

Article

GC-MS Non-Target Metabolomics-Based Analysis of the Volatile Aroma in *Cerasus humilis* After Grafting with Different Rootstocks

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Abstract

C. humilis is a small shrub belonging to the Rosaceae family, and grafting is one of the main ways for propagation. However, the influence of different rootstocks on volatile aroma is still unclear. In this study, an untargeted metabolomics approach based on gas chromatography–mass spectrometry (GC-MS) was utilized to analyze the volatile differential metabolites between the rootstock–scion combinations and self-rooted seedlings. Furthermore, metabolic pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. In total, 191,162 and 150 volatile differential metabolites were identified in different rootstock–scion combinations. The rootstock–scion combinations of ZG/MYT and ZG/BT could improve the volatile aroma in the fruit of *C. humilis* and made significant contributions to the rose and fruity flavors. KEGG pathway analysis indicated that the differential metabolites were mainly enriched in the butanoate metabolism and glycolysis/gluconeogenesis pathways, showing an increasing trend. *Prunus tomentosa* and *Amygdalus communis* can serve as preferred rootstocks for enhancing the aroma quality of *C. humilis* fruits. These results provide new insight into rootstock-based propagation and breeding and also offer some guidance for graft-based fruit production.

Keywords: Chinese dwarf cherry (*Cerasus humilis*); non-targeted metabolomics; aroma compounds; metabolic pathway



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1. Introduction

Aroma, as a key factor in fruit processing and fresh consumption, not only reflects the flavor characteristics and maturity of the fruits but also serves as a critical indicator for evaluating their commercial quality [1]. As consumers' demands for fruit quality continue to rise, research on aroma quality has also attracted growing attention. Chinese dwarf cherry (*C. humilis*), a deciduous shrub in the genus *Prunus* (*Cerasus*) of the *Rosaceae* family, is one of the unique wild fruit trees in China [2]. The fruit of *C. humilis* is shaped like a cherry, with a sweet and sour taste and a unique flavor [3–5]. In recent years, *C. humilis* fruits have been increasingly developed [3,4]. However, the root nodule disease in *C. humilis* also adversely impacts fruit quality to varying extents [6]. Grafting, a common method in

horticultural propagation and breeding, can mitigate the above problems. Xu et al. found that grafting *C. humilis* onto different rootstocks can mitigate root cancer in *C. humilis* and improve its fruit quality [6], but its impact on fruit aroma remains unclear. Some research has proved that using 110R, 1103P, and SO4 as rootstocks for the cultivar ‘Ruiduxiangyu’ of grape has been shown to improve its aroma profile [7]. Han et al. noted that different rootstocks have a more pronounced effect on fruit aroma. Specifically, the ‘140R’ rootstock promotes the synthesis of ester compounds in ‘Dana’ grape fruits [8]. Additionally, different rootstock–scion combinations affect both fruit quality and aroma components in apples [9].

Metabolomics is one of the four “omics” technologies. It is a systematic technical approach to studying metabolite concentrations and interactions in organisms [10]. Metabolomics is one of the emerging fields that has rapidly gained popularity and has been widely adopted across various scientific disciplines. The advantages of untargeted metabolomics include its comprehensive and unbiased nature, as well as its high throughput capability. Currently, several researchers have employed metabolomics-based detection and analytical approaches to elucidate the primary metabolic pathways involved in the synthesis of various volatile compounds in *C. humilis* fruits across different maturity stages [11]. However, the influence of different rootstocks on the volatile metabolites and aroma characteristics of the fruits of *C. humilis*, as revealed by non-targeted metabolomics, remains unclear.

Therefore, in this study, an untargeted metabolomics approach based on gas chromatography–mass spectrometry (GC-MS) was utilized to analyze the volatile differential metabolites between the rootstock–scion combinations and self-rooted seedlings. Furthermore, metabolic pathway enrichment analysis was performed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to elucidate the effects of different rootstocks on the volatile aroma components of *C. humilis*. Rootstocks that promote the accumulation of aroma compounds were selected to provide theoretical references and data support for the cultivation and fruit utilization of *C. humilis*.

2. Materials and Methods

2.1. Plant Materials

The experiment was conducted in the Yinchuan Botanical Garden in Ningxia (38°25′6″ N, 106°10′9″ E) at an altitude of 1089 m. The rootstocks used in this experiment were *Armeniaca sibirica* (L.) Lam, *Cerasus tomentosa* (Thunb.) Wall, and the long-stalked almond *Amygdalus communis* Pall. The rootstocks were all one-year-old seedlings. The scions for grafting and those from cuttings of self-rooted *C. humilis* ‘Nongda 4’ seedlings were collected from healthy 2–3-year-old mother plants in the same cutting nursery. All the above materials were provided by the State Key Laboratory of Efficient Production of Forest Resources. The specific experimental varieties are as follows (Table 1):

Table 1. Test variety and code name.

Number	Combinations	Code Name
1	<i>Cerasus humilis</i> Nongda 4/ <i>Amygdalus communis</i>	ZG/BT
2	<i>Cerasus humilis</i> Nongda 4/ <i>Cerasus tomentosa</i>	ZG/MYT
3	<i>Cerasus humilis</i> Nongda 4/ <i>Armeniaca sibirica</i>	ZG/SX
4	<i>Cerasus humilis</i> Nongda 4	ZG

2.2. Cultivation and Sampling

All rootstocks and self-rooted seedlings of *C. humilis* propagated by cutting nursery that year were transplanted in the greenhouse on 2–3 March 2017. The planting spacing was 0.5 m × 1 m with north–south row orientation. Bud grafting was performed on 25–26 June 2017. Three replicates were set for grafted seedlings from different rootstocks, with 50 grafted seedlings in each replicate. Take the self-rooted cutting nursery at the same time as the control. After transplantation, the seedlings were subjected to normal water and fertilizer management as well as pest and disease control. The pruning of the transplanted seedlings was intensified, with five fruiting branches retained per tree. In September 2022, fruit samples were collected for each rootstock combination, and five trees at the same position were selected for each repeat. For each tree, 200 g of fruit was collected from the top, middle, and bottom of the fruit cluster, and each combination was repeated three times. After the seeds were removed, they were immediately placed in liquid nitrogen for rapid freezing. The mixed samples were ground into powder using an automatic tissue grinder (Co. KG, Haan, Germany) and then stored in a refrigerator at −80 °C (Figure 1).



Figure 1. The growth of seedlings and fruits of *C. humilis* grafted on different rootstocks: (A) the first year after grafting; (B) the second year after grafting; (C) the rootstock of *C. humilis*; (D) ZG/MYT; (E) ZG/BT; (F) ZG/SX; (G) ZG.

2.3. Chemical Reagents

Regarding chemical reagents, NaCl (Beijing Chemical Works, Beijing, China), and 2-octanol (purity ≥ 99.5%, internal standard) was obtained from TCI Corporation (St Louis, MO, USA).

2.4. Headspace Solid-Phase Microextraction (HS-SPME)

The powder samples of fruit (2 g) were placed in 20 mL headspace containers with an internal standard of 10 µL 2-octanol (10 mg·L^{−1}). The headspace solid-phase microextraction (HS-SPME) (50/30 µm DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) was used to extract volatile compounds. The HS vial was sealed and left in the water bath (60 °C for 30 min) for equilibration. The aged extraction head (250 °C for 5 min) was thermostatically adsorbed at 60 °C for 40 min. During this time, the samples were stirred at a constant speed (300 rpm). The extraction head was immediately desorbed at the injection port for 3 min at 270 °C following adsorption.

2.5. Determination of Volatile Compounds Using Gas Chromatography–Mass Spectrometry (GC–MS)

The volatile compounds were determined using an Agilent 7890-5977B GC–MS system (Agilent Inc., Santa Clara, CA, USA) with a PAL automatic sample injection system, an automatic mass spectrometry deconvolution identification system, and NIST 11 mass spectrometry library. The analysis was performed using a DB-Wax capillary column (30 m × 250 µm × 0.25 µm, Agilent Inc., Santa Clara, CA, USA) at a flow rate of 3 mL min^{−1} (helium, 99.99%). The program began at 40 °C for 4 min, then ramped up to 245 °C at a pace of 5 °C min^{−1}, followed by a 10 °C·min^{−1} increase to 250 °C and was then held for 10 min. The MS was operated in an electron impact ionization mode at 70 eV. The mass spectrometry data were acquired in scan mode with the *m/z* range of 20–400, solvent delay of 0 min.

