Rootstock-Scion Interactions Affect Fruit Flavor in Grafted Tomato

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ABSTRACT

Vegetable grafting has become an important method for developing resistance to biotic and abiotic stresses and increasing potential yield in agronomic practice. To determine the effects of grafting on tomato taste, we examined the cultivated tomato 'Moneymaker' and the wild tomato *Solanum pimpinellifolium*, which have different fruit weights and qualities, to investigate the effect of grafting on sugars, acids, and volatiles in single-head and double-head grafted plants using GC-MS and LC-MS. We observed that the contents of sugars, acids and volatiles in heterografted tomato pericarps are influenced by rootstocks. Different metabolites exhibit distinct responses to the rootstock and might be associated with rootstock-scion interactions. Comparison of the metabolites in the heterogeneous fruit of the single- and double-head grafted plants indicates that the grafting method also influences the metabolic changes in grafted plants. Moreover, we also identified numerous mobile transcripts and determined that the mobile mRNAs are associated with metabolic processes in tomato leaves. Our work helps to elucidate the effect of genotype and graft method on fruit quality and may provide a basis for future research on rootstock breeding and selection in plants.

Keywords: tomato; grafting; fruit flavor; mobile mRNA; rootstock

1. Introduction

Grafting is a horticultural technique utilized since ancient times to improve plant uniformity and crop yield (Liu, 2006). In most cases, one plant is selected for its root to serve as the lower part, which is called the rootstock. The upper part of the combined plant is called the scion. This technique was initially used in woody parents to induce fruitfulness by shortening the juvenile phase and to enhance other characteristics of the scion (Mudge et al., 2009). To date, vegetable grafting has been increasingly employed for cultivation and has been successfully implemented to minimize the deleterious effects of abiotic stresses, such as salinity, heavy metals and temperature, or to control several soil-borne diseases and pathogens, especially Cucurbitaceae and Solanaceae Rootstock selection and breeding have been utilized primarily to improve productivity, endowing rootstock with abiotic and biotic resistance (Pang et al., 2020), and breeders have been less concerned with

selecting rootstock to improve fruit quality (Louws et al., 2010; Albacete et al., 2015). However, fruit quality is becoming increasingly important to consumers, and understanding the implications of grafting for fruit quality is highly important.

Previous studies conducted on several vegetables have indicated that grafting could affect characteristics of fruit quality, such as fruit color, texture, sweetness and aroma profile (Flores et al., 2010; Condurso et al., 2012; Krumbein and Schwarz, 2013; Djidonou et al., 2017; Wang et al., 2020). However, different rootstock-scion combinations have been observed to have differing effects on fruit quality. A study of watermelon plants grafted onto interspecific rootstocks, *Citrullus maxima* × *Citrullus moschata*, was shown to significantly raise lycopene levels during ripening in watermelon fruit, whereas the rootstock-scion combination involving *Lagenaria siceraria* and *Cucurbita argyrosperma* exhibited a decrease in lycopene levels in watermelon (Perkins-Veazie and Collins, 2006; Soteriou et al., 2014; Kyriacou et al., 2016). Therefore, the grafted combination might play an important role in determining fruit quality.

The interactions and communications between the rootstock and the scion are highly complex and bidirectional, affecting scion growth through the xylem and phloem. The rootstock and the scion may exchange water and metabolic substances, including nutrients, small organic molecules and signaling molecules, such as hormones, which may cause large biological effects (Ghanem et al., 2008, 2011a; Gregory et al., 2013; Savvas et al., 2017). In tomato, long-distance movement of gibberellic acid from the rootstock to the scion alters leaf morphology (Haywood et al., 2005). Moreover, large molecules, such as protein or RNA, can also be transported to affect the growth of scions (Kim et al., 2001; Paultre et al., 2016; Kehr and Kragler, 2018). In recent years, an increasing number of mobile protein-coding mRNAs and noncoding small RNAs have been identified by high-throughput sequencing(Molnar et al., 2010; Shen et al., 2012; Thieme et al., 2015). These RNAs play vital roles in various biological and metabolic processes to regulate plant development and enhance resistance to stress and pathogens (Kehr and Kragler, 2018; Thomas and Frank, 2019).

Tomato (*Solanum lycopersicum*) is a major horticultural crop that is widely grown and consumed throughout the world. However, tomato is susceptible to soil-borne diseases, which hinder the growth of tomato and cause significant economic losses. Grafting has been successfully implemented under adverse environments to increase the disease resistance of tomato and improve its productivity (Louws et al., 2010). Compared with other vegetables, tomato taste is critical for selection by consumers. Sweetness and acidity are the main factors influencing tomato taste and flavor. In tomato fruit, three main soluble sugars dynamically change during fruit development. Fructose and glucose are increased with fruit ripening and reach their highest levels in red ripe fruit, but sucrose decreases and is barely detected in red ripe fruit. Therefore, in mature fruit, fructose and glucose are the main soluble sugars affecting tomato taste (Quinet et al., 2019). The important acids in tomato fruit were malic and citric acid, and citric acid was more abundant than malic acid in the pericarp (Quinet et al., 2019 ; Wang et al., 2021). Beyond the foundation of sufficient sugars and acids, a fruit must also have sufficient volatile chemicals that endow tomato flavor. Over 400 volatiles have been detected in tomato, and 28 main volatiles affect tomato flavor (Carrari and Fernie, 2006; Tieman et al., 2017). Although some studies have explored the influence of grafting on tomato taste, they obtained notably discrepant findings, and the effect of grafting on the quality of tomato fruit have not been fully elucidated to date (Gajc-Wolska et al., 2012;

Nicoletto et al., 2013b; Schwarz et al., 2013; Rahmatian et al., 2014; Xu et al., 2020).

The rootstock-scion combination plays important roles in affecting most tomato quality characteristics, which is in keeping with the findings obtained for other vegetables (Gajc-Wolska et al., 2012; Nicoletto et al., 2013a; Schwarz et al., 2013; Riga, 2015). Thus, understanding the communication of metabolites and large molecules between the rootstock and the scion may help us to select rootstocks in tomato breeding. In this study, we analyzed such metabolites as sugars, acids, and volatiles, which may contribute to fruit taste in autografted and heterografted tomatoes. Our results demonstrated that the rootstock influenced the accumulation of sugars, acids and volatiles in the scion. These metabolites in the heterografted fruit displayed different responses to the *Solanum lycopersicum* Moneymaker (MM) and *S. pimpinellifolium* (PI365967, PP) rootstocks. Moreover, the differences observed in these metabolites between double-head and single-head grafted fruit indicated that the graft method also influenced fruit quality. We also observed that a large number of transcripts move directionally in the rootstock and scion, which might be associated with grafted-induced alterations of metabolites in fruit. The results of our study demonstrated that the rootstock and graft method affect fruit taste, which may facilitate future efforts to optimize rootstock selection in tomato breeding.

