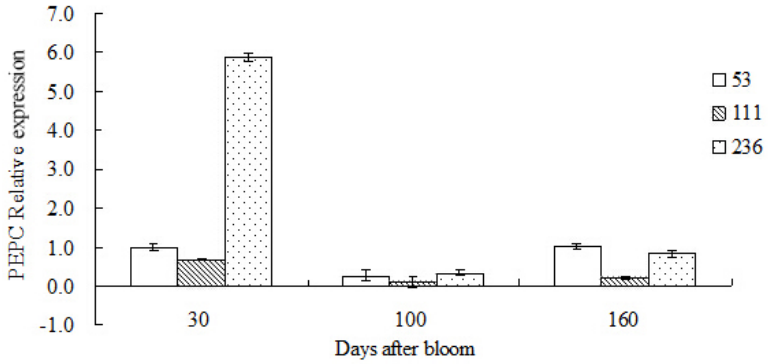

Figure 5. Relative expression of PEPC in Red Fuji apples on the different interstocks.

Changes in NADP-ME activity and gene expression

NADP-ME activity levels in the apples on the three interstocks generally showed an increasing trend over time (Figure 6). NADP-ME activities in all three interstocks were relatively

low from Day 20 to Day 30 after bloom. Following Day 40 after bloom, NADP-ME activity levels in apples on the No. 53 and No. 236 interstocks began to increase; NADP-ME activity in the No. 53 interstock apples was higher than in the No. 236 interstock apples, with significant differences on Day 40, Day 70, Day 100, and Day 130. NADP-ME activity in apples on the No. 111 interstock began to rise after Day 40 and reached a peak on Day 100. At this time, NADP-ME activity in the apples on the No. 111 interstock was considerably higher than in the other interstock apples.

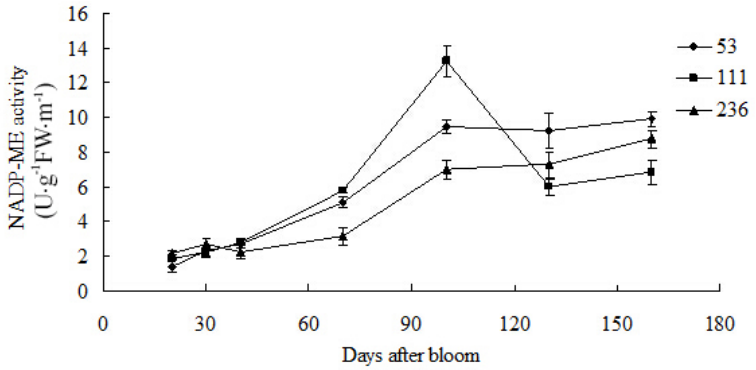

Figure 6. Changes of NADP-ME activity in Red Fuji apples on the different interstocks.

The results of real-time fluorescence quantitative PCR for the NADP-ME gene are shown in Figure 7. It can be seen that on Day 30 after bloom, the relative expression of the NADP-ME gene in the apples on the No. 236 interstock was significantly higher than in the No. 53 and No. 111 interstock apples, being 4.55 and 2.62 times the latter two, respectively; the difference in relative expression of the NADP-ME gene between the No. 53 and No. 111 interstock apples was not significant. On Day 100 after bloom, the relative expression of the NADP-ME gene in the apples on the No. 53 interstock was much higher than in those on the other interstocks; the relative expression of the NADP-ME gene in the apples on the No. 53 interstock was 2.83 and 4.69 times that in the No. 111 and No. 236 interstock apples, respectively. On Day 160 after bloom, the relative expression of the NADP-ME gene in the apples on the No. 236 interstock was highest, and there were significant differences among all the treatment groups.

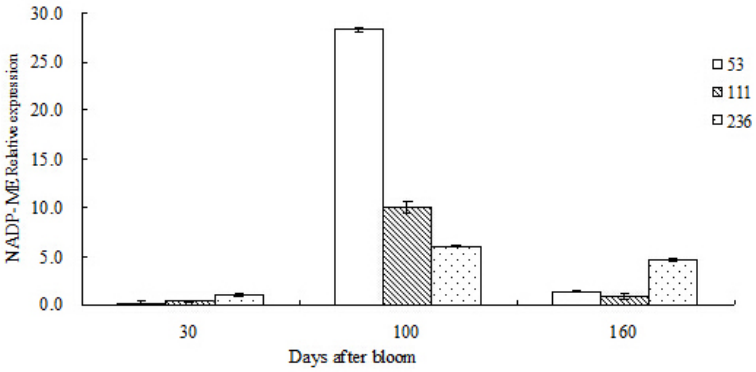

Figure 7. Relative expression of MADP-ME in Red Fuji apples on the different interstocks.