Chroma TOF 4.3X (LECO, VT, USA) and the NIST 11 database were used for raw peak extraction; the data underwent baseline filtering and calibration, peak alignment, deconvolution analysis, peak identification, integration, and spectrum match of the peak area, and qualitative analysis was conducted. The VOCs were analyzed semi-quantitatively based on peak areas and internal standards [12].

2.6. Data Preprocessing and Annotation

Data processing was performed using Microsoft Excel 2021. One-way ANOVA was performed using SPSS 25 (IBM, New York, NY, USA). PCA and O2PLS-DA were performed using Simca 14.1 (Umetrics, Umea, Sweden) software, and figures were generated with Origin 2024 (OriginLab, Northampton, MA, USA). The variable importance in projection (VIP) values were obtained using OPLS-DA.

3. Results

3.1. Characteristics of Volatile Compounds in Different Stock and Spike Combinations

A total of 275 volatile compounds were identified in three rootstock–scion combinations and self-rooted *C. humilis* seedlings. Based on structural characteristics, all the volatile compounds were divided into the following categories: terpenoids, esters, alkanes, aldehydes, alcohols, aromatic hydrocarbons, acids, heterocyclic compounds, nitriles, ethers, and ketones (Figure 2). Among these, esters were the most abundant, comprising 119 compounds, which accounted for 43.3% of the total identified volatiles. This was followed by aldehydes (34 compounds, 12.4%) and alcohols (33 compounds, 12%). Additionally, alkanes, ketones, terpenoids, acids, and ethers represented 7.3%, 6.9%, 5.0%, 4.7%, and 5.1% of the total compounds, respectively. In contrast, the remaining categories were detected in much lower quantities: aromatic hydrocarbons (only one compound), heterocyclic compounds (six compounds), and nitriles (two compounds).

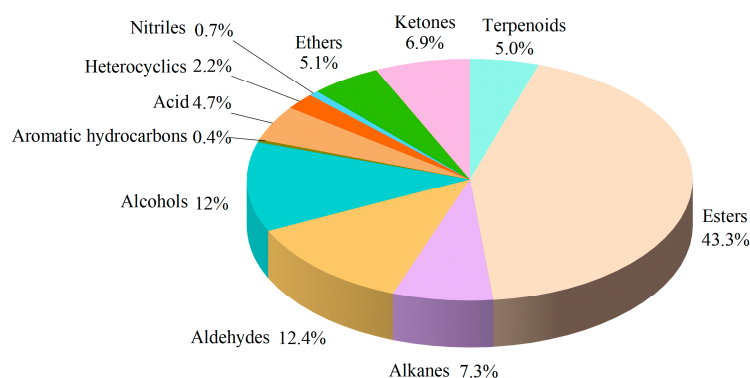


Figure 2. Classification and proportion of volatile compounds in the fruit of *C. humilis*.

The cumulative content of different volatile compound classes showed that esters were the most abundant in all combinations, representing 64.79–65.40% of the total content (Figure 3A). Aldehydes and alcohols followed, comprising 11.61–14.98% and 8.66–10.20% of the total, respectively. As shown in Figure 3B, except for heterocyclic, nitriles, and ethers, the contents of various volatile compounds in the rootstock–scion combination ZG/MYT were significantly higher than ZG/BT, ZG/SX, and ZG. Specifically, in the rootstock–scion combination ZG/MYT, the content of terpenoids was $47.06 \mu\text{g}\cdot\text{kg}^{-1}$, the content of esters was $723.6 \mu\text{g}\cdot\text{kg}^{-1}$, the content of alkanes was $3.340 \mu\text{g}\cdot\text{kg}^{-1}$, the content of alcohols was $114.0 \mu\text{g}\cdot\text{kg}^{-1}$, the content of aromatic hydrocarbons was $0.07 \mu\text{g}\cdot\text{kg}^{-1}$, the content of acids was $31.77 \mu\text{g}\cdot\text{kg}^{-1}$, and the content of ketones was $15.14 \mu\text{g}\cdot\text{kg}^{-1}$. In contrast, self-rooted seedlings (ZG) had lower contents of all compounds, with significant reductions ($p < 0.05$) observed in esters, alcohols, acids, heterocyclic compounds, and nitriles. Furthermore, the total volatile content varied significantly among the three rootstock–scion combinations and the self-rooted seedlings. Compared with the self-rooted seedlings (ZG), the contents of volatile compounds in rootstock–scion combination ZG/BT, ZG/MYT, and ZG/SX were all increased. Notably, the total aroma compound levels in ZG/MYT, ZG/BT, and ZG/SX surged by 248.2%, 184.2%, and 145.1%, respectively, relative to self-rooted seedlings. These findings demonstrate that grafting *C. humilis* onto these rootstocks significantly promotes the accumulation of volatile aroma compounds in fruits.

3.2. Characteristic Volatile Compounds of Different Rootstock–Scion Combinations

To investigate the characteristic volatile metabolites of different rootstock–scion combinations of *C. humilis*, two-way orthogonal partial least squares with discriminant analysis (OPLS-DA) was performed on the data that contained the volatile compound metabolites from the three rootstock–scion combinations (Table 2). Characteristic metabolites can be screened out for all three rootstocks. The results from the principal component analysis (PCA) (Figure S1) and hierarchical clustering analysis (HCA) revealed important distinctions between ZG and ZG/MYT, ZG/BT, and ZG/SX (Figure 4A–F). As shown in Table 2, the model's $R^2(X) = 0.761\text{--}0.81$, $R^2(Y) = 0.990\text{--}1.0$, and $Q^2 = 0.69\text{--}0.986$, indicating robust stability and predictive ability. To ensure model reliability, 200 permutation tests were conducted to prevent overfitting. Additionally, volcano plots were employed to visualize metabolic differences between groups, using screening thresholds of $p < 0.05$ and $|\log_2\text{FC}| > 1$.

Table 2. Validation of OPLS-DA models on volatile compounds for the comparison between each grafted and *C. humilis*.

OPLS-DA Model	Component	R2X (cum)	R2Y (cum)
ZG vs. ZG/BT	1 + 1 + 0	0.81	1
ZG vs. ZG/MYT	1 + 1 + 0	0.761	0.998
ZG vs. ZG/SX	1 + 1 + 0	0.8	1

Compared with ZG, ZG/MYT had 186 upregulated and 5 downregulated metabolites (Figure 4A). Applying screening criteria of $\text{VIP} > 1$ and $p < 0.05$, we identified 15 key differentially expressed volatile compounds, which are visualized in a heatmap (Figure 4B). The berries in ZG/MYT were characterized by more abundant compounds than ZG: propyl octanoate; hexanal; 4-hexen-1-ol, (4E), acetate; 1,5-hexanediol; hexanoic acid, butyl ester; fumaric acid, decyl 3-methylbut-3-enyl ester; isopentyl hexanoate; butyl caprylate; hexadecanoic acid, 1,1-dimethylethyl ester; and 3-hexen-1-ol, acetate, (Z)- were the main differentiating metabolites.