2. Materials and methods

2.1. Plant materials and grown conditions

All plants were grown in a greenhouse from March 15th to the Jane 30th at the Nankou experimental base of the Chinese Academy of Agricultural Sciences in Beijing, China. The cultivar *Solanum lycopersicum* Moneymaker (MM) and the wild tomato *S. pimpinellifolium* (PI365967, PP) were used for grafting as the scion or rootstock. After the appearance of the second true leaf at the seedling stage, grafting was performed by making a downward slanted incision above the first true leaf on the rootstock. The plastic clips were used to assemble the incisions and removed about 15 days after grafting. The grafted plants were transferred into the soil after 40 days. The leaves were trimmed with the plant growth. We used two strategies to generate autografted and heterografted plants and evaluated the effects of grafting on fruit quality. One method is to produce single-head grafted plants, which were generated by grafting PP shoots onto MM roots or *vice versa*. The second grafting method generated double-head grafted plants.

2.2. Fruit sample collection

The red ripen fruit were collected from ten different plants and more than 10 red ripen fruit were weighted as a replicate. The pericarps were immediately frozen and ground into powder in liquid nitrogen and subsequently weighed precisely for metabolite analysis. Five replicates were used to determine the fruit weight, the concentrations of sugars, acids and volatiles, and each sample was measured three times.

2.3. Measurement for sugars

The contents of fructose, glucose and sucrose were measured by UPLC-MS/MS (ACQUITY UPLC I-Class Xevo TQ-S Micro, Waters) according to a previous study with modification (Qi et al., 2020). Pericarp powder (0.1 g) was weighed, and water was added to a volume of 2 mL, with 1 mg · mL^{-1} arabinose serving as an internal standard. After sonication and centrifugation, the supernatant was filtered with a 0.22-μm polyethersulfone ultrafiltration membrane. The extraction solution was mixed with an equal volume of acetonitrile for further

analysis. A Waters ACQUITY UPLC BEH Amide 1.7 μm column was used (2.1 mm × 100 mm i.d., 1.7 μm) for the content analysis. The mobile phase was composed of acetonitrile as solvent A and 0.1% ammonium hydroxide in water as solvent B. The gradient run was performed at a flow rate of 0.2 mL · min^{-1} with initial 10% B. Mobile phase B changed from 10% to 20% in 2 min, maintained at 20% for 6 min, increased to 90% in 0.1 min, and was finally maintained at 90% for 6.9 min. The temperatures of the column and autosampler were 60 °C and 4 °C, respectively. Data analysis was performed using MassLynx V4.1 (Waters).

2.4. Measurement for citric and malic acids

The contents of malic and citric acid were measured by UPLC-MS/MS (ACQUITY UPLC I-Class Xevo TQ-S Micro, Waters) according to a previous study with several modifications (Qi et al., 2020). Pericarp powder (0.2 g) was weighed, and water was added to a volume of 2 mL, with 0.5 mg · mL^{-1} lactic acid serving as an internal standard. After sonication and centrifugation, the supernatant was filtered with a 0.22-μm polyethersulfone ultrafiltration membrane and subsequently diluted 20-fold with water. The diluted solution was further diluted 50-fold with water/acetonitrile (1:1) for analysis. A Waters ACQUITY UPLC HSS T3 (2.1 mm × 100 mm i.d., 1.8 μm) column was used for acid analysis. Mobile phases A and B for acid separation were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. The acid elution was performed at a flow rate of 0.1 mL · min^{-1} with 90% B for 5 min. The temperatures of the column and autosampler were 25 °C and 4 °C, respectively. Data analysis was performed using MassLynx V4.1 (Waters).

2.5. Measurement for volatile metabolites analysis

Volatile compounds were analyzed based on previously reported method (Wang et al., 2018). The volatiles were enriched by headspace solid phase microextraction (HS-SPME). Two grams of pericarp powder and 0.6 g sodium chloride were weighed and placed into a closed 20-mL glass bottle. Next, 2.05 μg 2-nonanone was added as an internal standard. The sealed glass bottle was placed into a water bath at 50 °C for 10 min to reach equilibrium with agitation. Next, the SPME fiber was inserted into the bottle. After 40 min, the SPME fiber was quickly taken out and inserted into the GC injection port for measurement. The chromatographic analysis was performed using an Agilent 7890B-5977A GC-MS with a split-splitless injector. The operation was performed in split mode with 2:1 split ratio. The capillary column was HP-5MS with a 30-m length, 0.25-mm I.D., and 0.25-μm film thickness. The injector temperature was 250 °C, and the carrier gas was helium at 1 mL · min^{-1}. The temperature program was as follows: 40 °C for 5 min; ramped to 120 °C at a step of 2 °C · min^{-1}; ramped to 280 °C at a step of 10 °C · min^{-1} and held at 280 °C for 29 min. Identified compounds were compared with the NIST database 14 to obtain volatile information. The sensitivity of GC-MS was controlled with standards.

2.6. Transcriptome analysis and mobile transcript prediction

For transcriptome analysis, the fifth true leaves of two-month grafted MMr:PPs_MMr, MMr:PPs_PPs, PPr:MMs_PPr and PPr:MMs_MMs plants were collected. The RNA-Seq libraries were sequenced using the Illumina HiSeq 2500 platform. The analysis process was modified based on a previously published method (Thieme et al., 2015). Paired-end Illumina RNA-Seq data (150 bp) were mapped to tomato cDNA sequences (ITAG2.4 version) using bwa v0.7.16a (Grabherr et al., 2011). DNA-Seq reads of MM and PP were mapped to tomato chromosomes (SL2.50 version). All optimal matches (reported by BWA's X0 and XA tags) and mate pair

reads mapped to the same gene were retained. SNPs were detected in DNA sequences and RNA sequences using SAMtools mpileup (version 1.9)(Li et al., 2009), respectively. All SNPs in RNA-Seq results were confirmed with DNA-Seq data to eliminate other sources of sequence variation, such as RNA editing or other sequence modifications. Each SNP was required to show exactly two different and homozygous alleles in MM and PP DNA-Seq data. Next, the SNPs were filtered according to the published method (Thieme et al., 2015), including the criteria that mapped reads supporting a SNP were required to have an average Phred base mapping quality (Q) above 45 and at least 3 reads supporting each nucleotide position. Heterozygous alleles were required to have more than 5% of all reads mapped as alternative genotypes to support a SNP. After all these filter criteria were applied, the remaining SNPs were called informative SNPs. A gene was considered mobile if at least one informative SNP was heterozygous in any one of the heterografted plants. GO analysis was performed using their best homologous genes in *Arabidopsis* with agriGo (the Database for Annotation, Visualization and Integrated Discovery, http://systemsbiology.cau.edu.cn/agriGOv2/index.php)(Tian et al., 2017). Sequence data from this article can be found in the Sequence Read Archive under accession numbers PRJNA684928, SRR2391865 and SRR2391866.

