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Assessment of fruit quality and volatile profiles in watermelons grafted onto various rootstocks

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Abstract

Grafting is a common technique used to enhance watermelon yield under biotic and abiotic stress conditions; however, it may influence fruit development and quality. This study evaluated the impact of grafting on the fruit quality attributes and volatile profiles of 'Ningnongke huadai' watermelon using 11 commercial rootstocks, encompassing wild watermelons, bottle gourds, and pumpkins. Significant variations were observed in fruit weight, length, rind thickness, firmness of rind and flesh, and central soluble solids content. Notably, wild watermelon grafting increased central soluble solids, while pumpkin-grafted fruits exhibited higher fruit weight but lower central soluble solids. The analysis identified 122 volatile compounds in watermelon samples, primarily ketones, aldehydes, and alcohols. Regarding volatile composition, bottle gourd and wild watermelon grafts did not significantly differ from non-grafted watermelons, whereas pumpkin-grafted fruits showed distinctive volatile profiles characterized by higher aldehyde content and lower levels of alcohols and aromatic hydrocarbons. In conclusion, wild watermelon rootstocks increased soluble solids and had minimal impact on the volatile profiles of grafted fruits, making them potentially suitable for commercial production of 'Ningnongke huadai' watermelon. The evident variability in volatile profiles among grafting combinations underscores the need for watermelon rootstock breeding programs to account for the influence of rootstocks on fruit aroma.

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Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*], originally from Africa, is a member of the Cucurbitaceae family and constitutes an economically significant crop in China[1]. Grafting technology has become instrumental in addressing the challenges of continuous cropping, enhancing plant stress tolerance, and achieving high yields in watermelon cultivation[2]. Despite the growth in protected cultivation and the increased area dedicated to watermelon farming, grafted watermelons only represent about 20% of the cultivation area in China[3], primarily due to a shortage of suitable rootstocks. These rootstocks are in demand for their compatibility, quality, and multi-resistance to diseases such as fusarium wilt and anthracnose, as well as to adverse conditions like low temperatures and insufficient light.

The selection of rootstocks is paramount for successful watermelon grafting and achieving high yields. The ideal rootstocks should exhibit strong compatibility with the scion, resistance to soil-borne pathogens, vigorous growth, and promote high yields without compromising fruit quality[4]. Currently, the rootstocks used in watermelon production encompass wild watermelon (*C. lanatus* subsp. *lanatus*), citron watermelon (*C. amarus*), *C. colocynthis*, pumpkin (*Cucurbita maxima*), butternut squash (*C. moschata*), hybrids of *C. maxima* × *C. moschata*, and bottle gourd (*Lagenaria siceraria* Standl.)[5−7]. Among these, hybrids of *C. maxima* × *C. moschata* and *L. siceraria* are preferred for their positive impact on fruit yield and quality[8]. Watermelon grafting significantly influences fruit quality, with varying outcomes[9−12]. Grafts involving *Lagenaria* hybrids show high survival rates[13]. Compared to non-grafted watermelons, those grafted onto bottle gourd and pumpkin rootstocks, particularly the latter, have larger single fruit weights but lower total soluble solid content (TSS) and taste quality[14]. Contrary findings suggest that grafting onto interspecific hybrid squash and gourd rootstocks does not adversely affect fruit quality, including TSS, titratable acidity, pH, and sensory properties[11,15]. The variability in fruit quality of grafted watermelons may be attributed to environmental conditions, rootstock-scion combinations, and delayed ripening[4,14]. Consequently, further research is necessary to understand the effects of scion-rootstock interactions on fruit quality in watermelon.

Watermelon is widely consumed for its refreshing quality, and its fruit flavor quality is a crucial determinant of its market value[16]. The unique aroma profile of watermelon, attributed to its aromatic volatiles, has significant sensory value, enhancing consumer appeal and differentiating it in the marketplace[17]. These aroma compounds, a complex blend of volatiles including alcohols, aldehydes, aromatic hydrocarbons, ketones, and terpenes, are integral to the sensory experience of watermelon[18]. In watermelon juice, the dominant aromatic volatiles are primarily C_6 and C_9 alcohols, aldehydes, and ketones, which are characterized by their low olfactory thresholds[19]. Grafting has been shown to significantly modify volatile composition. For example, citron melon-grafted watermelons displayed only minor changes in their volatile profiles compared to non-grafted ones[20]. Guler et al.[21] found that among various local and commercial bottle gourd rootstocks, two local bottle gourds were identified as the most suitable for yielding desirable volatile compounds in grafted watermelon, particularly affecting the concentrations of (*Z*)-6-nonenal and 6-methyl-5-hepten-2-one. Furthermore, watermelons grafted onto pumpkin interspecific hybrids exhibited increased levels of (*E*)-2-nonenal compared to those that were not grafted[22].

Current research on the impact of various rootstocks on grafted watermelon fruit quality has predominantly examined parameters such as fruit weight, firmness, soluble sugars, organic acids, vitamin C, and carotenoids[10,22−29]. Comparative studies on the aroma quality of watermelons grafted onto different rootstocks, however, are less common. Moreover, identifying rootstock-scion combinations that enhance the sensory quality of grafted watermelons remains a significant challenge. In this study, headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) techniques were employed to analyze the volatile compounds in the 'Ningnongke huadai' watermelon grafted onto 11 commercial rootstocks, which include wild watermelon, bottle gourd, and pumpkin. How grafting impacts fruit quality traits was also assessed. The present findings illuminate the influence of these rootstock types on the sensory quality attributes of watermelon and provide an empirical foundation for selecting optimal rootstocks in watermelon cultivation.

Materials and methods

Plant materials

The study was conducted in a plastic tunnel at the Ningxia Academy of Agriculture and Forestry Sciences in Yinchuan, China, during the spring of 2021. The scion used was the commercial watermelon variety 'Ningnongke huadai,' with a selection of three wild watermelons, two bottle gourds, and six pumpkin rootstocks (Table 1). Rootstock seeds were planted 5 d before the scion seeds in an organic substrate using polystyrene trays. Grafting was performed using the hole insertion method at the emergence of the first true leaf, following the procedure outlined by Guan & Zhao[30]. Standard horticultural practices for drip irrigation, fertilization, and pest management were adhered to. Plants were spaced at 1.0 m between rows and 0.5 m within rows. Only one fruit per plant was allowed to develop, selected from the same node. The experimental design was a randomized complete block with three replicates, each consisting of 20 plants. Fruits were harvested upon reaching visual maturity, indicated by senescent tendrils, ground spot color, and external fruit color. In total, six fruits from each replicate (18 fruits per grafting combination) were collected at maturity in June 2021, transported to the laboratory at a controlled low temperature, and immediately processed for sampling.