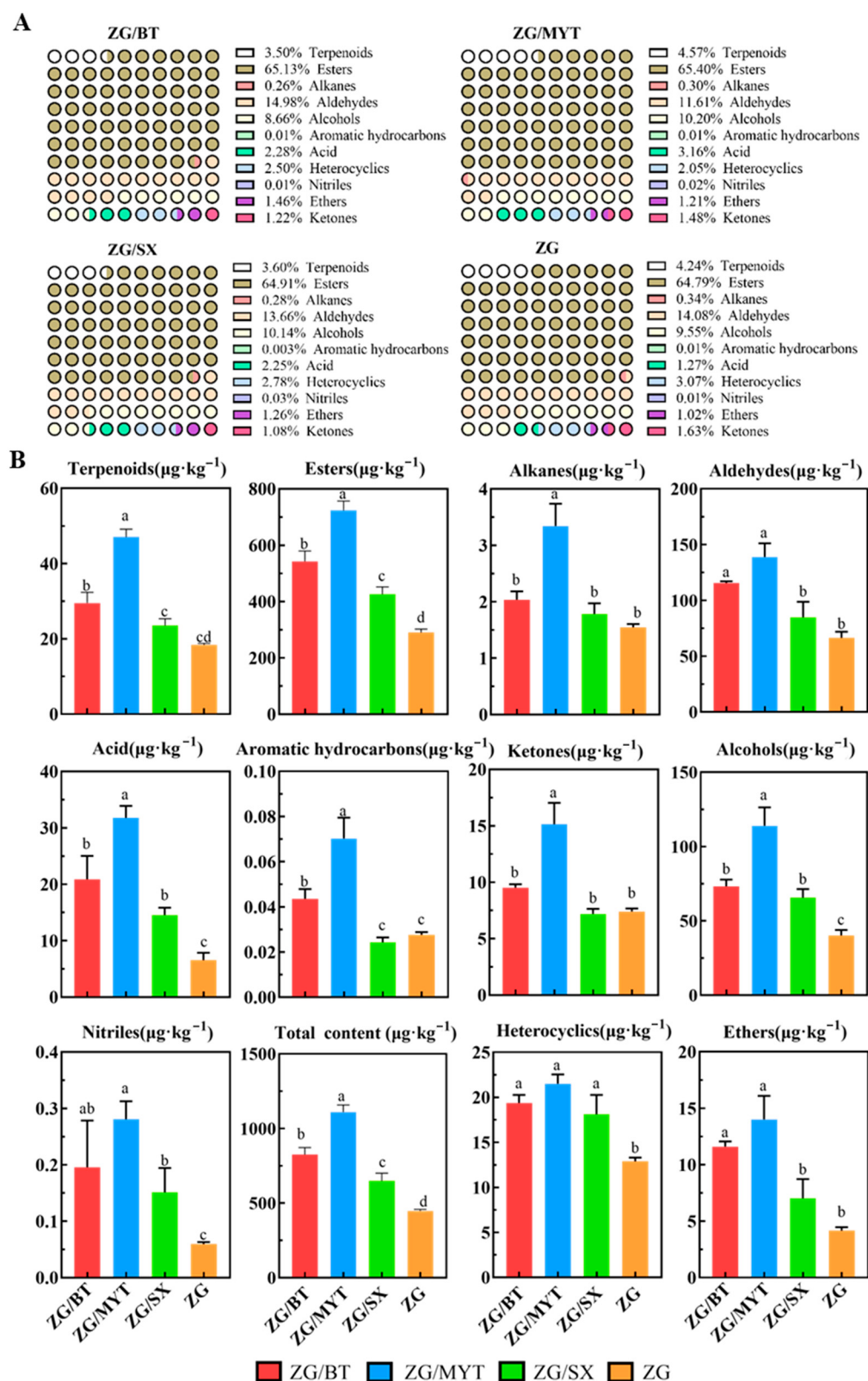


Figure 3. The differences in the content of volatile compounds in the fruits of self-rooted *C. humilis* seedlings and different combinations of rootstock: (A) the proportion of aroma compounds' content; (B) the differences in the content of various volatile compounds. Lowercase letters are used to identify whether there are significant differences in the mean values between different treatments.

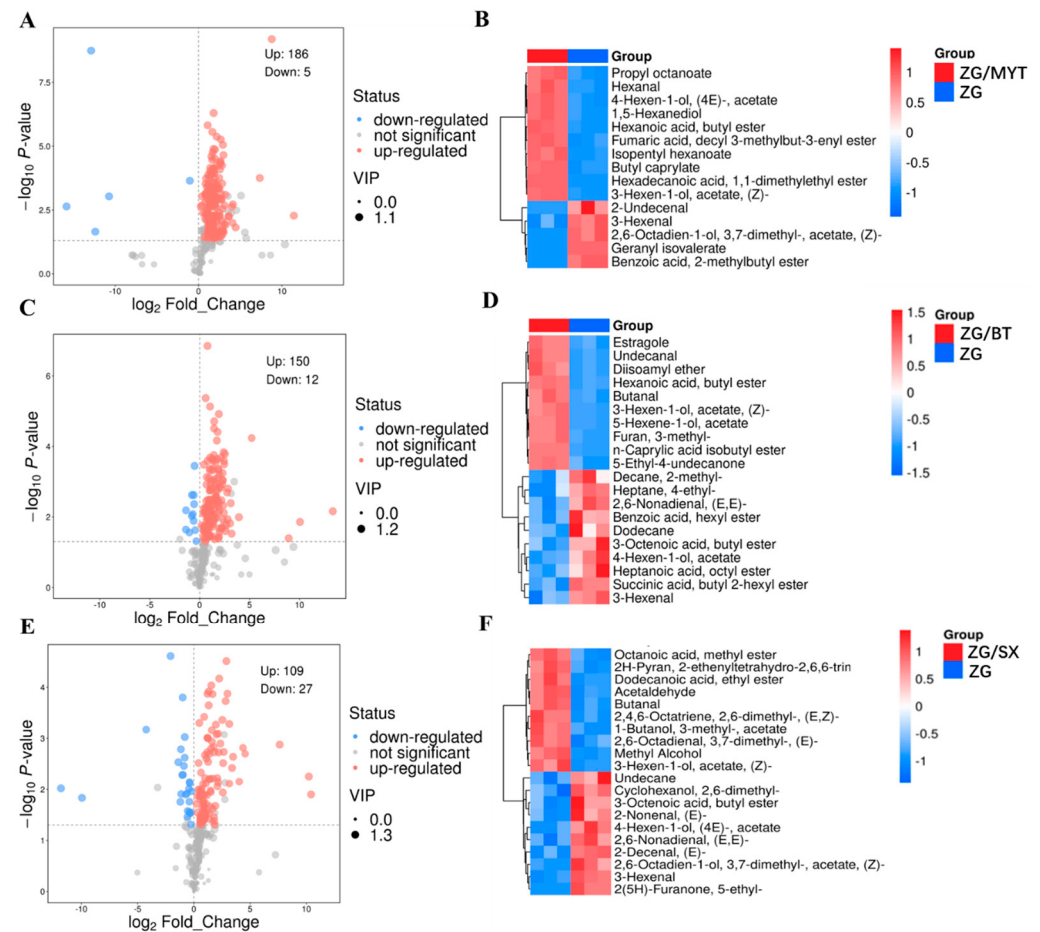


Figure 4. Analysis of differential compounds in self-rooted *C. humilis* seedlings and different combinations of rootstock: (A) volcano plot analysis of volatile compounds between ZG and ZG/MYT; (B) heatmap between ZG and ZG/MYT; (C) volcano plot between ZG and ZG/BT; (D) heatmap between ZG and ZG/BT; (E) volcano plot between ZG and ZG/SX; (F) heatmap between ZG and ZG/SX.

Compared with ZG, ZG/BT had 150 upregulated metabolites and 12 downregulated metabolites (Figure 4C). The OPLS-DA results showed significant separation between ZG and ZG/BT, indicating significant changes in VOCs in ZG/BT. Estragole; undecanal; diisoamyl ether; hexanoic acid, butyl ester; butanal; 3-hexen-1-ol, acetate, (z)-; 5-hexene-1-ol, acetate; furan,3-methyl; n-caprylic acid isobutyl ester; and 5-ethyl-4-undecanone were more abundant in ZG/BT than those grown in ZG (Figure 4D).

Compared with ZG, ZG/SX had 109 upregulated and 27 downregulated metabolites (Figure 4E). The grafted combination was characterized by higher levels of methyl octanoate, 2-ethenyltetrahydro-2,6,6-trimethyl-2H-pyran, ethyl dodecanoate, acetaldehyde, butanal, (E,Z)-2,6-dimethyl-2,4,6-octatriene, 3-methylbutyl acetate, (E)-3,7-dimethyl-2,6-octadienal, methanol, and (Z)-3-hexen-1-yl acetate (Figure 4F).

The results demonstrated that rootstock–scion combination (ZG/MYT) exhibited the strongest aroma enhancement effect, and it upregulated 186 metabolites (e.g., propyl octanoate, hexanal, etc.) that significantly enhance the floral and fruity characteristics. ZG/BT and ZG/SX upregulated 150 and 109 compounds, respectively. The compound 3-hexen-1-ol, acetate, (Z)- displayed consistent upregulation across all graft combinations, while 3-hexenal showed persistent downregulation. These findings clearly indicate that grafting with different rootstocks induces significant alterations in the volatile organic compound (VOC) profiles of *C. humilis*.