3. Results

3.1. Fruit weight was affected by grafting

To determine the effects of grafting on fruit quality, we chose two tomato species, the wild relative PP and the cultivar MM, to generate heterografted plants. These two tomato species both have red and edible fruit but with different sizes and tastes. The fruit of PP is small, weighing only 1 – 2 g, and MM has fruit that weighs 80 – 100 g (Zhang et al., 2018). One of our grafting method is to produce single-head grafted plants, which were generated by grafting PP shoots onto MM roots or *vice versa*, and these grafted plants were named MMr:PPs or PPr:MMs, respectively (Fig. 1, A and Fig. S1, A). The second grafting method generated double-head grafted plants. One of the heads consisted of the lateral branches grown from the first leaf axil of the rootstock after grafting, and the other head was the scion shoot grafted on the rootstock. MMr:PPs_PPs, MMr:PPs_MMr, MMr:MMs_MMs or MMr:MMs_MMr were two double-head grafted plants grown on MM roots (Fig. 1, B and Fig. S1, B).

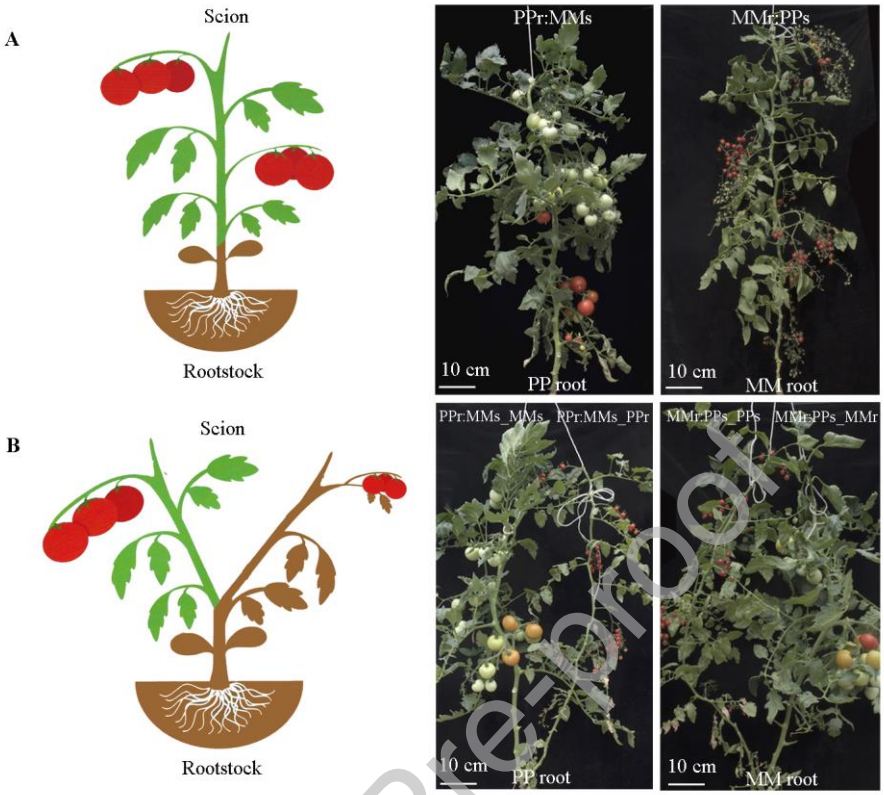


Fig. 1 Diagram of the grafting methods and grafted plants

(A) Diagram and photos of single-head grafted plants. PPr:MMs indicates the plant with MM shoots grafted on PP roots; MMr:PPs indicates the heterogeneous plant with PP shoots grafted on MM roots. (B) Diagram and photos of double-head grafted plants. One of the heads consisted of the lateral branches grown from the first leaf axil of the rootstock after grafting, and the other head was the scion shoot grafted on the rootstock. MMr:PPs_MMr and MMr:PPs_PPs represent the two shoots on the MM rootstocks; PPr:MMs_PPr and PPr:MMs_MMs represent the two shoots on the PP rootstocks. MM, Moneymaker; PP, PI365967.

Because PP and MM have distinct fruit sizes, we first tested whether fruit weight is affected by grafting. Comparing fruit weight between the heterografted and autografted plants, we found that the fruit weights of both PP and MM scions on the single-head grafted plants decreased. The mean weights of MM and PP fruit decreased approximately 10% (Fig. 2, A, B). Different from the single-head grafted fruit, the MM fruit (MMr:PPs_MMr and PPr:MMs_MMs) on the MM or PP rootstocks of double-head heterogeneous grafted plants were larger than the fruit of self-grafted MM (Fig. 2, C). However, the fruit weights of PPr:MMs_PPr and MMr:PPs_PPs were decreased by approximately 30% compared to the self-grafted PP fruit (Fig. 2, D). These results suggested that grafting does not always increase productivity in tomato.

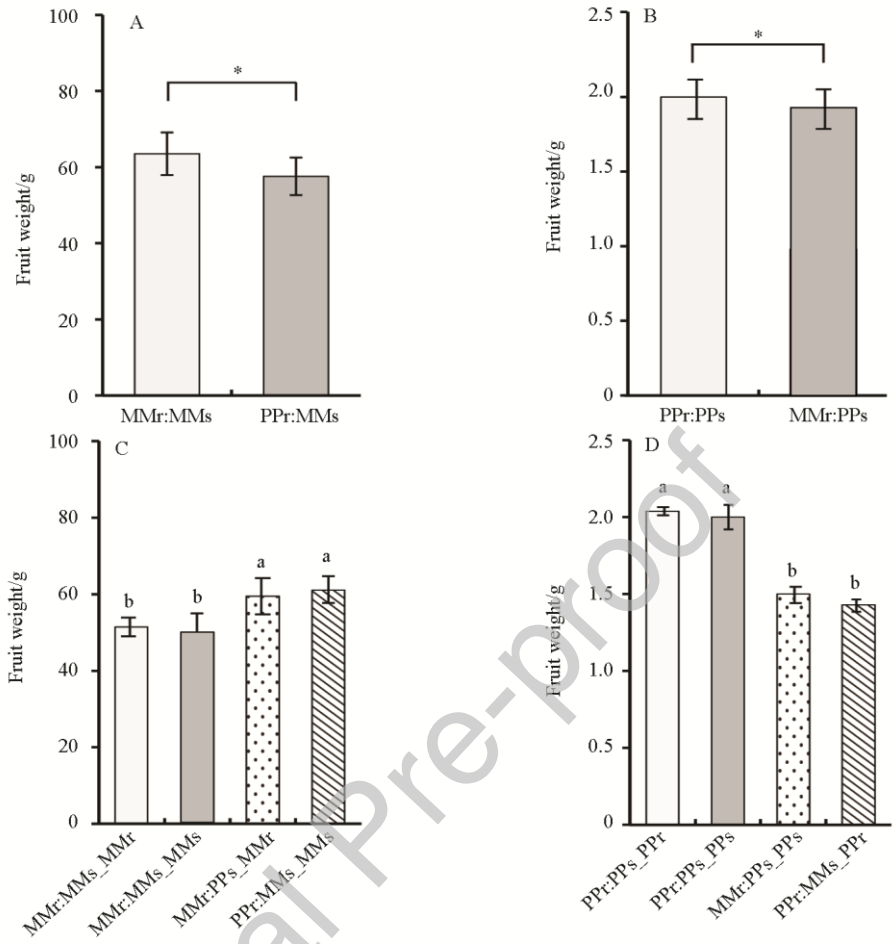


Fig. 2 Fruit weights of different grafted plants

(A) Weight of MM fruit on the single-head grafted plants. (B) Weight of PP fruit on the single-head grafted plants. (C) Weight of MM fruit on the double-head autografted and heterografted plants. (D) Weight of PP fruit on the double-head autografted and heterografted plants. Student's *t*-test and Turkey's test were used for statistical analyses. Student's *t*-test (**, $P < 0.01$; *, $P < 0.05$). The different letters indicate a significant difference between the samples ($P < 0.05$). Five biological replicates were used. Error bar, SD.