Table 1. Eleven rootstocks for 'Ningnongke huadai' watermelon.

Rootstock	Abbreviation	Type
Yongshi	YS	Wild watermelon (*Citrullus lanatus* subsp. *Lanatus*)
Ningzhen 101	NZ101	Wild watermelon (*Citrullus lanatus* subsp. *Lanatus*)
Yezhuang 1	YZ1	Wild watermelon (*Citrullus lanatus* subsp. *Lanatus*)
Jingxinzhen 1	JX1	Bottle gourd (*Lagenaria siceraria* Standl.)
Sizhuang 111	SZ111	Bottle gourd (*Lagenaria siceraria* Standl.)
Jingxinzhen 2	JX2	Pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)
Zhuangshi	ZS	Pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)
Ningzhen 1	NZ1	Pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)
Mingxiu	MX	Pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)
Jinchengxuefeng	JC	Pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)
Qingyou 1	QY1	Pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)

Chemicals and reagents

Hexane, ethanol, and sodium chloride were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The internal standard, 3-hexanone, and the n-alkanes standard mix (C_8–C_{20}) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Thirty-six authentic standards were sourced from three companies: Yuanye Bio-Technology Co., Ltd., Rhawn Co., Ltd., and ZZBIO Co., Ltd., all located in Shanghai, China. These standards were solubilized in ethanol for subsequent GC-MS analysis.

Measurement of fruit quality characteristics

The quality characteristics of each fruit (18 fruits per grafting combination) were assessed, including weight (kg), dimensional attributes such as length and width (cm), rind thickness (cm), and firmness of both rind and flesh (kg/cm²). Additionally, soluble solids content was measured at both central and edge locations (°Brix). Fruit weight was determined using an electronic balance, while dimensions and rind thickness were gauged with a vernier caliper. Firmness was evaluated in the watermelon's rind and central flesh, excluding seed locations, using an FT 011 penetrometer (Effegi, Japan). The concentration of soluble solids was quantified using a PAL-1 handheld digital refractometer (ATAGO, Japan), taking readings at the fruit's central flesh and 1 cm from the rind edge.

Extraction of volatiles and GC-MS analysis

For each replicate, six mature fruits were harvested. The central flesh from these fruits was combined, finely chopped, and homogenized to prepare a uniform sample. Samples were immediately frozen at −20 °C until analysis. Volatile compounds extraction from the watermelon samples employed the HS-SPME method as outlined by Yu et al[31]. In a 20 mL headspace vial, 6 mL of watermelon juice and 1.5 g of sodium chloride were mixed thoroughly. To this mixture, 6 μL of 3-hexanone (0.163 g/L), serving as an internal standard was added. The vial was then sealed with a magnetic crimp cap and a silicone/PTFE septum (Gerstel, Linthicum, MD, USA). Vials were placed into an autosampler (Model MPS2, Gerstel) with a cooling holder (Laird Tech, Gothenburg, Sweden). The samples were incubated at 40 °C for 30 min. Subsequently, a triphase SPME fiber (50/30 μm DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) was exposed to the headspace for 60 min at the same temperature to absorb analytes. The SPME fiber was then inserted into the injection port of a GC (7890B, Agilent Technologies, Santa Clara, CA, USA) interfaced with a Time-of-Flight MS (LECO, Saint Joseph, MI, USA), where it was equipped with a DB-5 column (30 m × 0.25 μm × 0.25 μm, Rxi-5 Sil MS, Restek, Bellefonte, PA, USA). The volatile compounds were desorbed at 250 °C for 5 min. The GC oven temperature began at 40 °C, held for 5 min, then ramped up to 230 °C at 3 °C/min, followed by an increase to 260 °C at 15 °C/min, and maintained at 260 °C for a final 5 min. Helium served as the carrier gas at a constant flow rate of 1 mL/min. The MS operated with an electron ionization energy of 70 eV, scanning from 30 to 500 m/z, with the ion source kept at 230 °C.

Identification of volatile compounds

Volatile compounds in watermelon flesh were identified by comparing their mass spectra and retention time against those of authentic standards or by referencing retention indices (RIs) and mass spectra from the NIST05.L (National Institute of Standards and Technology Mass Spectral Library, Gaithersburg, MA, USA) and ADAMS.L[32]. The RIs for these compounds were determined using a series of n-alkane standards (C_8–C_{20}) and were calculated daily under consistent conditions. The relative content of each volatile compound, including both identified and unidentified peaks was quantified by comparing its peak area with that of the internal standard.

Statistical analysis

The study employed a completely randomized design, with three replicates per grafting combination. Statistical analysis was performed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA), JMP Pro 13 (SAS, Cary, NC, USA), and Origin 8.0 (Origin, Northampton, MA, USA). Comparative profiles of volatile compounds from different rootstock-scion combinations were illustrated using Venn diagrams and Principal Component Analysis (PCA). Hierarchical Cluster Analysis (HCA), based on Ward's method, was conducted using R software (v4.2.1) to discern patterns in the volatile profiles across combinations. Significant differences were assessed using one-way ANOVA, Student's t-test, and Tukey's Honestly Significant Difference test, with a significance level set at $p < 0.05$.

Result and discussion

Grafting effect on fruit quality characteristics

Grafting significantly influenced watermelon quality traits, including fruit weight and length, rind thickness, flesh firmness, and central soluble solids (Table 2). Consistent with previous studies[33,34], grafting onto *Cucurbita* spp., *Lagenaria* spp., and *Citrullus* spp. rootstocks increased average fruit weight. Specifically, pumpkin-grafted watermelons (11.5–14.22 kg) weighed more than non-grafted ones (NG, 10.41 kg). Pumpkin rootstocks enhanced leaf photosynthesis and increased expression levels of *ClCWIN4*, *ClAGA2*, and *ClVST1*, as well as CWIN activity, contributing to weight gain[35]. Rind thickness, crucial for transport, was reduced in watermelons grafted onto 'Zhuangshi' (ZS, 0.73 cm), 'Jingxinzhen 2' (JX2, 0.75 cm), and 'Ningzhen 1' (NZ1, 0.66 cm) compared to NG (1.05 cm). In contrast, watermelons grafted onto wild rootstocks, such as 'Yongshi' (YS, 1.15 cm), 'Ningzhen 101' (NZ101, 1.13 cm), and 'Yezhuang 1' (YZ1, 1.3 cm), had thicker rinds. While citron or *Cucurbita* rootstocks increased rind thickness and fruit size compared to NG[20], but this study found that wild watermelon rather than pumpkin rootstocks led to even larger fruits with thicker rinds.