3.3. Analysis of Aromatic Series

The odor threshold refers to the lowest concentration of specific VOCs that can be perceived by humans [13]. It is also a routine indicator for evaluating the contribution of volatile components in fruits to aroma [7]. Typically, VOCs with an odor activity value (OAV) ≥ 1 are deemed critical [14]. The larger the value is, the greater the contribution. By referring to the aroma characteristics of volatile components, the contributions of compounds to fruit aroma can be evaluated by the aromatic series. Based on the threshold value, a total of 22 volatile metabolites were greater than the threshold (OAV > 1) in the fruit aroma compounds of the three rootstocks and self-rooted seedlings (Table 3). They were characterized by nine primary series: fruity, floral, herbaceous, sweet, mushroom, aldehyde, citrus, balsamic, and woody. Notable examples include linalool, hexyl acetate, ethyl octanoate, 1-octen-3-one, and (4E)-4-hexen-1-ol acetate, which possess high OAVs and contribute to fruity, sweet, rose-like, herbaceous, and mushroom aromas.

Table 3. Odor thresholds and aromatic description of volatile compounds.

Taste Component	Thresholds ($\mu\text{g}\cdot\text{kg}^{-1}$)	Odor Description	OAVs				Aroma Series
			ZG/BT	ZG/MYT	ZG/SX	ZG	
Butanoic acid, butyl ester	10	Banana, strawberry, apple, grape	1.13	2.19	0.94	0.84	1
Hexanoic acid, ethyl ester	0.01	Pineapple	71.01	127.25	80.39	18.00	1
Acetic acid, hexyl ester	2	Pears, berries	62.06	77.06	48.61	34.53	1
Octanoic acid, ethyl ester	0.58	Sweet, pineapple	20.36	46.31	23.23	3.70	1
Octanal	0.7	Fruit	4.35	11.31	4.38	4.71	1
2-Vinylethyl acetate	1	Pears, apples	10.37	17.44	7.47	4.95	1
Hexanal	4.5	Green leaves	3.56	5.48	2.46	1.70	2
3-Hexenal	0.25	Green flavor	10.51	7.67	3.76	15.82	2
2-Hexenal, (E)-	17	Grassy green	0.95	1.34	0.63	0.67	2
3-Hexen-1-ol, acetate, (Z)-	1	Grassy green	6.64	8.50	6.05	2.43	2
2,4-Hexadienal, (E,E)-	0.05	Grassy green	8.79	11.32	6.47	4.04	2
Linalool	0.22	Rose, fresh floral-woody	80.95	162.21	69.46	60.24	3
trans-Calamenene	0.005	Piney, woody	0.91	0.00	0.79	1.27	4
2-Nonenal, (E)-	0.08	Cucumber, green leaves	2.54	3.88	1.74	3.22	5
Estragole	10	Fennel, sweet	1.03	1.15	0.59	0.30	5
1-Octen-3-one	0.001	Mushroom-like	37.93	79.43	38.49	32.05	6
trans-3-Nonen-2-one	0.25	Mushroom-like	0.93	2.07	1.09	0.68	6
1-Heptanol, 4-methyl-	1	Fatty, cheese-like	7.26	8.72	4.54	3.65	9
1-Butanol, 3-methyl-, acetate	2	Sweet, banana	9.34	8.18	5.95	1.89	1,5
4-Hexen-1-ol, acetate	1	Grassy green, fruity	15.41	40.62	26.55	22.65	1,2
Decanal	0.1	Sweet, citrus, dairy	0.91	2.15	1.08	1.27	5,8,9
Nonanal	1	Aldehyde, citrus note, dairy	16.78	30.05	13.32	9.32	7,8,9

Note: Odor threshold, odor description, odor series [15–36]. Odor series: 1. fruity; 2. herbaceous; 3. floral; 4. woody; 5. sweet; 6. mushroom; 7. aldehyde; 8. citrus; 9. balsamic.

As shown in Figure 5A, the aroma profiles of grafted and self-rooted seedlings are similar, mainly comprising fruity, floral, herbaceous, mushroom, and other aroma categories. The rootstock combination ZG/MYT significantly suppressed woody aroma flavors, while the other eight aroma series were markedly higher than those in ZG and the other two rootstock combinations. In the rootstock combination ZG/MYT, linalool and hexanoic acid, ethyl ester were the main compounds attributed to the floral and fruity flavors. The aroma values were as high as 162.21 and 127.51, respectively, which were 2.7 times and 7.1 times that of the self-rooted seedlings. However, both ZG/BT and ZG/SX rootstock combinations significantly enhanced the fruity, floral, and mushroom flavor of *C. humilis*.

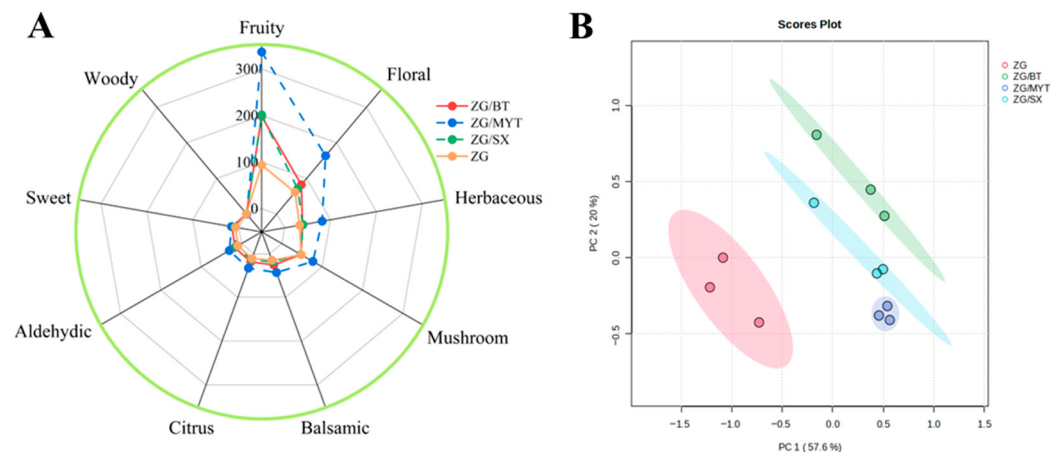


Figure 5. Aromatic series values in different scion–rootstock combinations: (A) the aroma profile of aromatic compounds; (B) aromatic series values PCA.

Compared with self-rooted seedlings, the aroma values of compound 3-hexenal exhibited a significant decreasing trend in the three rootstock combinations, which was consistent with the previous analysis of the differences in aroma compounds. This observed reduction may reflect grafting-induced modulation of key metabolic pathways and regulatory genes governing aroma biosynthesis in *C. humilis* fruits.

3.4. KEGG Classification and Enrichment Analysis of Differential Metabolites

Metabolic pathway analysis was performed using the KEGG database (<https://www.kegg.jp/kegg>) [37], with differential metabolites categorized according to KEGG pathway classification (Figure 6A). Compared with *C. humilis* self-rooted seedlings, differential metabolites from the three rootstock groups commonly annotated to 15 metabolic pathways: Butanoate metabolism (33.33%, the most abundant pathway) played a key role in fruit ripening, flavor formation, and postharvest storage [38]; glycolysis/gluconeogenesis (25%) served as the core energy metabolism process, synergizing with butanoate metabolism during fruit development [39]; pyruvate metabolism (20.45%) served as the critical junction connecting glycolysis and the TCA cycle [40], while phenylalanine metabolism (8.83%) was involved in amino acid metabolism [41]. Other pathways (carbon metabolism, propanoate metabolism, sulfur metabolism, etc.) accounted for 8.33–16.66%, encompassing energy, lipid, terpene, and polyketone metabolism [42–48].

Additionally, KEGG enrichment analysis was conducted on the differential metabolites of grafted *C. humilis* fruit samples from the different rootstocks (Figure 6B). The color of the dots represents the significance of *p* value, where redder dots indicate more significant enrichment. The size of the dots reflects the number of enriched differential metabolites [35]. The analysis revealed that 26 differential metabolites were enriched among the three rootstock combinations and *C. humilis*, including butanoate metabolism, glycolysis/gluconeogenesis, pyruvate metabolism, phenylalanine metabolism, etc. Notably, butanoate metabolism showed the highest enrichment degree, containing the majority of differentially accumulated metabolites. Importantly, all 15 identified pathways exhibited overall upregulation patterns in grafted plants compared to self-rooted controls.