3.2. Sugar was affected by grafting

To investigate how the grafting to affect fruit sweetness, we explored the contents of fructose and glucose in red ripen fruit. PP red fruit of autografted plants had higher contents of fructose and glucose than MM fruit did (Fig. 3). When we grafted PP onto MM roots, we observed that the concentrations of fructose and glucose were increased by approximately 30% in the heterogenous PP fruit (MMr:PPs) of single grafted plants compared with self-grafted PP fruit. Slight but significant increases in fructose and glucose were also observed in the MM fruit (PPr:MMs) on the PP roots compared to the autografted MM fruit (Fig. 3, A, B). All these results suggested that single-head grafting could enhance the sugar content of scion fruit.

Similar to the single-head self-grafted MM and PP fruit, the contents of fructose and glucose were not notably different in the fruit of double-head autografted plants (Fig. 3, C, D). However, the glucose and fructose

contents of double-head grafted fruit displayed different responses to heterografting. The concentration of glucose increased in the PP fruit but decreased in the MM fruit of double-head heterogeneous grafted plants (Fig. 3, C). However, no significant changes in fructose content were observed in the grafted fruit, except for the fruit of MMr:PPs_PPs (Fig. 3, D). The different effects on sugar content observed in single- and double-head grafted fruit indicated that the different plant grafting might be an important factor affecting sugar content in fruit.

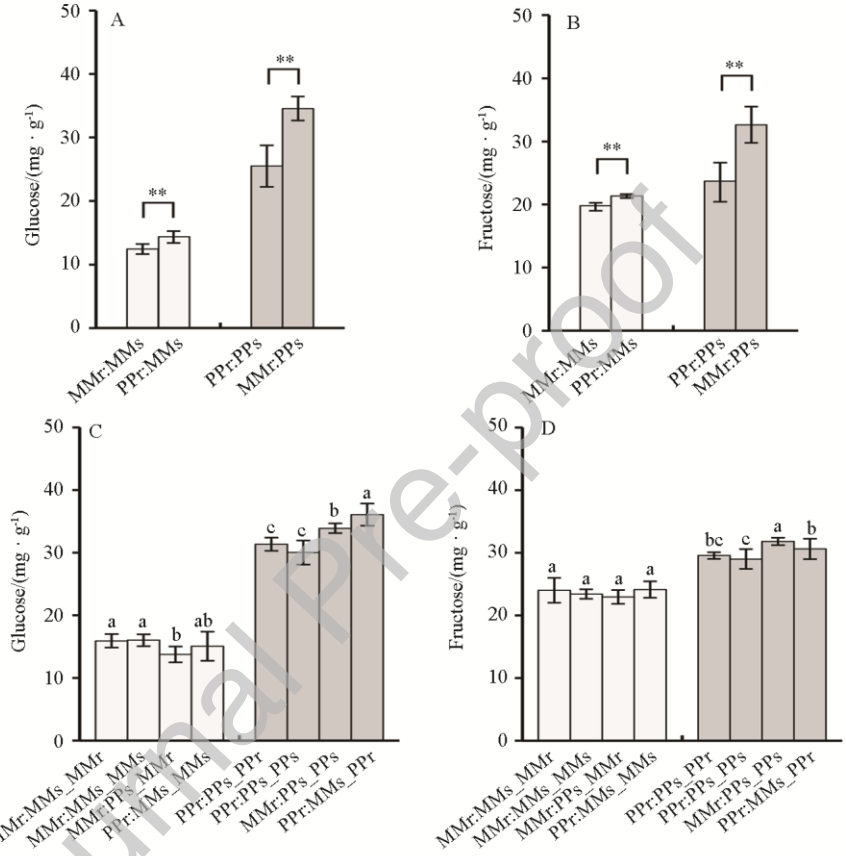


Fig. 3 Sugar concentrations in different grafted fruit

(A, B) Concentrations of glucose (A) and fructose (B) in the fruit of single-head grafted plants. (C, D) Concentrations of glucose (C) and fructose (D) in the fruit of double-head grafted plants. Student's *t*-test (**, $P < 0.01$; *, $P < 0.05$). The different letters indicate a significant difference between the samples ($P < 0.05$). Five biological replicates were used. Error bar, SD.

3.3. MM could enhance the contents of citric and malic acids in PP heterografted fruit

MM and PP have different concentration of citric and malic acid in red ripen fruit. The autografted PP pericarps contained higher citric acid and lower malic acid than self-grafted MM pericarps (Figs. 4, A, B). In contrast to the increase in sugar content observed in all the single-head grafted PP and MM fruit, the concentrations of citric and malic acids were clearly enhanced in the PP pericarps of the MMr:PP fruit but reduced in the MM pericarp of PPr:MM fruit compared with autografted PP or MM fruit (Figs. 4, A, B). Notably,

malic acid was dramatically affected by PP rootstock, and its content decreased by approximately 30% in the MM fruit of PPr:MMs (Fig. 4, B), suggesting that MM and PP rootstock have different effects on the accumulation of malic and citric acids in heterogeneous scion fruit.

In line with the results obtained from single-head grafted plants, self-grafting did not affect the contents of malic and citric acid in the MM or PP pericarps of double-head grafted plants (Fig. 4, C, D). In contrast to the single-head grafted plants, the concentrations of malic and citric acids in MM fruit of MMr:PPs_MMr and PPr:MMs_MMs did not change regardless of whether MM or PP was the rootstock. However, MM affected their contents in heterografted PP fruit of MMr:PPs_PPs and PPr:MMs_PPr plants (Fig. 4, C, D). The contents of these two acids in the heterografted PP fruit were increased by nearly 30% compared to the self-grafted PP fruit. All these results indicated that MM rootstock could enhance the fruit acidity of PP heterografted fruit and that this effect was not associated with the grafting method.