Pumpkin rootstocks, specifically 'Qingyou 1' (QY1, 1.19 kg/cm²), ZS (1.08 kg/cm²), and NZ1 (1.14 kg/cm²), significantly enhanced flesh firmness in watermelon compared to NG (0.63 kg/cm²). Flesh firmness is a critical sensory attribute of watermelon quality, with numerous studies documenting increased firmness in grafted fruits[5,12,36,37]. TSS is key to consumer acceptance. Wild watermelon-grafted fruits had the highest TSS, ranging from 11.67 °Brix in YZ1 to 12.17 °Brix in NZ101. Conversely, bottle gourd- (10.4–10.8 °Brix) and pumpkin-grafted (9.98–10.87 °Brix) watermelons had lower central soluble solids compared to NG (11.3 °Brix). The effects of grafting on watermelon TSS vary across rootstock-scion combinations. *Citrullus* spp. rootstocks increase, while *Lagenaria* spp. rootstocks decrease, TSS in watermelon 'NS-295'[34]. Sun et al.[35] found that *C. maxima* × *C. moschata* rootstocks altered sugar profiles by increasing glucose and fructose and reducing sucrose, likely due to up-regulated *ClVIN2* expression and increased VIN activity. Several genes related to glucose and sucrose metabolism (*FBA2*, *FK*, *SuSy*, *SPS*, *IAI*, *AI*, *SWT3b*) may play a central role in regulating TSS in pumpkin-grafted watermelon[3].

PCA and HCA were utilized to assess differences in fruit quality between NG and their grafted counterparts. PC1 accounted for 46.1% of the variance, effectively separating pumpkin-grafted watermelons from NG and other grafted types based on flesh firmness, central soluble solids, and fruit weight (Fig. 1a & b). HCA also grouped all pumpkin-grafted watermelons into a distinct cluster (Fig. 1c). Wild watermelon-grafted fruits excelled in TSS, while pumpkin-grafted watermelons had better texture and size but lower TSS, supporting previous findings[38,39].

Table 2. Fruit quality characteristics of 'Ningnongke huadai' watermelon grafted onto different rootstocks.

Type	NG	Wild watermelon			Bottle gourd		Pumpkin					
		NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
Fruit weight (kg)	10.41^{h}	11.76^{ef}	12.01^{de}	10.94^{fgh}	11.36^{efg}	10.78^{gh}	14.22^{a}	14.08^{a}	12.64^{cd}	13.54^{ab}	11.50^{efg}	12.97^{bc}
Fruit length (cm)	22.62^{cd}	24.17^{bcd}	23.77^{cd}	26.32^{ab}	23.50^{cd}	22.80^{cd}	27.12^{a}	24.38^{bc}	23.97^{bcd}	21.81^{d}	23.78^{cd}	23.30^{cd}
Fruit width (cm)	29.65^{c}	32.99^{abc}	31.98^{abc}	31.38^{bc}	35.00^{ab}	34.05^{abc}	35.68^{ab}	37.03^{a}	35.15^{ab}	31.33^{bc}	35.61^{ab}	34.55^{abc}
Rind thickness (cm)	1.05^{bc}	1.13^{b}	1.15^{b}	1.30^{a}	1.12^{b}	0.95^{cd}	0.87^{def}	0.92^{cd}	0.88^{de}	0.73^{fg}	0.75^{efg}	0.66^{g}
Rind firmness (kg/cm^2)	15.21^{abc}	16.65^{a}	14.69^{bc}	14.90^{abc}	14.56^{bcd}	16.02^{ab}	14.90^{abc}	14.88^{bc}	14.02^{cd}	12.82^{d}	15.84^{ab}	14.32^{bcd}
Flesh firmness (kg/cm^2)	0.63^{e}	0.53^{e}	0.59^{e}	0.61^{e}	0.77^{cde}	0.70^{de}	1.03^{ab}	0.97^{abc}	1.19^{a}	1.08^{ab}	0.93^{bcd}	1.14^{ab}
Central soluble solids (°Brix)	11.30^{bcd}	12.17^{a}	11.93^{ab}	11.67^{abc}	10.40^{ef}	10.80^{def}	9.98^{f}	10.48^{def}	10.30^{ef}	10.85^{cde}	10.72^{def}	10.87^{cde}
Edge soluble solids (°Brix)	8.05^{abc}	8.02^{abc}	8.40^{a}	7.47^{bc}	7.65^{abc}	7.72^{abc}	8.17^{abc}	7.43^{bc}	7.37^{c}	7.83^{abc}	8.27^{ab}	8.42^{a}

All values are mean of three biological replicates (six fruits each) per grafting combination. Distinct letters within the same row indicate statistically significant differences, as determined by Student's t test at $p < 0.05$.