DISCUSSION

Stocks influence organic acid content in fruits to a certain extent. In this study, the malic acid content in Red Fuji apples on different dwarfing interstocks varied significantly. After maturity, the malic acid content in the apples on the No. 53 interstock was much higher than in the No. 111 interstock apples. It has been discovered that a pair of major genes related to the low-acid trait in fruits has a regulatory effect on the key enzymes of malic acid metabolism. Therefore, the relationship between malic acid accumulation and the expression of key enzymes can be analyzed by detecting the level of transcription, the protein content, and the enzyme activity (Maliepaard et al., 1998; Yao et al., 2008a). MDH gene expression is associated with malic acid accumulation in fruits at all stages (Yao et al., 2008b). NAD-MDH is the key enzyme of malic acid production in fruits. Iannetta et al. (2004) found that NAD-MDH activity was greatly enhanced with increasing malic acid content as strawberries mature. Our study suggested that the NAD-MDH activity in the apples on the No. 53 interstock was highest on Day 30 after bloom, corresponding to the highest malic acid content; moreover, the difference was significant compared with the No. 111 interstock apples. Before maturity, NAD-MDH activity levels in all the treatment groups declined gradually. On Day 100 and Day 160 after bloom, the NAD-MDH activity in the apples on the No. 53 interstock was markedly higher than in the No. 111 interstock apples; in addition, the relative expression of the NAD-MDH gene in the former was much higher than in the latter. Zhao et al. (2007) divided the changes in organic acids in nectarines into two stages, namely, organic acid synthesis and organic acid decomposition. Our research indicated that at the later growth stages of apples, the significant difference in NAD-MDH activity and the relative expression of the NAD-MDH gene in alternative treatment groups was the main reason for the difference in malic acid content in mature Red Fuji apples in the different treatment groups.

Luo et al. (2003) believed that PEPC activity in citrus fruits had an impact on organic acid metabolism. Wang et al. (2013) studied *Cerasushumilis* fruits with different acid content, and found that the enhanced PEPC activity at the later growth stages promoted malic acid accumulation. It was also found that the PEPC gene was expressed differentially in fruits of high- and low-acid genotypes (Yao et al., 2009). In our experiment, PEPC activity in the No. 53 interstock treatment was highest on Day 30 after bloom, corresponding to the highest malic acid content. Although the relative expression of the PEPC gene in apples on the No. 53 interstock was not the highest, it was significantly higher than in the No. 111 interstock apples. On Day 160, the relative expression of the PEPC gene in apples on the No. 53 interstock was much higher than in the No. 111 and No. 236 interstock apples, respectively. Thus, the relative expression of the PEPC gene in the different treatment groups was positively correlated with malic acid content. In the early growth stages, PEPC promoted malic acid accumulation in the apples. As the apples matured, PEPC activities influenced malic acid accumulation in the different treatment groups.

The existing studies on grapes (Ruffner et al., 1984), pears (Sha, 2012), and nectarines (Wang et al., 2013) all demonstrated that NADP-ME activity was negatively correlated with malic acid content. Dong et al. (2013) cloned two NAD-ME genes from Fuji apples, namely MdNAD-ME1 and MdNAD-ME2, which were proved to play different roles at different growth stages. In the present study, on Day 30 after bloom, the relative expression of the NADP-ME gene in apples on the No. 236 interstock was significantly higher than in No. 53 interstock apples, and the enzyme activity of the former was also higher than in the latter. From Day 70 to Day 100 after bloom, NADP-ME activity in the apples on the No. 111 interstock was much higher than in the No. 53 interstock apples, and the malic acid content was also higher. This may be the critical stage of NADP-ME

action, resulting in much higher malic acid content in ripe fruits on the No. 53 interstock compared with the No. 111 interstock.

Conflicts of interest

The authors declare no conflict of interest.

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