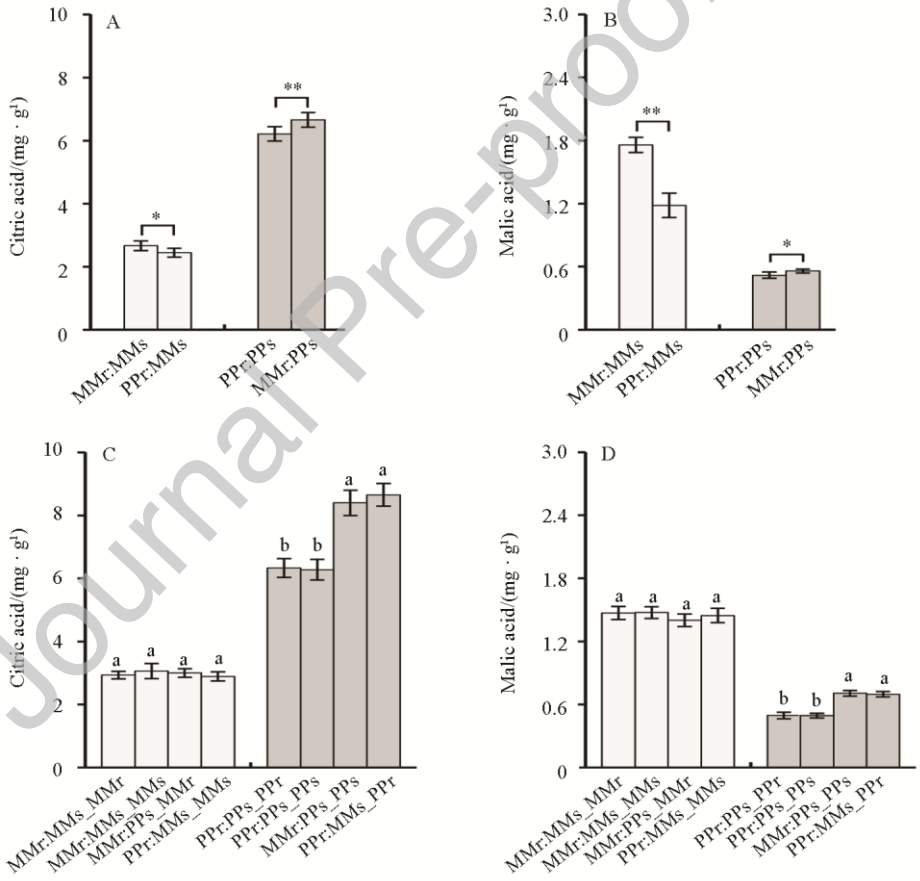


Fig. 4 Acid concentrations in different grafted fruit

(A, B) Concentrations of citric acid (A) and malic acid (B) in the fruit of single-head grafted plants. (C, D) Concentrations of citric acid (C) and malic acid (D) in the fruit of double-head grafted plants. Student's *t*-test (**, $P < 0.01$; *, $P < 0.05$). The different letters indicate a significant difference between the samples ($P < 0.05$). Five biological replicates were used. Error bar, SD.

3.4. Accumulation of distinct volatiles was influenced by grafting

In our experiments, a total of 33 volatile chemicals were stably detected in the tomato pericarp using GC-MS, including 19 of the 28 volatiles that mainly influence tomato fruit flavor (Table 1) (Tieman et al., 2017). According to the properties of these volatiles, they were divided into five groups: alcohols, ketones, aldehydes, esters and others. Except for 6 volatiles, the concentrations of 27 volatiles were significantly different between PP and MM fruit. The concentrations of 6 volatiles were higher in MM fruit than in PP fruit, including linalool and benzaldehyde, which were only detected in autografted MM pericarps, and 3-methyl-1-butanol, 6-methyl-5-hepten-2-ol, (*E*)-citral and (*E*)-2-nonenal. 23 of these volatiles displayed higher concentrations in the PP pericarp (Table 1).

Table 1 Content of volatiles in the heterogeneous fruit of the single-head grafted plants (ng · g^{-1})

Variety	Compound	MMr:MMs	PPr:MMs	PPr:PPs	MMr:PPs
Alcohol	3-Methyl-1-butanol	12.13 ± 1.46 c	31.82 ± 3.73 a	9.70 ± 2.74 c	19.83 ± 4.83 b
	6-Methyl-5-hepten-2-ol	89.87 ± 15.35 a	52.44 ± 11.69 b	9.23 ± 1.85 c	32.87 ± 9.96 b
	1-Pentanol	19.07 ± 5.51 c	16.22 ± 3.05 c	39.98 ± 5.79 b	54.27 ± 4.15 a
	2-Phenylethanol	9.01 ± 9.12 c	9.04 ± 8.88 c	92.37 ± 37.90 b	148.57 ± 21.28 a
	Hexanol	38.22 ± 11.86 b	44.21 ± 9.15 b	84.87 ± 9.41 a	62.49 ± 20.76 ab
	(*Z*)-3-Hexen-1-ol	195.85 ± 62.20 b	249.38 ± 61.27 ab	363.16 ± 78.89 a	347.75 ± 97.34 a
	Linalool	59.98 ± 10.34 a	51.5 ± 8.06 a	N.D.	N.D.
	1-Octen-3-ol	18.25 ± 4.00 b	18.39 ± 2.50 b	25.01 ± 3.28 ab	26.94 ± 7.46 a
Aldehyde	Benzaldehyde	150.29 ± 41.44 b	125.77 ± 45.19 b	N.D.	326.65 ± 69.78 a
	Heptanal	87.00 ± 9.60 b	152.77 ± 16.58 a	38.97 ± 4.23 c	46.54 ± 8.29 c
	Hexanal	4 182.80 ± 283.47 c	4 466.85 ± 449.40 c	10 910.46 ± 847.09 b	13 724.29 ± 1 727.92 a
	(*E*)-2-Pentenal	46.02 ± 9.66 c	33.74 ± 5.58 c	62.86 ± 1.89 b	94.77 ± 13.87 a
	(*E,E*)-2,4-Nonadienal	60.70 ± 4.80 b	77.59 ± 6.05 b	185.53 ± 14.74 a	200.76 ± 29.71 a
	(*Z*)-3-Hexenal	103.69 ± 13.25 b	85.9 ± 13.10 b	103.22 ± 8.88 b	145.89 ± 15.87 a
	(*E*)-2-Hexenal	4 451.39 ± 208.51 c	4 787.25 ± 322.86 c	11 767.14 ± 1013.23 b	13 650.23 ± 1 420.04 a
	Phenylacetaldehyde	25.76 ± 12.22 c	35.16 ± 6.71 c	102.59 ± 19.68 b	147.00 ± 29.14 a
	β-Cyclocitral	24.04 ± 3.30 b	21 ± 3.58 b	65.50 ± 15.97 a	66.87 ± 4.85 a
	(*E*)-Citral	102.41 ± 26.73 a	96.74 ± 20.72 a	78.27 ± 24.04 a	87.82 ± 14.18 a
	(*Z*)-Citral	16.89 ± 5.21 a	17.18 ± 2.60 a	20.57 ± 8.56 a	23.54 ± 3.56 a
	(*E*)-2-Heptenal	276.72 ± 36.53 b	249.66 ± 46.72 b	675.4 ± 67.07 a	669.76 ± 55.58 a
	(*E*)-2-Octenal	155.29 ± 17.09 b	147.39 ± 13.25 b	349.97 ± 30.65 a	379.44 ± 24.84 a
	Decanal	49.28 ± 4.38 a	51.54 ± 7.30 a	71.55 ± 19.36 a	77.1 ± 31.64 a
	(*E*)-2-Nonenal	89.39 ± 17.87 a	80.15 ± 13.73 a	17.75 ± 2.90 b	16.96 ± 6.32 b
	Nonanal	145.22 ± 19.95 b	131.35 ± 12.80 b	226.52 ± 43.05 a	241.85 ± 57.75 a
	Octanal	51.58 ± 7.24 b	44.47 ± 4.16 b	72.79 ± 12.85 a	78.79 ± 13.71 a
	1-Pentanal	41.73 ± 7.39 b	42.66 ± 6.91 b	108.11 ± 12.15 a	132.23 ± 21.87 a
Ester	Methyl salicylate	158.04 ± 34.79 b	143.55 ± 40.94 b	263.92 ± 43.69 a	251.8 ± 29.17 a
Ketone	1-Penten-3-one	65.73 ± 11.24 c	49.91 ± 9.57 c	124.02 ± 12.84 b	185.82 ± 23.87 a
	1-Octen-3-one	87.06 ± 13.94 b	100.29 ± 7.39 ab	115.83 ± 8.59 a	115.20 ± 8.57 a
	Geranylacetone	298.95 ± 59.43 b	429.33 ± 71.03 ab	574.23 ± 147.29 a	564.34 ± 75.00 a
	6-Methyl-5-hepten-2-one	749.83 ± 60.73 b	828.4 ± 63.54 b	1 347.63 ± 183.72 a	1 475.88 ± 134.44 a
	β-Ionone	74.41 ± 23.55 b	58.60 ± 7.06 b	162.62 ± 35.19 a	145.78 ± 25.20 a
Other	2-Isobutylthiazole	624.24 ± 42.75 a	656.25 ± 51.43 a	128.08 ± 28.85 b	96.45 ± 42.48 b