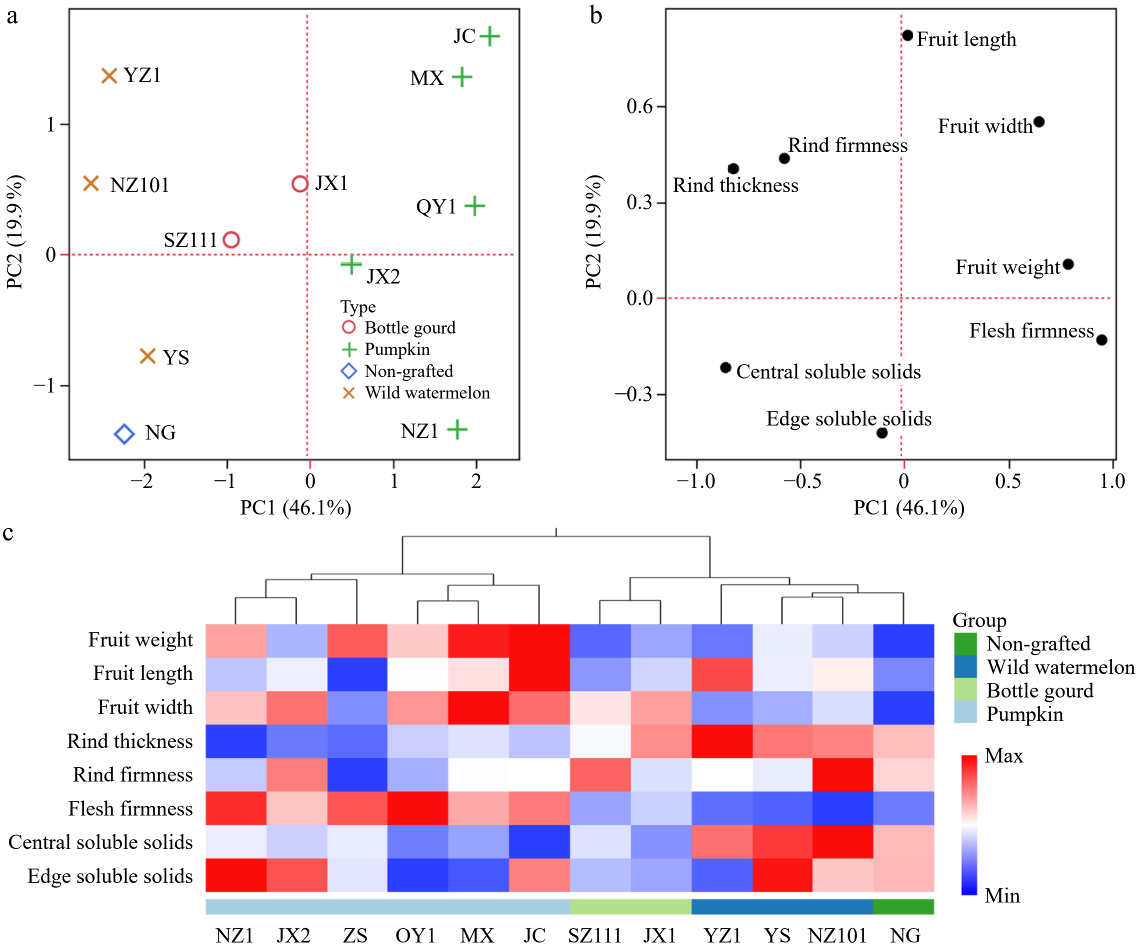

Fig. 1 PCA and HCA of fruit characteristics for 'Ningnongke huadai' watermelon grafted onto various rootstocks. (a) PCA score, and (b) loading plots of fruit characteristics. (c) HCA of fruit characteristics. Each row in the figure corresponds to a distinct fruit trait and each column to a specific sample. The color gradient represents the relative magnitudes of the fruit characteristics across the sample groups. The dendrogram at the top indicates the clustering of samples, with the sample names listed below.

Fruit volatile profile of NG watermelon

HS-SPME coupled with GC-MS facilitated the extraction and quantification of volatile constituents within the watermelon flesh. A spectrum of 122 volatile compounds was identified, comprising one acid, 28 alcohols, 35 aldehydes, 10 aromatic hydrocarbons, one epoxide, five esters, four furans, 20 ketones, one lactone, two monoterpenes, one phenol, and three sulfides (Tables 3 & 4, Fig. 2a). These compounds align with those previously reported in watermelon literature[40,41]. Predominantly, ketones were the most prevalent group, with 6-methyl-5-hepten-2-one ranging from 8.68 to 15.05, contributing to the characteristic herbaceous, fruity, and oily green aromas (Tables 3 & 5, Fig. 2b). This ketone is speculated to arise from the degradation of lycopene, a highly unstable carotenoid abundant in watermelon[20,42]. Geranyl acetone (0.84–1.67), the subsequent most abundant ketone, noted for its floral and fruity scent, is a byproduct of β-carotene breakdown. Despite their abundance, neither 6-methyl-5-hepten-2-one nor geranyl acetone significantly contribute to watermelon aroma due to their high odor thresholds, at 50 μg/L and 186 μg/L, respectively[43]. Additionally, β-ionone, with its violet-like fragrance, was detected and is also a metabolite of β-carotene catabolism[43].

Aldehydes, ranging from 5.23 to 12.9, and alcohols, between 4.44 and 11.55, were prominent volatile constituents in the watermelon samples (Table 5 & Fig. 2b). Notably, C_6 and C_9 aldehydes and alcohols are recognized as key aroma volatiles of the Cucurbitaceae family[7]. According to Yang et al.[44], C_9 saturated and unsaturated linear aldehydes and alcohols, including (*E*)-2-nonenal, (*Z*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, (*Z*)-3-nonenol, (*E*)-6-nonenol, (*E*,*E*)-3,6-nonadienol, (*E*,*Z*)-3,6-nonadienol, and (*Z*,*Z*)-3,6-nonadienol, are representative of watermelon's distinctive aroma. Additionally, aldehydes and alcohols comprised the largest groups, with 34 to 35 and 25 to 28 volatile compounds, respectively. Among the aldehydes, hexanal, nonanal, (*E*,*Z*)-2,6-nonadienal, geranial, and octanal were the most abundant, with concentrations detailed in Table 3. Hexanal, a C_6 aldehyde resulting from linoleic acid oxidation, imparts a green and floral aromatic quality[45]. Conversely, the C_9 aldehydes, nonanal and (*E*,*Z*)-2,6-nonadienal, are pivotal in defining watermelon's aroma, lending melon, citrus peel, and cucumber-like scents[46,47]. Interestingly, (*Z*,*Z*)-3,6-nonadienal was not detected in this study, although frequently reported in prior research, possibly due to its quick isomerization to (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal[42].

The watermelon samples demonstrated significant levels of various alcohols, with nonanol and its homologues being particularly abundant. Concentrations of (*E*,*Z*)-3,6-nonadienol ranged from 2.23 to 6.32, while hexanol, nonanol, 1-octen-3-ol, and (*Z*)-

Table 3. Volatile compounds of 'Ningnongke huadai' watermelon grafted onto different rootstocks.