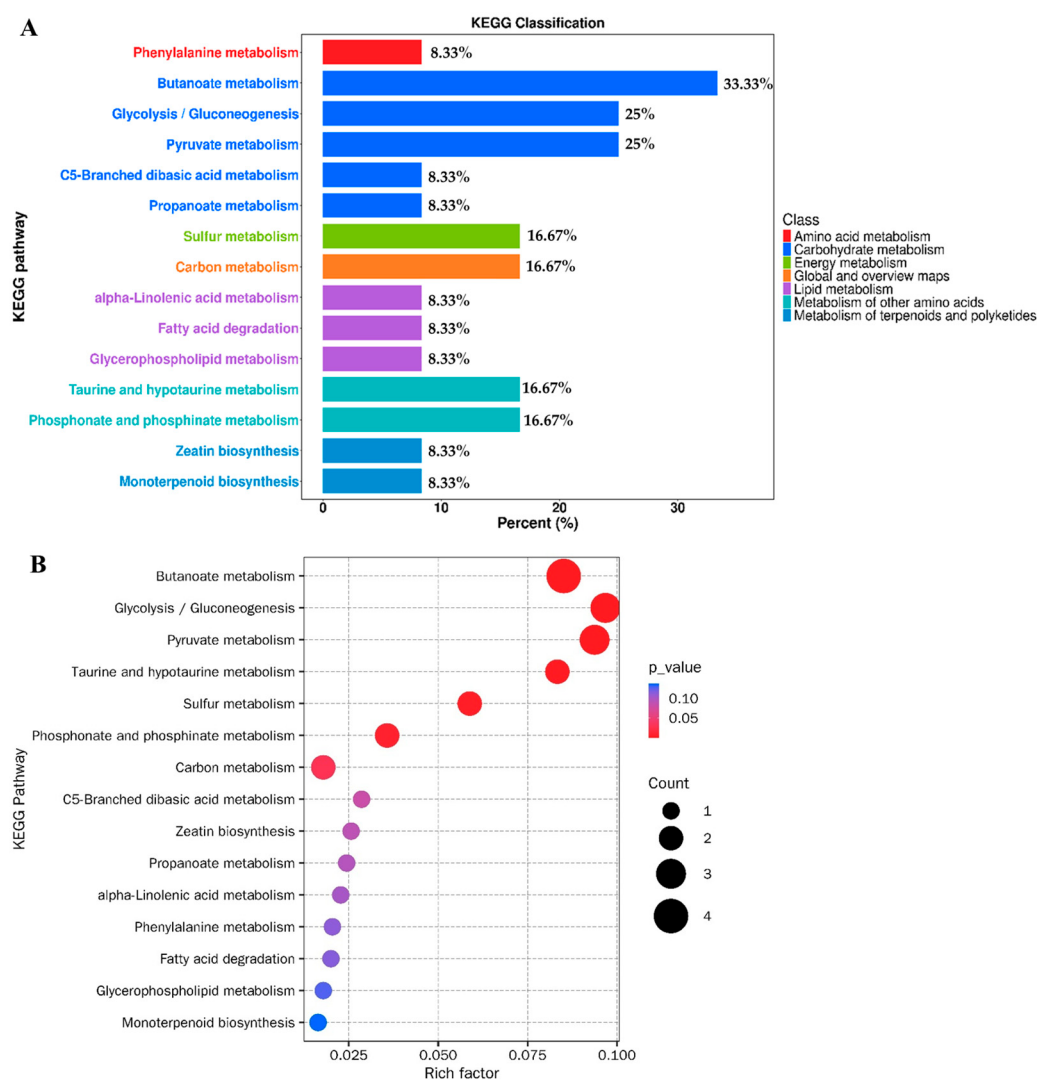


Figure 6. KEGG classification and enrichment analysis of differential metabolites: (A) KEGG classification of differential metabolites; (B) bubble diagram of the metabolic pathway enrichment.

4. Discussion

4.1. Regulatory Effect of Rootstocks on the Composition of Volatile Compounds in *C. humilis* Fruits

C. humilis is a small shrub belonging to the *Prunus* (*Cerasus*) of the Rosaceae family. It is an ancient tree species unique to China and widely distributed in northern China [49]. This economically important species produces fruits renowned for their distinctive flavor profile, nutritional value, and diverse volatile organic compounds (VOCs) [50,51]. Currently, there are no reports on the influence of rootstock grafting on the aroma of *C. humilis* fruits. However, there are quite a few research reports on the aroma quality of other fruits grafted onto rootstocks. Studies on apples [52], kiwis [53], sweet cherry [54], and grapes have shown that grafting on different rootstocks can all affect the aroma compounds and flavors of the fruits. Wang et al. [55] studied the effects of different rootstocks on the aroma components of the wine grape ‘Cabernet Sauvignon’. The results showed that the rootstocks 110R, Riparia Gloire, and SO4 reduced the content of ester compounds, while 101-14, Ganzin 1, 110R, and 5BB increased the content of isoprene compounds. Sun Lei et al. demonstrated that three rootstocks, 1103P, 110R, and SO4, could significantly enhance the rose aroma of grapes [7]. Niu S.K. et al. [56] reported that Cabernet Sauvignon grafted on rootstocks 1616C, SO4, 125AA, Cosmo2, and 5BB had a higher quality and a higher content of aromatic substances, while Cabernet Sauvignon grafted on rootstocks

5A, Freedom, 1447P, 5C, 99R, and 1103P had a lower quality and a lower content of aromatic substances. This study reveals that grafting on different rootstocks significantly changed the composition of volatile compounds in *C. humilis* fruits. The ZG/MYT and ZG/BT combinations showed particularly pronounced increases in ester content ($p < 0.05$), consistent with patterns observed in other fruit species. These results demonstrate that rootstock selection can effectively enhance aromatic compound accumulation in *C. humilis*.

Ester compounds are mainly formed by alcohols produced through fatty acid and amino acid metabolic pathways under the action of alcohol acyltransferases (AATs) [57]. Our findings demonstrate that rootstock grafting significantly influences this process, likely through modulation of fatty acid metabolic pathways [58]. In this study, the aroma value of ethyl hexanoate in the ZG/MYT combination was 7.1 times higher than that of self-rooted seedlings. As a characteristic compound of the pineapple-flavored type, it directly enhances the sweet and refreshing aroma of the fruit [59].

The synthesis of monoterpene volatile compounds mainly relies on the MEP pathway, which has a low aroma threshold and high aromatic contribution, with linalool and limonene being important sources of the characteristic aroma of the fruit [60,61]. Terpenoids such as linalool and limonene may be associated with the activation of secondary metabolism induced by rootstocks. In this study, the linalool content in the ZG/MYT and ZG/BT combinations was higher than that of self-rooted seedlings. These aroma metabolites endow the fruit with a rose fragrance and a fresh herbal aroma [19]. This might be linked to the upward transport of signal molecules such as hormones or secondary metabolites secreted by the rootstock's root system, activating the expression of key genes for terpene synthesis in scion fruits to promote the synthesis of terpene compounds [62,63]. Interestingly, the apricot rootstock ZG/SX showed a weaker aroma enhancement effect, which may reflect differences in root physiological activity, metabolic regulation capacity [64], or other unidentified factors. Therefore, further multi-perspective verification is required in future research.

4.2. Differences in Key Aroma Compounds and Their Flavor Contributions

Based on the analysis of aroma value (OAV), 22 key volatile compounds were identified that play a key role in the flavor of *C. humilis* in this study, among which linalool, ethyl hexanoate, and 1-octen-3-one had OAVs > 100 , serving as core components for the fruit's floral, fruity, and mushroom aromas. Comparative analysis revealed that the ZG/MYT and ZG/BT combinations markedly enhanced the "fruity-floral" flavor profile relative to self-rooted seedlings, while ZG/SX demonstrated greater efficacy in enhancing "herbaceous" characteristics. This difference may be related to the preference of metabolic pathways regulated by rootstocks; for instance, the rootstock of *Prunus chinensis* significantly promotes fatty acid β -oxidation and alcohol acyltransferase (AAT) activity, thereby accelerating the synthesis of esters [65]. Conversely, the rootstock of Hyacinth may activate the methyl valeric acid (MVA) pathway to promote terpene precursor IPP/DMAPP accumulation [66].

Furthermore, green leaf volatile compounds (e.g., 3-hexenal) were consistently down-regulated in the grafted combinations, suggesting rootstock-mediated inhibition of the lipoxygenase (LOX) pathway. The LOX pathway is a key pathway for the formation of green leaf odor in plants, and its reduced activity decreases fruit raw green notes while enhancing mature fruit aromas [67]. This discovery provides a new perspective for improving the flavor and texture of fruits via rootstock selection.