Note: Values represent the average ± *SE*.; N.D., not detected. ANOVA with Turkey post-hoc test were used for statistical analyses. The different letters indicate significance between the samples, $P < 0.05$.

To evaluate the grafting effect on fruit flavor, we tested the contents of volatiles in the pericarp of grafted plants. In the single-head grafted plants, 20 of 33 chemicals were not influenced by grafting (Table 1). The

concentrations of these volatiles were not different in the MM or PP fruit of PPr:MMs or MMr:PPs compared with their corresponding autografted MM or PP fruit. An extreme example was linalool, which was not detected in the PP fruit but accumulated in the MM fruit. After grafting, linalool was still not identified in PP fruit of MMr:PPs, and its concentration was not affected in PP fruit on the MM rootstocks (Table 1). Unlike these volatiles, the concentrations of 13 volatiles were affected by grafting, and most of them were observed at higher concentrations in the heterografted fruit. Among these volatiles, 11 volatiles were affected by MM rootstock and increased in the PP fruit of MMr:PPs and only 3 volatiles appeared increase in the heterografted MM fruit on the PP roots, including heptanal, 1-Octen-3-one and 3-Methyl-1-butanol. One interesting example is benzaldehyde, which was only detected in MM fruit. After grafting, benzaldehyde was detected in the heterografting PP fruit of MMr:PPs. The concentration of 3-methyl-1-butanol was significantly increased after grafting in both heterogeneous MM and PP fruit. Different with these volatiles, the concentrations of 6-methyl-5-hepten-2-ol in the heterogeneous fruit of MM or PP scions were observed to be close to those in the rootstock fruit, which accumulated in the PP and were reduced in MM fruit (Table 1). Taken together, these results indicate that grafting could change the concentrations of volatiles in the grafted plants to affect tomato fruit flavor.

3.5. Fruit volatiles were affected by rootstock genotype and different grafting

To further determine how the concentrations of volatiles are affected by grafting method, we explored the grafting effect on volatiles in fruit of double-head heterografted plants. Identical amounts of 33 volatiles were detected in both the double-head grafted MM and PP fruit, and 29 volatiles showed different concentrations between PP and MM fruit (Table S1). The same as the single-head grafted plant, 6 volatiles exhibited higher concentrations in MM fruit, such as linalool. A total of 23 volatiles largely accumulated in PP fruit that are in keeping with 21 volatiles detected in the single-head grafted fruit (Table S1).

In self-grafted MM or PP fruit, except for (*Z*)-3-hexen-1-ol, the concentrations of the other 32 volatiles were not influenced by grafting in the double-head autografted fruit, indicating that self-grafting has almost no effect on fruit volatiles (Table S1). We further compared the volatile concentration of the double-head heterogeneous grafted fruit with that of the autografted fruit and found that 18 volatiles were not affected by the double-head grafting, including the 15 same volatiles unaffected in the single-head grafted fruit. 6 volatiles were increased in the PP fruit of MMr:PPs_PPs, compared to PPr:MMs_PPr plants (Table S1), such as benzaldehyde which was only detected in the PP fruit (MMr:PPs_PPs) grafted on the MM root (Fig. 5, A). There are 5 volatiles accumulated and 3 volatiles reduced in the MM fruit on the MMr:PPs_MMr compared to PPr:MMs_MMs plants, such as 1-octen-3-one (Fig. 5, B), which was still affected by the PP rootstock, and its concentration increased in the MM fruit, consistent with the changes observed in the single-head heterologous MM fruit (Table 1). In addition, similar to the single-head grafted fruit, the concentration of 6-methyl-5-hepten-2-ol (Fig. 5, C) in the scion fruit was also significantly decreased in MM but increased in PP fruit and 3-Methy-1-butanol accumulated in both double-head grafted MM and PP fruit after grafting (Fig. 5, D). Moreover, we also observed that 3 volatiles, (*E*)-2-heptenal, hexanal and *β*-ionone, were only increased in the MM fruit grafted on the PP rootstocks. Our observation further confirmed that the rootstock influenced fruit flavors by changing the accumulation of volatiles in the scion fruit.

Notably, some volatiles displayed different changes in the double-head and single-head grafted plants. The concentrations of 10 volatiles whose contents were not affected in single-head plants were affected by double-head grafting. Three of these volatiles, namely, decanal (Fig. 5, E), nonanal (Fig. 5, F) and 6-methyl-5-hepten-2-one (Fig. 5, G), were significantly increased in the PP fruit of MMr:PPs_PPs and PPr:MMs_PPr regardless of the rootstock. Another two volatiles, (*Z*)-citral (Fig. 5, H) and 2-isobutylthiazole (Fig. 5, I), were decreased in the MM fruit and increased in the PP fruit compared with their corresponding autografted plants. 3-Methy-1-butanol accumulated in both double-head grafted MM and PP fruit after grafting (Fig. 5, D). These results suggest that the volatiles in fruit are also affected by the aerial parts of the double-head grafted plants, indicating that the graft method could also influence fruit flavor in tomato.