Code	Compound	Class	RI (Caculated)	RI (Published)[b]	NG	NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
3	2-methyl-1-butanol	alcohol	726	724	0.14^{z}	0.08^{zy}	0.11^{zy}	0.10^{zy}	0.11^{zy}	0.13^{zy}	0.05^{zy}	0.05^{zy}	0.05^{zy}	0.06^{zy}	0.04^{zy}	0.04^{y}
6	dimethyl disulfide	sulfide	728	785[c]	0.02	0.06	0.04	0.02	0.22	0.39	0.02	0.03	0.02	tr	0.19	tr
10	2-methyl-3-pentanone	ketone	731	745[d]	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.04	0.02	0.01	0.01
13	(*E*)-2-pentenal[a]	aldehyde	734	744	0.07^{zy}	0.08^{zy}	0.07^{y}	0.07^{zy}	0.06^{y}	0.08^{zy}	0.07^{zy}	0.09^{zy}	0.16^{z}	0.11^{zy}	0.06^{y}	0.07^{zy}
17	pentanol[a]	alcohol	741	762	0.26	0.25	0.19	0.23	0.19	0.19	0.17	0.18	0.29	0.24	0.15	0.18
20	(*Z*)-2-pentenol[a]	alcohol	743	765	0.01	0.01	0.01	0.01	tr	0.01	0.01	0.01	0.01	0.01	tr	0.01
21	2-methyl-3-pentanol	alcohol	745	775[d]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
29	2-methyl-4-pentenal	aldehyde	757	793[d]	tr	tr	tr	0.01	tr	0.01	0.01	0.02	0.02	0.02	tr	tr
31	2,3-butanediol	alcohol	760	785	tr	0.02	nd	0.04	tr	0.01	0.03	0.01	0.03	0.02	nd	0.01
33	hexanal[a]	aldehyde	764	801	2.88^{zy}	2.86^{zy}	2.02^{y}	2.56^{zy}	2.52^{zy}	2.90^{zy}	2.93^{zy}	2.97^{zy}	5.35^{z}	3.91^{zy}	2.75^{zy}	2.70^{zy}
38	2,4-dimethyl-heptane	aromatic hydrocarbon	783	822[d]	tr	tr	tr	tr	0.01	0.01	tr	tr	tr	tr	tr	tr
41	pentyl formate	ester	789	797[d]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
43	propylcyclopentane	aromatic hydrocarbon	792	834[d]	nd^{x}	nd^{x}	nd^{x}	tr^{yx}	tr^{x}	tr^{yx}	tr^{zyx}	0.01^{zy}	0.01^{z}	tr^{zyx}	tr^{yx}	tr^{zyx}
47	2-ethyl-butanal	aldehyde	797	762[d]	0.02^{y}	0.01^{y}	0.01^{y}	0.02^{y}	0.03^{zy}	0.02^{zy}	0.02^{y}	0.02^{zy}	0.06^{z}	0.03^{zy}	0.02^{zy}	0.02^{y}
48	2,4-dimethyl-1-heptene	aromatic hydrocarbon	801	840[d]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
50	(*E*)-2-hexenal[a]	aldehyde	807	846	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
51	(*E*)-3-hexenol	alcohol	812	844	tr^{zyx}	0.01^{zy}	0.01^{z}	tr^{zyx}	tr^{zy}	tr^{x}	nd^{x}	nd^{x}	nd^{x}	nd^{x}	nd^{x}	tr^{x}
52	2-hexenal	aldehyde	813	856[c]	0.08^{y}	0.10^{y}	0.09^{y}	0.11^{y}	0.09^{y}	0.15^{zy}	0.12^{zy}	0.14^{zy}	0.22^{z}	0.19^{zy}	0.11^{y}	0.10^{y}
53	1-(2-methyl-1-cyclopenten-1-yl)-ethanone	ketone	813		tr	tr	tr	tr	tr	tr	tr	tr	0.01	tr	tr	tr
55	(*Z*)-3-hexenol[a]	alcohol	816	850	0.22^{z}	0.21^{z}	0.23^{z}	0.19^{z}	0.18^{z}	0.13^{zy}	0.04^{y}	0.06^{y}	0.04^{y}	0.07^{y}	0.05^{y}	0.03^{y}
60	ethylbenzene[a]	aromatic hydrocarbon	819	850[d]	0.11^{zy}	0.13^{zy}	0.12^{zy}	0.15^{zy}	0.12^{zy}	0.18^{z}	0.06^{y}	0.07^{y}	0.10^{zy}	0.06^{y}	0.07^{zy}	0.05^{y}
61	4-methyl-octane	aromatic hydrocarbon	825	863[d]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	nd
65	hexanol[a]	alcohol	832	863	1.12	0.93	0.69	0.88	1.03	0.69	0.54	0.39	0.83	0.64	0.55	0.33
70	styrene[a]	other	851	893[c]	0.33^{zy}	0.45^{z}	0.43^{z}	0.51^{z}	0.31^{zy}	0.51^{z}	0.02^{y}	0.03^{y}	0.02^{y}	0.03^{y}	0.02^{y}	0.02^{y}
73	2-heptanone[a]	ketone	853	889	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.04	0.01	0.01	tr
75	2-butylfuran	furan	854	892[d]	0.01	0.01	tr	0.01	0.01	0.02	0.01	0.01	0.03	0.02	0.01	tr
80	(*Z*)-4-heptenal	aldehyde	864	893	tr^{y}	tr^{y}	tr^{y}	tr^{y}	tr^{y}	tr^{zy}	tr^{zy}	0.01^{zy}	0.02^{z}	0.01^{zy}	0.01^{zy}	0.01^{zy}
82	heptanal[a]	aldehyde	866	901	0.16	0.14	0.10	0.14	0.10	0.13	0.12	0.13	0.34	0.20	0.12	0.11
84	methional	sulfide	871	909[c]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
85	(*E,E*)-2,4-hexadienal[a]	aldehyde	876	907	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
89	amyl acetate	ester	883	911	tr	tr	tr	0.01	tr	0.01	tr	tr	0.01	0.01	tr	tr
92	cumene	aromatic hydrocarbon	889	924	tr^{zy}	tr^{z}	tr^{zy}	tr^{z}	tr^{zy}	tr^{z}	nd^{y}	nd^{y}	nd^{y}	nd^{y}	nd^{y}	nd^{y}
102	4-methyl-2-heptanone	ketone	908	918	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
106	RI918	other	918		0.04^{zy}	0.03^{zy}	0.02^{y}	0.04^{zy}	0.05^{zy}	0.04^{zy}	0.03^{zy}	0.04^{zy}	0.11^{z}	0.05^{zy}	0.04^{zy}	0.03^{zy}
110	6-methyl-2-heptanone	ketone	929	955[d]	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.01
111	(*E*)-2-heptenal[a]	aldehyde	931	947	0.13	0.12	0.10	0.13	0.10	0.10	0.09	0.09	0.22	0.14	0.08	0.09

(to be continued)

Table 3. (continued)