4.3. The Regulatory Mechanism of Metabolic Pathways on the Formation of Aroma Induced by Rootstocks

KEGG analysis indicates that butanoate metabolism and glycolysis/gluconeogenesis serve as central metabolic pathways mediating rootstock-induced differential metabo-

lite accumulation in *C. humilis* fruits. As a branch of fatty acid degradation, butanoate metabolism generates acetyl-CoA, which directly supplies precursors for ester and terpenoid synthesis [68]. In the combinations of ZG/MYT and ZG/BT, the activities of enzymes related to butanoate metabolism (e.g., butyrate CoA transferase) may be induced and upregulated by the rootstock, promoting derivatization of fatty acids like caproic and caprylic acids [69]. Concurrently, the enhancement of the glycolytic pathway provides more energy (ATP) and carbon skeleton (pyruvate) for fruit metabolism. Pyruvate is converted to acetyl-CoA via the pyruvate dehydrogenase complex, further driving aroma compound synthesis [70]. Notably, the phenylalanine metabolic pathway was also activated in the grafted combinations [71], with its characteristic products (phenylacetaldehyde and phenylethanol) making notable contributions to sweet and floral aroma notes [72]. These findings collectively suggest that rootstocks may synergistically affect the composition of aroma substances by regulating the cross-network of amino acid metabolism and secondary metabolism [73]. Future studies should integrate transcriptomic and metabolomic analyses to explore the relationship between key enzyme gene expressions (e.g., AAT, TPS, etc.) and aroma phenotypes.

5. Conclusions

The results showed that compared with self-rooted seedlings, the total aroma compounds of different rootstock–scion combinations in the *C. humilis* fruit significantly increased, with esters and terpenoids, in particular, showing the most notable enhancement. The rootstock–scion combinations ZG/MYT and ZG/BT significantly enhanced the floral aroma of *C. humilis*. Butanoate metabolism and glycolysis/gluconeogenesis likely serve as the key pathways underlying rootstock-induced aroma differences: the former serves as a carbon precursor source for aroma synthesis, while glycolysis/gluconeogenesis regulates metabolic flux via energy metabolism. Further research combining transcriptomic analysis is needed to better understand the regulatory mechanisms of these metabolic pathways.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11080972/s1>, Figure S1: Analysis of differential compounds self-rooted *Cerasus humilis* seedlings and different combinations of rootstock: (A) principal component analysis (PCA) of volatile compounds between ZG and ZG/MYT; (B) volatile compounds between ZG and ZG/SX; (C) volatile compounds between ZG and ZG/BT. Table S1: Analysis of variance for all compounds; Table S2: Statistical table of upward and downward adjustment of differential compounds; Table S3: Differential compounds between ZG/BT and ZG; Table S4: Differential compounds between ZG/MYT and ZG; Table S5: Differential compounds between ZG/SX and ZG; Table S6: KEGG pathway; Table S7: PCA analysis.

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Abbreviations

VOCs	Volatile organic compounds
OAV	Odor activity value
AAT	Amino acid transporter
MEP	2-C-methyl-D-erythritol 4-phosphate pathway
MVA	Mevalonate pathway
IPP	Isopentenyl pyrophosphate
DMAPP	Dimethylallyl pyrophosphate
LOX	Lipoxygenase
ATP	Adenosine triphosphate
TPS	Terpene synthase

References

- Hua, Q.Y.; Wei, T.; Han, W.; Wu, G.H.; Liu, Q. Progress in the study of composition, volatile flavor compounds and bioactivity of Chinese dwarf cherry. *Food Mach.* **2024**, *40*, 186–192.
- Liu, M.; Du, J.J. Distribution of European Plum germplasm resources and expression of variety Traits. *Deciduous Fruit Tree* **2010**, *3*, 18–21.
- Khoo, G.M.; Clausen, M.R.; Pedersen, B.H.; Larsen, E. Bioactivity and total phenolic content of 34 sour cherry cultivars. *J. Food Compos.* **2011**, *24*, 772–776. [[CrossRef](#)]
- Zhang, Y.Q.; Si, Q.; Zhai, R.; Zhou, Y.B.; Zhang, X. Comparison of nutrients in wild and cultivated Chinese dwarf cherry. *Food Res. Dev.* **2017**, *38*, 104–108.
- Liu, J.Y.; Xin, X.L.; Lan, R. Study on detection of amino acids in Chinese dwarf cherry juice with isotope internal standard method. *Food Res. Dev.* **2010**, *31*, 140–143.
- Xu, M.L.; Qiao, G.X.; Liu, Y.J. The effects of different rootstocks on the survival and fruit quality of European plum grafting. *J. Nucl. Agric. Sci.* **2023**, *37*, 2503–2509.
- Sun, L.; Wang, X.Y.; Wang, H.L.; Yan, A.L.; Zhang, G.J.; Ren, J.C.; Xu, H.Y. The Influence of Rootstocks on the Growth and Aromatic Quality of Two Table Grape Varieties. *Sci. Agric. Sin.* **2021**, *54*, 4405–4420.
- Han, X.; Yang, H.Y.; Chen, W.K. Comparison of Aroma Compounds in Fruits of *Vitis vinifera* cv. Danna Grafted on Different Rootstocks. *Food Sci.* **2022**, *43*, 223–231.
- Zhao, L.; Jiang, Z.; Song, L. Effects of Different Rootstocks on Fruit Quality and Aroma Compounds of Red Chief Apples. *J. North China Agric. Sci.* **2014**, *29*, 234–238.
- Selamat, J.; Rozani, N.A.A.; Murugesu, S. Application of the Metabolomics Approach in Food Authentication. *Molecules* **2021**, *26*, 7565. [[CrossRef](#)]
- Hew, P.S.; Jinap, S.; Jambari, N.N.; Murugesu, S.; Sanny, M.; Khatib, A.; Sukor, R. Quality and safety of food product—Current assessment, issues, and metabolomics as a way forward. *Food Chem Adv.* **2024**, *4*, 100–632. [[CrossRef](#)]
- Duan, W.; Guan, Q.; Zhang, H.L.; Wang, F.Z.; Lu, R.; Li, D.M.; Geng, Y.; Xu, Z.-H. Improving flavor, bioactivity, and changing metabolic profiles of goji juice by selected lactic acid bacteria fermentation. *Food Chem.* **2023**, *408*, 135–155. [[CrossRef](#)]
- He, W.; Pan, H.L.; Pan, T.F.; Tang, H.R.; Wang, X.R.; Pan, D.M. Research Progress on the Interaction Between Scion and Rootstock in Fruit Trees. *Acta Hortic. Sin.* **2017**, *44*, 1645–1657.
- Zhang, J.; Ai, D.; Meng, J.X.; Wei, Y.C.; Zhang, Y. Research Progress on the Interaction Mechanism between Rootstock and scion in Plant grafting. *J. Northwest AF Univ. (Nat. Sci. Ed.)* **2022**, *50*, 139–145.
- Zhao, Z.H.; Hao, Y.F.; Liu, Y.J.; Shi, Y.S.; Lin, X.; Wang, L.; Wen, P.; Hu, X.P.; Li, J.X. Comprehensive evaluation of aroma and taste properties of different parts from the wampee fruit. *Food Chem.* **2023**, *19*, 100–835. [[CrossRef](#)] [[PubMed](#)]
- Yang, C.X.; Wang, Y.J.; Wu, B.H.; Fang, J.B.; Li, S.H. Volatile compounds evolution of three table grapes with different flavour during and after maturation. *Food Chem.* **2011**, *128*, 823–830. [[CrossRef](#)]
- He, J.J.; Zhou, Y.Z.; Li, Y.L.; Zhou, H.J. Aroma effects of key volatile compounds in Keemun black tea at different grades: HS-SPME-GC-MS, sensory evaluation, and chemometrics. *Food Chem.* **2022**, *373*, 131–587.
- Wen, Y.Q.; Zhong, G.Y.; Gao, Y.; Lan, Y.B.; Duan, C.Q.; Pan, Q.H. Using the combined analysis of transcripts and metabolites to propose key genes for differential terpene accumulation across two regions. *BMC Plant Biol.* **2015**, *15*, 240. [[CrossRef](#)]
- Noguerol-Pato, R.; Gonzalez-Barreiro, C.; Cancho-Grande, B.; Martinez, M.C.; Santiago, J.L.; Simal-Gandara, J. Floral, spicy and herbaceous active odorants in Gran Negro grapes from shoulders and tips into the cluster, and comparison with Brancellao and Mouratón varieties. *Food Chem.* **2012**, *135*, 2771–2782. [[CrossRef](#)] [[PubMed](#)]
- Pino, J.A.; Mesa, J. Contribution of volatile compounds to mango (*Mangifera indica* L.) aroma. *Flavour Fragr.* **2006**, *21*, 207–213. [[CrossRef](#)]