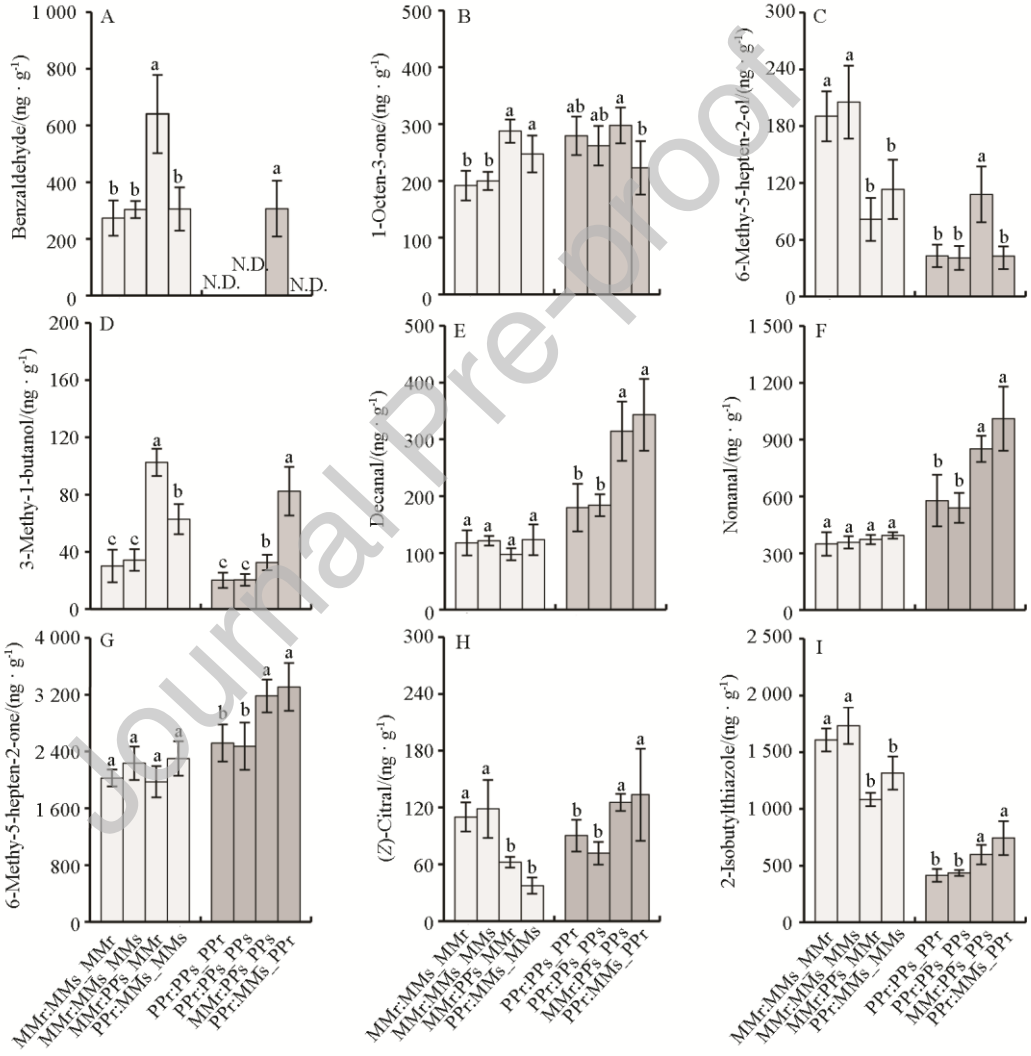


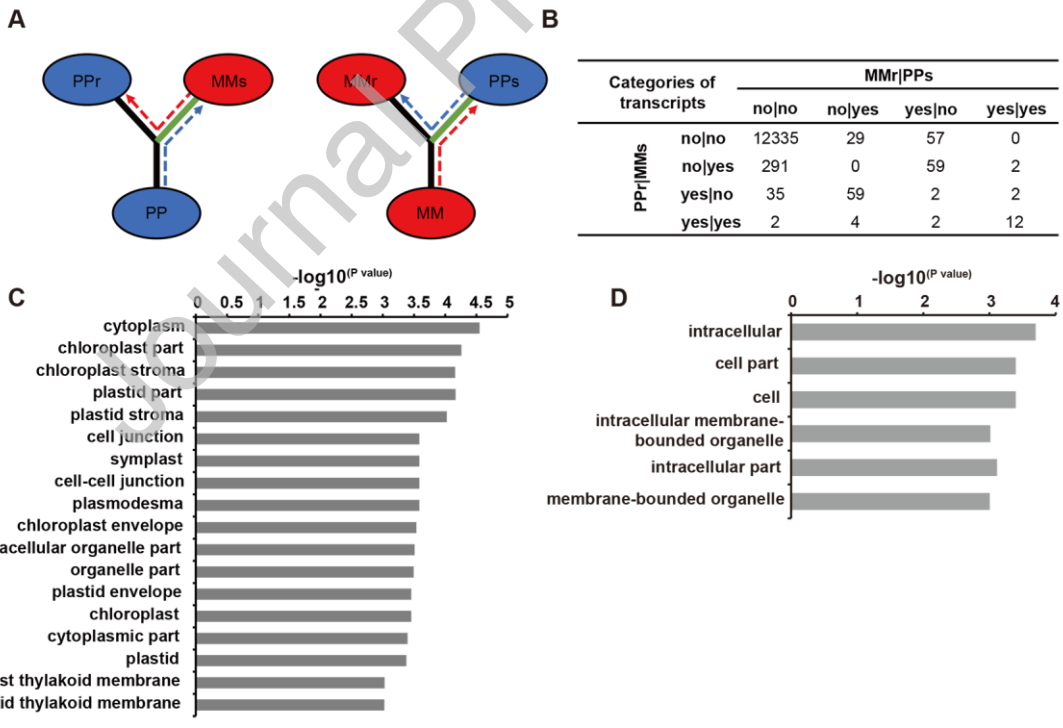
Fig. 5 Concentrations of volatiles in the fruit of the double-head grafted plants

Benzaldehyde (A), 1-octen-3-one (B),6-methyl-5-hepten-2-ol (C), 3-methyl-1-butanol (D), decanal (E), nonanal (F), 6-methyl-5-hepten-2-one (G), (Z)-citral (H), and 2-isobutylthiazole (I) . Turkey's test was used for statistical analyses. The different letters indicate a significant difference between the samples ($P < 0.05$). N.D., not detected. Five biological replicates were used. Error bar, SD.

3.6. Shoot-root interaction is important for mRNA movement

In order to explore the mRNA transportation between the gradfted plants, we detected mobile mRNA in double-head grafted plants through large-scale transcriptome analyses (Table S2). The cultivated MM and wild species PP displayed high frequencies of single nucleotide polymorphisms (SNPs) in the genome, as we described previously (Zhang et al., 2018), which enabled us to identify the heterogeneous mRNA in the grafted plants. We further determined the SNPs that presented the transcripts of PP or MM to identify the mobile mRNA after grafting. A total of 12891 transcripts expressed in leaves differed between PP and MM, including 556 mobile transcripts, which moved upward or downward in the grafted plants (Table S3 and Fig. 6, A).