Code	Compound	Class	RI (Caculated)	RI (Published)[b]	NG	NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
113	benzaldehyde[a]	aldehyde	933	952	0.05	0.07	0.05	0.06	0.09	0.06	0.05	0.06	0.14	0.07	0.05	0.04
116	dimethyl trisulfide[a]	sulfide	939	974[c]	0.01	0.05	0.02	0.01	0.11	0.20	0.02	0.02	0.01	tr	0.11	tr
117	2-methyl-1-hepten-6-one	ketone	943	966[d]	0.02	0.01	0.01	0.01	0.01	0.01	tr	tr	0.01	0.01	0.01	tr
118	(*Z*)-3-heptenol	alcohol	944	959[d]	0.02	0.01	tr	tr	0.01	tr	tr	tr	tr	tr	tr	tr
119	(*E*)-2-heptenol[a]	alcohol	948	958	tr	tr	nd	tr	tr	tr	tr	tr	tr	tr	tr	tr
121	heptanol[a]	alcohol	952	959	0.15	0.11	0.06	0.09	0.09	0.07	0.06	0.05	0.15	0.09	0.06	0.04
122	3,5,5-trimethyl-2-hexene	aromatic hydrocarbon	954	968[d]	tr^{y}	0.02^{zy}	0.01^{zy}	0.01^{zy}	tr^{y}	0.02^{zy}	0.01^{zy}	0.02^{zy}	0.02^{z}	0.02^{zy}	0.01^{zy}	0.01^{y}
123	1-octen-3-one	ketone	957	972	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.03	0.02	0.02	0.02
127	1-octen-3-ol	alcohol	965	974	0.42	0.50	0.41	0.54	0.39	0.47	0.35	0.34	0.72	0.51	0.35	0.32
128	phenol	phenol	968	980[c]	0.08	0.05	0.05	nd	nd	nd	nd	0.02	nd	nd	nd	0.01
129	6-methyl-5-hepten-2-one[a]	ketone	969	981	13.09	13.80	13.98	14.53	10.54	15.05	9.19	8.68	10.15	11.61	9.72	9.76
131	3-octanone[a]	ketone	970	979	0.04	0.05	0.04	0.05	0.03	0.04	0.04	0.05	0.10	0.06	0.04	0.04
133	2-pentylfuran	furan	973	984	0.61	0.89	0.62	0.93	0.42	1.09	0.58	0.63	1.01	0.89	0.62	0.61
136	1-cyclohexyl-ethanone	ketone	978	963[d]	tr	tr	0.01	0.01	tr	tr	tr	tr	tr	tr	tr	tr
137	6-methyl-5-hepten-2-ol[a]	alcohol	978	989	0.05	0.04	0.06	0.09	0.04	0.07	0.02	0.02	0.02	0.04	0.02	0.02
138	(*E,Z*)-2,4-heptadienal,	aldehyde	980	1,000[c]	0.06^{zy}	0.06^{zy}	0.05^{y}	0.06^{zy}	0.05^{y}	0.05^{y}	0.06^{zy}	0.07^{zy}	0.14^{z}	0.08^{zy}	0.04^{y}	0.06^{zy}
139	RI980	other	980		0.03^{yx}	0.03^{yx}	0.02^{yx}	0.01^{x}	0.01^{x}	0.04^{yx}	0.06^{zyx}	0.09^{zyx}	0.16^{z}	0.12^{zy}	0.05^{yx}	0.07^{zyx}
141	trans-2-(2-pentenyl)furan	furan	983	984[d]	tr	0.01	0.01	0.01	tr	0.02	0.01	0.01	0.02	0.02	0.01	tr
142	hexanoic acid	acid	985	967	nd	tr	tr	tr	nd	nd	nd	nd	nd	nd	nd	nd
144	octanal[a]	aldehyde	989	998	0.27	0.29	0.20	0.26	0.15	0.22	0.25	0.28	0.60	0.46	0.23	0.28
149	(*E,E*)-2,4-heptadienal	aldehyde	997	1,005	0.09^{y}	0.09^{y}	0.07^{y}	0.08^{y}	0.07^{y}	0.08^{y}	0.08^{y}	0.09^{y}	0.31^{z}	0.14^{zy}	0.05^{y}	0.07^{y}
152	hexyl acetate[a]	ester	1,002	1,007	tr	tr	tr	0.01	tr	tr	nd	nd	nd	tr	nd	nd
154	RI1008	ketone	1,008		tr^{w}	tr^{w}	tr^{w}	tr^{w}	tr^{w}	0.01^{xw}	0.02^{yxw}	0.03^{zyx}	0.05^{z}	0.03^{zy}	0.02^{yxw}	0.02^{yxw}
155	3-ethyl-4-methylpentanol	alcohol	1,011	1,023[d]	tr	tr	tr	tr	tr	tr	tr	nd	tr	tr	tr	tr
156	*p*-cymene	monoterpene	1,012	1,020	tr	tr	tr	tr	tr	tr	tr	tr	tr	nd	nd	nd
158	RI1018	aromatic hydrocarbon	1,017		0.01	nd	0.01	0.01	tr	0.02	tr	nd	nd	nd	tr	nd
159	limonene[a]	monoterpene	1,018	1,024	0.01^{zy}	0.01^{zy}	tr^{y}	0.01^{zy}	tr^{y}	0.01^{zy}	0.01^{zy}	0.01^{zy}	0.04^{z}	0.02^{zy}	tr^{zy}	0.01^{zy}
161	(*Z*)-2-decene	aromatic hydrocarbon	1,020	1,009[d]	0.57^{zyx}	0.98^{z}	0.84^{zyx}	0.86^{zyx}	0.61^{zyx}	0.91^{zy}	0.49^{zyx}	0.49^{zyx}	0.46^{yx}	0.72^{zyx}	0.48^{yx}	0.40^{x}
163	2-ethyl-1-hexanol[a]	alcohol	1,022	1,032[c]	tr^{z}	tr^{zy}	nd^{y}	nd^{y}	nd^{y}	nd^{y}	nd^{y}	tr^{zy}	nd^{y}	nd^{y}	nd^{y}	nd^{y}
165	2,2,6-trimethyl-cyclohexanone	ketone	1,023	1,036[d]	tr	tr	0.01	0.01	tr	0.01	tr	tr	tr	tr	tr	tr
166	benzyl alcohol[a]	alcohol	1,024	1,026	0.18	0.18	0.24	0.31	0.44	0.19	0.16	0.22	0.34	0.26	0.17	0.15
170	3-octen-2-one	ketone	1,031	1,030	nd^{y}	nd^{y}	nd^{y}	tr^{zy}	nd^{y}	nd^{y}	nd^{y}	nd^{y}	tr^{z}	tr^{zy}	tr^{zy}	tr^{zy}
172	benzeneacetaldehyde[a]	aldehyde	1,033	1,036	0.02	0.03	0.03	0.04	0.02	0.03	0.01	0.02	0.03	0.03	0.01	0.02
175	RI1040	ketone	1,040		0.04	0.04	0.03	0.04	0.05	0.03	0.02	0.03	0.09	0.04	0.03	0.03
180	2,6-dimethyl-2,6-octadiene	aromatic hydrocarbon	1,047	980[d]	0.03	0.04	0.04	0.04	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.03
181	bergamal	aldehyde	1,049	1,051	0.01	0.02	0.02	0.02	tr	0.01	tr	tr	tr	0.01	tr	0.01
183	(*Z*)-3-octenol	alcohol	1,051	1,047	0.01	0.02	0.01	0.01	0.01	0.02	tr	tr	tr	tr	tr	tr
185	RI1053	other	1,053		0.06	0.08	0.06	0.08	0.02	0.05	0.03	0.02	0.02	0.04	0.02	0.02