21. Zhang, W.W.; Wu, Y.S.; Chen, Y.J.; Zheng, Q.Z.; Ma, C.; Xu, W.P.; Zhang, C.X.; Wang, S.P. Aroma characteristics of three Kyoho grapevine series. *Shanghai Jiao Tong Univ. (Agric. Sci.)* **2018**, *36*, 51–59, 66.
22. Wu, Y.S.; Duan, S.Y.; Zhao, L.P.; Gao, Z.; Luo, M.; Song, S.R.; Xu, W.P.; Zhang, C.X.; Ma, C.; Wang, S.P. Aroma characterization based on aromatic series analysis in table grapes. *Sci. Rep.* **2016**, *6*, 31–116. [[CrossRef](#)]
23. Pineau, B.; Barbe, J.C.; Van Leeuwen, C.; Dubourdieu, D. Which impact for β -damascenone on red wines aroma. *Agric. Food Chem.* **2007**, *55*, 4103–4108. [[CrossRef](#)]
24. Fenoll, J.; Manso, A.; Hellin, P.; Ruiz, L.; Flores, P. Changes in the aromatic composition of the *Vitis vinifera* grape Muscat Hamburg during ripening. *Food Chem.* **2009**, *114*, 420–428. [[CrossRef](#)]
25. Cai, J. Study on Aroma Quality Improvement for Cabernet Sauvignon Wines Based on Pre-Fermentation Technology from North Slope of Tianshan Mountains. Doctoral Thesis, China Agricultural University, Beijing, China, 2014.
26. Jogaiah, S.; Oulkar, D.P.; Banerjee, K.; Sharma, J.; Patil, A.G.; Maske, S.R.; Somkuwar, R.G. Biochemically induced variations during some phenological stages in Thompson Seedless grapevines grafted on different rootstocks. *S. Afr. J. Enol. Vitic.* **2013**, *34*, 36–45. [[CrossRef](#)]
27. Qiu, L.L.; Jiang, B.; Fang, J.; Shen, Y.K.; Fang, Z.X.; RM, S.K.; Yi, K.K.; Shen, C.J.; Yan, D.L.; Zheng, B.S. Analysis of transcriptome in hickory (*Carya cathayensis*), and uncover the dynamics in the hormonal signaling pathway during graft process. *BMC Genom.* **2016**, *17*, 935. [[CrossRef](#)] [[PubMed](#)]
28. Warschefsky, E.J.; Klein, L.L.; Frank, M.H.; Chitwood, D.H.; Londo, J.P.; Von-Wettberg, E.J.B.; Miller, A.J. Rootstocks: Diversity, domestication, and impacts on shoot phenotypes. *Trends Plant Sci.* **2016**, *21*, 418–437. [[CrossRef](#)]
29. He, L.; Xu, X.Q.; Wang, Y.; Chen, W.K.; Sun, R.Z.; Cheng, G.; Liu, B.; Chen, W.; Duan, C.Q.; Wang, J.; et al. Modulation of volatile compound metabolome and transcriptome in grape berries exposed to sunlight under dry-hot climate. *BMC Plant Biol.* **2020**, *20*, 59. [[CrossRef](#)]
30. Burdack-Freitag, A.; Schieberle, P. Changes in the key odorants of Italian Hazelnuts (*Coryllus avellana* L. var. Tonda Romana) induced by roasting. *Agric. Food Chem.* **2010**, *58*, 6351–6359. [[CrossRef](#)] [[PubMed](#)]
31. Sun, B.G.; Chen, H.T. *The Technology of Food Flavor*, 3rd ed.; Chemical Industry Press: Beijing, China, 2016.
32. Van Gemert, L.J. *Compilations of Odour Threshold Values in Air, Water and Other Media*; Oliemans, Punter and Partners BV: Zeist, The Netherlands, 2011.
33. Giri, A.; Osako, K.; Ohshima, T. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chem.* **2010**, *120*, 621–631. [[CrossRef](#)]
34. Du, X.F.; Finn, C.E.; Qian, M.C. Volatile composition and odour-activity value of thornless ‘Black Diamond’ and ‘Marion’ blackberries. *Food Chem.* **2010**, *119*, 1127–1134. [[CrossRef](#)]
35. Spillman1, P.J.; Sefton, M.A.; Gawel, R. The contribution of volatile compounds derived during oak barrel maturation to the aroma of a Chardonnay and Cabernet Sauvignon wine. *Aust. J. Grape Wine Res.* **2004**, *103*, 227–235. [[CrossRef](#)]
36. Buhr, K.; Köhnhofer, B.; Heilig, A.; Hinrichs, J.; Schieberle, P. Behaviour of selected flavour compounds in dairy matrices: Stability and release. *Food Chem.* **2010**, *408*, 165–168.
37. Ogata, H.; Goto, S.; Sato, K.; Fujibuchi, W.; Bono, H.; Kanehisa, M. KEGG Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **1999**, *27*, 29–34. [[CrossRef](#)] [[PubMed](#)]
38. Jiang, L.L.; Lu, M.; An, H.M. Comparative analysis of chemical components of traditional Chinese medicine and antioxidants between *Rosa roxburghii* and *R. sterilis* fruits. *Fruit Sci.* **2023**, *40*, 111–125.
39. Negri, A.S.; Prinsi, B.; Rossoni, M.; Failla, O.; Scienza, A.; Cocucci, M.; Espen, L. Proteome changes in the skin of the grape cultivar Barbera among different stages of ripening. *BMC Genom.* **2008**, *9*, 378. [[CrossRef](#)]
40. Liu, Q.; Zhu, A.; Chai, L.; Zhou, W.; Deng, X. Transcriptome analysis of a spontaneous mutant in sweet orange citrus sinensis osbeck during fruit development. *Exp. Bot.* **2009**, *60*, 801–813. [[CrossRef](#)] [[PubMed](#)]
41. Feng, X.; Li, Y.; Wu, D.; Qin, C.; Yang, C.; Jiang, Y.; Hou, Z. Enhancement of antioxidant capacity and preservation of quality in postharvest apricots through led light quality treatment. *Hortic. Environ. Biotechnol.* **2025**, *66*, 253–268. [[CrossRef](#)]
42. Luo, C.D.; Feng, C.M.; Li, J.Q.; Li, X.R.; Wei, Y.; Yang, L.Y.; Liu, X.J.; Tan, H.; Ren, E.F.; Luo, X.J. Analysis of differential aroma volatiles of tainong no.1 mango of different ripeness by non-targeted metabolomics based on gas chromatography-mass spectrometry. *Sci. Agric. Sin.* **2025**, *58*, 564–581.
43. Terzoudis, K.; Hertog, M.L.A.T.M.; Nicolaï, B.M. Dynamic labelling reveals central carbon metabolism responses to stepwise decreasing hypoxia and reoxygenation during postharvest in pear fruit. *Postharvest Biol. Technol.* **2022**, *186*, 111–816. [[CrossRef](#)]
44. Wang, S.F.; Deng, Y.P.; Zhang, K.M.; Jiang, Z.R.; Chen, Z.Y.; Miao, Y.; Huang, K.L.; Hu, C.; Wang, Z. Volatile fatty acids production and sulfur metabolism pathways altered in a two-phase anaerobic sulfate-reducing system due to high organic and sulfate loading rates. *Water Process Eng.* **2025**, *70*, 106948. [[CrossRef](#)]
45. Ma, Y.; Yu, K.J.; Wang, N.; Xiao, X.J.; Leng, Y.J.; Fan, J.; Du, Y.; Wang, S.H. Sulfur dioxide-free wine with polyphenols promotes lipid metabolism via the Nrf2 pathway and gut microbiota modulation. *Food Chem. X* **2024**, *21*, 101079. [[CrossRef](#)]