According to the heterogeneous mRNA, a total of 444 transcripts moved from root to shoot in the grafted plants, including 352 mobile mRNAs that moved from PP rootstock to MM scion and 92 that moved from MM root to PP shoot (Fig. 6, B). Conversely, 218 transcripts moved downward from the leaves of the heterografted plants; specifically, 120 mobile mRNAs moved from PP and 98 moved from MM to the other aerial parts of grafted plants. In all the movement mRNAs, 59 mRNAs were always transported from PP to MM, and 59 mobile transcripts were transported from MM to PP. Twelve transcripts appeared in all MM or PP leaves of every aerial part of the grafted plant, suggesting that these mRNAs were transported along all directions. In addition, no transcripts simultaneously moved from MM and PP rootstocks and aerial leaves (Fig. 6, B and Table S3). All these



Categories of transcripts		MMr|PPs			
		no|no	no|yes	yes|no	yes|yes
PPr|MMs	no|no	12335	29	57	0
	no|yes	291	0	59	2
	yes|no	35	59	2	2
	yes|yes	2	4	2	12

Fig. 6 Analysis of identified mobile transcripts in double-head grafted plants

(A) Schematic representation of mobile transcripts. (B) Number and categories of mobile transcripts in double-head grafted plants. (C) Graphical results for GO terms in cellular processes of the mRNAs moved out from PP to MM (the cluster of 59 mobile mRNAs in (B)). (D) Graphical results for GO terms in cellular

processes of the mRNAs removed from MM to PP (the cluster of 59 mobile mRNAs in (B)). 'no'indicates the mRNAs do not move; 'yes' indicates the mRNAs are movable.

results indicate that transportation of mRNA is related to the plant genotype but is not determined by the rootstock or the scion.

To further evaluate the functions of the mobile annotated transcripts, we performed Gene Ontology (GO) enrichment analysis using the different category mobile mRNAs. Notably, we found that the 59 mobile RNAs moved from PP to MM and were enriched in cellular components related to "chloroplasts" and "cell junctions" (Fig. 6, C). In contrast to these mobile mRNAs, the 59 mRNAs moving from MM were enriched in "intracellular", "cell part" and "cell", although they were also clustered in the cell components (Fig. 6, D). Moreover, for all mobile mRNAs from PP to MM, the most correlated biological processes were "generation of precursor metabolites", "energy response to chemical" and "photosynthesis" (Fig. S2, A); whereas the transported mRNA coming from MM enriched in various metabolic processes, such as "single-organism metabolic process" and "single-organism catabolic process" (Fig. S2, B). Thus, these results suggested that the categorized mobile transcripts conferring relative degrees of function might act as signals to affect plant substance metabolism.

4. Discussion

Grafting has become highly important for intensive vegetable production in recent decades. However, the effects of rootstock on vegetable fruit quality are puzzling because of conflicting observations, which have been attributed to the different combinations of rootstock and scion (Kyriacou et al., 2017). In our work, two edible tomato species, one cultivated tomato and one wild relative, which have different fruit qualities and weights (Zhang et al., 2018), were used to explore the effects of grafting on tomato fruit. The cultivar MM has thicker stems and darker green leaves than PP, implying vigorous growth. We found that the mean fruit weight of PP and MM was significantly reduced in the single-head heterografted plants. Thus, the vigorous roots of MM did not enhance the fruit weight of PP in our work. Different with fruit weight, the concentrations of glucose and fructose in the PP and MM fruit of single-head heterografted plants increased significantly, especially in the heterogeneous PP fruit. So grafting was observed to enhance the sugar concentration of tomato fruit in our work in contrast with previous studies that concluded sugar concentration decreased in many grafted fruit of different tomato root-scion combinations (Gajc-Wolska et al., 2012; Nicoletto et al., 2013b). Interestingly, pronounced sugar enhancement was observed in single-head heterografted PP fruit on MM root that might be caused by the MM vigorous rootstock. One possible reason is that the vigorous root of MM could promote the development of the PP canopy by enhancing assimilate supply in PP leaves (Ghanem et al., 2011b; Gregory et al., 2013). Notably, an increase in MM fruit weight and a decrease in PP fruit weight were observed in the double-head heterogeneous plants regardless of rootstocks, implying potential competition for assimilative products between MM and PP scions in the heterografted plants. Moreover, the increase of sugar in the PP fruit of double-head grafted plants was obviously reduced compared with their increase in single-head grafted fruit, further supporting that the interaction of scions influenced fruit quality by redistribution of assimilative products. Different with the sugar concentrations, the citric and malic acid concentrations in PP scion fruit of double-head grafted plants were dramatically increased, but there were only small changes in the single-head grafted plants,

suggesting that interactions of rootstock and scion influenced fruit quality characteristics through different mechanisms (Albacete et al., 2015).

Odor-active volatiles contribute to tomato flavor, but their concentrations and whether they are influenced by grafting have not been thoroughly elucidated (Goff and Klee, 2006; Kyriacou et al., 2017). One previous study indicated that grafting could induce the enhancement or reduction of several aromas in tomato fruit when the two different cultivars were grafted onto two commercial rootstocks, “Brigeor” and “Maxifort”(Krumbein and Schwarz, 2013). In line with this finding, we also found that different responses of distinct volatiles to grafting and the rootstock will be an important factor to affect the fruit flavor, such as 2-phenylethanol. It was only increased in single-head and double-head scion PP fruit. Different from it, 1-octen-3-one only increased in MM scion fruit. More convincing evidence was obtained for benzaldehyde, which was only detected in the MM pericarps and could be induced in the PP fruit when we used MM as the rootstock but not in the double-head heterografted PP fruit on the PP root. In our work, we also confirmed that the different plant grafting influences fruit flavor. The contents of sugar, acid and some volatiles show different responses to single-head and double-head grafting. However, the various mechanisms by which rootstocks determine volatiles in fruit have not been elucidated (Albacete et al., 2015). Identifying the substrates and genes involved in the volatile biosynthesis pathways may help us to determine the rootstock effects on fruit flavor.

Many studies have detected abundant exchanges of transcripts between roots and scions, and these transcripts play critical roles in influencing scion development and stress responses (McGarry and Kragler, 2013; Thieme et al., 2015; Kehr and Kragler, 2018). We analyzed the mobile mRNA in leaves of the rootstock and scion, which represents the spatial gene function and might coordinate growth and metabolic activity. The GO analysis indicates that some mobile mRNAs are related to metabolite processes, suggesting that they may be involved in the changes in metabolites of the grafted fruit. In a departure from previous works that generally identified the mobile mRNA transported from the rootstock to the grafted aerial part (Thieme et al., 2015), we identified the mobile transcripts that moved downward from the scion leaves in the double-head grafting plants. Interestingly, we found that the mobile mRNAs are determined by the plant genotypes but not by which plant is the rootstock. Fifty-nine mobile PP mRNAs can always be identified from MM, but few common mobile transcripts can be identified from PP or MM at the same time. Thus, similar to metabolites, the plant genotype is important for mRNA movement. Taken together, the results of this study indicate that the genotypes of rootstock and the graft method can affect metabolite accumulation and mRNA movement in heterografted plants, which may influence plant growth and fruit quality. Thus, the selection of a suitable combination of rootstock and scion is important to optimize fruit quality in tomato.

mmc1.pdf

mmc2.pdf

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