(to be continued)

Table 3. (continued)

Code	Compound	Class	RI (Caculated)	RI (Published)[b]	NG	NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
187	(*E*)-2-octenal[a]	aldehyde	1,054	1,060[c]	0.09	0.09	0.07	0.10	0.06	0.06	0.05	0.05	0.13	0.08	0.04	0.06
191	acetophenone	ketone	1,059	1,059	tr^{zy}	tr^{zy}	tr^{zy}	tr^{z}	tr^{zy}	tr^{zy}	tr^{zy}	tr^{y}	tr^{zy}	nd^{y}	nd^{y}	nd^{y}
192	6-methyl-3,5-heptadiene-2-one	ketone	1,061	1,105[d]	tr^{zyxw}	tr^{zyx}	tr^{z}	tr^{zy}	tr^{zyxw}	tr^{zyx}	tr^{xw}	tr^{w}	tr^{yxw}	tr^{zyxw}	tr^{xw}	nd^{w}
195	(*E*)-2-octenol[a]	alcohol	1,068	1,060	0.04	0.06	0.05	0.07	0.04	0.05	0.05	0.04	0.07	0.06	0.04	0.04
196	(*E,E*)-3,5-octadien-2-one	ketone	1,068	1,083[d]	0.02	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.04	0.03	0.02	0.03
199	RI1072	other	1,073		0.05	0.06	0.04	0.04	0.04	0.04	0.03	0.05	0.08	0.08	0.02	0.04
200	octanol[a]	alcohol	1,073	1,063	0.17	0.19	0.11	0.14	0.10	0.11	0.10	0.11	0.21	0.19	0.12	0.10
211	2-nonanone	ketone	1,095	1,087	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
217	(*E*)-4-nonenal	aldehyde	1,098	1,105[d]	tr	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	tr	0.01
223	perillene	furan	1,103	1,102	0.04	0.06	0.06	0.08	0.03	0.07	0.04	0.03	0.03	0.05	0.03	0.04
227	(*Z*)-6-nonenal	aldehyde	1,108	1,097	0.24^{x}	0.43^{yx}	0.30^{x}	0.25^{x}	0.13^{x}	0.73^{zyx}	0.87^{zyx}	1.5^{zy}	1.16^{zyx}	1.67^{z}	0.73^{zyx}	1.24^{zyx}
228	nonanal[a]	aldehyde	1,112	1,100	0.71^{zy}	1.04^{zy}	0.82^{zy}	0.83^{zy}	0.34^{y}	1.06^{zy}	1.04^{zy}	1.39^{zy}	1.28^{zy}	1.81^{z}	0.99^{zy}	1.48^{zy}
239	RI1137	other	1,137		0.16	0.15	0.10	0.16	0.14	0.13	0.08	0.10	0.29	0.16	0.10	0.11
246	RI1153	ketone	1,153		0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.04	0.02	0.02	0.02
247	(*E,E*)-2,6-nonadienal	aldehyde	1,154	1,152[d]	0.02^{zy}	0.03^{zy}	0.02^{zy}	0.03^{zy}	0.01^{y}	0.02^{zy}	0.03^{zy}	0.03^{zy}	0.04^{zy}	0.04^{z}	0.02^{zy}	0.03^{zy}
254	(*E,Z*)-2,6-nonadienal	aldehyde	1,166	1,150	0.54^{yx}	0.99^{zyx}	0.71^{zyx}	0.89^{zyx}	0.36^{x}	0.86^{zyx}	1.24^{zyx}	1.42^{zy}	1.28^{zyx}	1.65^{z}	0.70^{zyx}	1.17^{zyx}
256	(*Z*)-3-nonenol	alcohol	1,176	1,152	0.29^{zyxw}	0.48^{z}	0.46^{zyx}	0.48^{zy}	0.33^{zyxw}	0.34^{zyxw}	0.17^{zyxw}	0.17^{xw}	0.15^{w}	0.23^{zyxw}	0.20^{zyxw}	0.17^{yxw}
257	(*E*)-2-nonenal	aldehyde	1,177	1,157	0.25	nd	nd	nd	0.47	nd	0.59	1.29	0.68	0.94	0.82	0.80
258	(*E,Z*)-3,6-nonadienol	alcohol	1,178	1,149	3.77^{yxw}	5.97^{zy}	6.32^{z}	4.94^{zyx}	4.35^{zyxw}	4.42^{zyxw}	2.73^{xw}	2.82^{xw}	2.71^{xw}	3.21^{xw}	2.32^{w}	2.23^{w}
259	(*E,Z*)-2,6-nonadienol	alcohol	1,183	1,159	tr	0.03	0.02	0.05	tr	0.02	0.06	0.04	0.02	0.03	tr	0.02
261	(*E*)-2-nonenol	alcohol	1,187	1,163	0.02	0.07	0.05	0.17	0.03	0.04	0.14	0.06	0.03	0.06	0.02	0.04
263	(*Z*)-6-nonenol	alcohol	1,191	1,164	0.25^{zyx}	0.50^{z}	0.41^{zy}	0.33^{zyx}	0.22^{yx}	0.39^{zyx}	0.17^{yx}	0.24^{zyx}	0.22^{yx}	0.33^{zyx}	0.17^{yx}	0.14^{x}
265	nonanol[a]	alcohol	1,193	1,165	1.00^{zy}	1.84^{z}	1.51^{zy}	1.41^{zy}	0.69^{y}	1.36^{zy}	0.58^{y}	0.63^{y}	0.57^{y}	1.02^{zy}	0.64^{y}	0.53^{y}
268	1-(4-methylphenyl)-ethanone	ketone	1,200	1,191[c]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
277	2,4-nonadienal	aldehyde	1,214	1,200[c]	tr	0.01	tr	0.01	tr	0.01	tr	tr	0.01	0.01	tr	tr
286	decanal[a]	aldehyde	1,228	1,201	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.04	0.03	0.03	0.02
293	9-decenol	alcohol	1,234	1,262[d]	tr	0.01	0.01	0.02	tr	0.01	tr	tr	tr	tr	tr	tr
295	(*E,E*)-2,4-nonadienal	aldehyde	1,236	1,210	0.10	0.12	0.09	0.14	0.07	0.08	0.07	0.08	0.16	0.13	0.07	0.09
296	*β*-cyclocitral[a]	aldehyde	1,238	1,217	0.01	0.02	0.02	0.02	tr	0.02	tr	tr	0.01	0.01	tr	0.01
302	2-ethylhexyl acrylate	ester	1,252	1,220[d]	tr	tr	tr	tr	tr	nd	nd	nd	nd	nd	nd	tr
304	3-methyl-3-(4-methyl-3-pentenyl)-2-oxiranecarbaldehyde	aldehyde	1,254	1,236[d]	0.04	0.06	0.05	0.05	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.04
309	neral	aldehyde	1,261	1,235	0.15	0.20	0.22	0.23	0.12	0.20	0.11	0.10	0.11	0.15	0.11	0.15
314	RI1268	other	1,268		tr	tr	tr	tr	tr	tr	0.03	0.02	0.05	0.02	0.02	0.02
316	1,3-bis(1,1-dimethylethyl)-benzene	aromatic hydrocarbon	1,272	1,249[d]	0.02	0.02	0.02	0.03	0.01	0.03	tr	tr	tr	tr	tr	tr
317	geraniol[a]	alcohol	1,277	1,249	tr	0.01	0.01	0.01	tr	tr	tr	tr	tr	tr	tr	tr
319	(*E*)-2-decenal	aldehyde	1,288	1,260	0.04	0.04	0.03	0.04	0.02	0.02	0.02	0.02	0.04	0.03	0.01	0.02
322	geranial	aldehyde	1,294	1,264	0.34	0.43	0.45	0.47	0.25	0.39	0.22	0.21	0.23	0.31	0.24	0.31
329	decanol	alcohol	1,301	1,266	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
339	(*E*)-2,7-octadien-1-yl acetate	ester	1,323	1,388[d]	0.01	0.02	0.02	0.06	tr	0.02	0.02	tr	tr	tr	tr	0.01