46. Ji, X.A.; Yu, X.W.; Xu, Y.; Wu, Q.; Madadi, M.; Mousavi Khaneghah, A. Initial acidity regulates microbial sulfur metabolism in the spontaneous fermentation of sesame flavor-type baijiu. *Int. J. Food Microbiol.* **2025**, *435*, 111182. [\[CrossRef\]](#)
47. Pinsorn, P.; Sanachai, K.; Rungrotmongkol, T.; Hoefgen, R.; Watanabe, M.; Brueckner, F.; Nakabayashi, R.; Mori, T.; Oikawa, A.; Sasaki, R.; et al. Sulfur metabolism in durian pulps: Factors contributing to the production of volatile sulfur compounds during fruit ripening. *Postharvest Biol. Technol.* **2023**, *206*, 112533. [\[CrossRef\]](#)
48. Song, X.S.; Shang, Z.W.; Yin, Z.P.; Ren, J.; Sun, M.C.; Ma, X.L. Mechanism of xanthophyll-cycle-mediated photoprotection in *Cerasus humilis* seedlings under water stress and subsequent recovery. *Photosynthetica* **2011**, *49*, 523–530. [\[CrossRef\]](#)
49. Dong, S.; Zhou, Y.; Wang, J.C.; Zhuo, Y.L.; Sun, Y.H.; Wang, J.G.; Zhang, G.G. Four new compounds from the fruits of Chinese dwarf cherry (*Prunus humilis* Bunge). *Nat. Prod. Res.* **2024**, *38*, 1207–1215. [\[CrossRef\]](#)
50. Li, L.Y.; Chen, C.Y.; Wang, W.L.; Jin, J.H.; Ma, M.S.; Jin, J.Z. Evaluation of volatile compounds from Chinese dwarf cherry (*Cerasus humilis* (Bge.) Sok.) germplasms by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Food Chem.* **2017**, *217*, 389–397.
51. Zhang, L.; Han, X.; Zhang, S.; Du, J.; Zhang, J.; Gao, Y.G.; Wang, P.; Mu, X. A systematic profiling of the volatile compounds in 53 *Cerasus humilis* genotypes using headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Horticulturae* **2023**, *9*, 806. [\[CrossRef\]](#)
52. Wang, H.; Shi, Z.X.; Jiang, H.Y.; Wang, C.Z.; Chen, L.L.; Li, L.G. *Effect of Different Rootstocks on Volatile Components in Fruits of 'Fuji' Apple*; Shandong Academy of Agricultural Sciences: Jinan, China, 2018.
53. Zhang, H.; Li, X.L.; Li, Y.; Chen, Z.W.; Le, Y.Z.; Zhai, J.H. The influence of rootstock on the fruit quality of Chinese kiwifruit “Jianxiang”. *Fruit Trees S.* **2024**, *53*, 161–168.
54. Wang, N.; Zhang, X.; Guo, Q.; Yan, G.; Wang, J.; Wu, C.; Zhou, Y.; Zhou, J.; Zhang, K.; Li, T.; et al. Effects of different rootstocks on fruit quality and non-volatile flavor-related compounds of sweet cherry ‘summit’. *Food Chem.* **2025**, *463 Pt 4*, 141512. [\[CrossRef\]](#)
55. Wang, Y.; Chen, W.K.; Gao, X.T.; He, L.; Yang, X.H.; He, F.; Duan, C.Q.; Wang, J. Rootstock-mediated effects on Cabernet Sauvignon performance: Vine growth, berry ripening, flavonoids, and aromatic profiles. *Int. J. Mol. Sci.* **2019**, *20*, 401. [\[CrossRef\]](#)
56. Niu, S.K.; Yang, L.L.; Sun, C.W. Grape aromatic substance factors affecting research progress. *China Foreign Grape Wine* **2017**, *4*, 100–103.
57. El-Sharkawy, I.; Manríquez, D.; Flores, F.B.; Regad, F.; Bouzayen, M.; Latché, A.; Pech, J.C. Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme. *Plant Mol. Biol.* **2005**, *59*, 345–362. [\[CrossRef\]](#)
58. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of plant-derived flavor compounds. *Plant J.* **2008**, *54*, 712–732. [\[CrossRef\]](#)
59. Luan, F.; Mosandl, A.; Munch, A.; Wust, M. Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: Demonstration of stereoselective reduction, E/Z-isomerization, oxidation and glycosylation. *Phytochemistry* **2005**, *66*, 295–303. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Lund, S.T.; Bohlmann, J. The molecular basis for wine grape quality—A volatile subject. *Science* **2006**, *311*, 804–805. [\[CrossRef\]](#)
61. Albacete, A.; Martínez-Andújar, C.; Martínez-Pérez, A.; Thompson, A.J.; Dodd, I.C.; Pérez-Alfocea, F. Unravelling rootstock × scion interactions to improve food security. *J. Exp. Bot.* **2015**, *66*, 2211–2226. [\[CrossRef\]](#)
62. Harada, T. Grafting and RNA transport via phloem tissue in horticultural plants. *Sci. Hortic.* **2010**, *125*, 545–550. [\[CrossRef\]](#)
63. Huang, Q.; Liu, H.; Shen, Q.; Yuan, H.; Cui, F.; Yan, D.; Ding, W.; Wang, X.; Zheng, B. Hormone Fluctuation and Gene Expression During Early Stages of the Hickory Grafting Process. *Plants* **2025**, *14*, 2229. [\[CrossRef\]](#)
64. Miroslav, B.; Jana, R.; Miroslav, P. Comparative analysis of genetic diversity in *Prunus* L. as revealed by RAPD and SSR markers. *Sci. Hortic.* **2006**, *108*, 253–259. [\[CrossRef\]](#)
65. Li, X.Y.; Song, L.Q.; Li, M.Y.; Wang, H.J.; Liu, J.Z.; Wu, J.K.; Zhang, L.B. Advances in Biosynthesis and Metabolism Regulation of Fruit Volatile Compounds. *J. Agric. Biotechnol.* **2023**, *31*, 629–642.
66. Pan, F.; Zhao, X.; Liu, F.; Luo, Z.; Chen, S.; Liu, Z.; Zhao, Z.; Liu, M.; Wang, L. Triterpenoids in jujube: A review of composition, content diversity, pharmacological effects, synthetic pathway, and variation during domestication. *Plants* **2023**, *12*, 1501. [\[CrossRef\]](#)
67. Hao, H.; Xue, Z.; Li, Y.; Ma, H.; Wen, Q.; Lin, L.; Zhu, H. Genome-wide identification and characterization of lipoxygenases gene family in *Luffa aegyptiaca* revealed downregulation of lox genes under heat stress. *Sci. Rep.* **2025**, *15*, 17696. [\[CrossRef\]](#)
68. Huang, M.L.; Li, K.; Cheng, Y.; Li, M.Y.; Liu, L.; Mur, L.A.J.; Luo, J.; Fang, C.Y. PeWRKY20 represses PeMDH1 to modulate malic acid metabolism and flavor formation in postharvest passion fruit. *Postharvest Biol. Technol.* **2024**, *218*, 113164. [\[CrossRef\]](#)
69. Zhang, C.; Wang, C.C.; Wang, H.J.; Ablimit, A.; Sun, Q.; Dong, H.J. Identification of characteristic volatiles and metabolomic pathways during the fermentation of red grapefruit by *Monascus purpureus* using HS-SPME-GC–MS and metabolomics. *Food Chem.* **2025**, *464 Pt 3*, 141786. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Nishimura, T.; Yoshinari, Y. Carbohydrate and protein metabolism. In *Reference Module in Life Sciences*; Elsevier: Amsterdam, The Netherlands, 2025.

71. Liu, R.H.; Jia, J.W.; Wang, C.W.; Wu, Q.P.; Du, L.; Li, W.Q.; Yang, W.W.; Ma, J.J.; Zhang, D.; Xing, L.B.; et al. Transcriptomic and primary metabolic profiles reveal the mechanism of development and maturation of fuji apple grafted onto different dwarfed intermediate rootstocks. *Sci. Hortic.* **2025**, *343*, 114060. [[CrossRef](#)]
72. Wei, J.; Guo, R.F.; Zhou, Y.; Shi, B.S.; Liu, Y.G.; Li, J.Q.; Zhou, X.H. Phenylacetaldehyde reductase gene (SpPAR) regulates the biosynthesis of Phenylethanol and Benzylalcohol in *Syringa pubescens*. *Sci. Hortic.* **2025**, *343*, 114075. [[CrossRef](#)]
73. Jahan, T.; Huda, M.N.; Zhang, K.X.; He, Y.Q.; Lai, D.L.; Dhami, N.; Quinet, M.; Ali, M.A.; Kreft, I.; Woo, S.H.; et al. Plant secondary metabolites against biotic stresses for sustainable crop protection. *Biotechnol. Adv.* **2025**, *79*, 108520. [[CrossRef](#)] [[PubMed](#)]

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