(to be continued)

Table 3. (continued)

Code	Compound	Class	RI (Caculated)	RI (Published)^b	NG	NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
342	4-ethyl-2-hexynal	aldehyde	1,328		0.02	0.05	0.05	0.09	0.01	0.02	0.02	0.01	0.01	0.02	tr	0.01
345	undecanal	aldehyde	1,338	1,305	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
349	(*E*,*E*)-2,4-decadienal	aldehyde	1,346	1,315	0.01	0.01	tr	0.01	tr	tr	tr	tr	0.03	0.01	tr	0.01
358	γ-nonalactone	lactone	1,386	1,358	0.03^{zy}	0.06^{zy}	0.07^{zy}	0.09^{z}	0.04^{zy}	0.04^{zy}	0.02^{y}	0.02^{y}	0.02^{y}	0.03^{zy}	0.02^{y}	0.01^{y}
359	(*E*)-2-undecenal	aldehyde	1,395	1,357	0.01	0.01	0.01	0.02	tr	tr	tr	tr	0.02	0.01	tr	tr
374	geranyl acetone^a	ketone	1,478	1,453	1.21	1.44	1.27	1.54	0.84	1.67	0.98	1.02	1.20	1.28	0.95	1.20
377	(*E*)-β-ionone	ketone	1,505	1,487	0.02	0.02	0.02	0.03	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01
380	β-ionone epoxide	epoxide	1,507	1,473^d	0.02	0.02	0.02	0.03	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.02
391	trans-ψ-ionone	ketone	1,601	1,589^d	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr

The volatile relative content was determined by normalizing the areas of the compound peaks to that of the internal standard. Mean values were obtained from three biological replicates for each grafting combination. Retention indices (RI) were calculated using a standard mixture of alkanes ranging from C_8 to C_{20}. The abbreviation 'RI' stands for retention index; 'tr' indicates that the peak was detected, but the value was below 0.0095; 'nd' denotes that the compound was not detected. ^{a}Compounds were identified using authentic volatile standards. ^{b}Published RI for the DB-5 column, unless otherwise stated, were sourced from Adams[32]. ^{c}Published RI on DB-5 column reported on Flavornet and Human Odor Space[58]. ^{d}Published RI on DB-5 column reported on PubChem[59]. $^{z–w}$Different letters in the same rows indicate significant differences according to Tukey's Honestly Significant Difference test at $p < 0.05$.

3-nonenol were present in lower quantities (Table 3). (*E*,*Z*)-3,6-Nonadienol was notably the second most prevalent volatile, contributing green and cucumber-like aromas to the watermelon. This compound is especially influential in the aroma profile of fresh watermelon due to its low olfactory perception threshold[48] and is also a common constituent in the scent profiles of melons (*Cucumis melo*) and cucumbers (*C. sativus*)[49,50]. Other key aroma components in watermelon include (*E*,*Z*)-2,6-nonadienal, nonanal, (*Z*)-3-nonenol, (*E*)-2-nonenal, and (*Z*)-6-nonenal, which are characterized by their low odor detection thresholds and thereby define the fruit's characteristic flavor[19]. Additionally, sulfides, with concentrations ranging from 0.01 to 0.6, are known for imparting the distinctive flavors of onion, garlic, cabbage, and other vegetables and may influence the flavor profile of watermelon[42,51].

Grafting effect of different rootstock types on fruit volatile profiles

A comprehensive analysis of fruit volatile profiles revealed that 119, 121, 118, and 120 volatile compounds were identified in NG, wild watermelon-, bottle gourd-, and pumpkin-grafted watermelons, respectively. Pairwise comparisons were conducted to ascertain the differential accumulation of volatile compounds between NG and grafted watermelons, resulting in the identification of 21 distinct metabolic features (Table 6). Relative to NG, none, five and six volatile compounds were more abundantly accumulated in bottle gourd-, pumpkin- and wild watermelon-grafted fruits, respectively. Conversely, the relative content of two aroma volatiles in bottle gourd-grafted, 10 in pumpkin-grafted, and one in wild watermelon-grafted samples was significantly lower (Fig. 3a). Grafting with different rootstocks did not alter the basic volatile composition of the watermelon fruits, which primarily consisted of ketones, aldehydes, and alcohols. Notably, wild watermelon-grafted fruits exhibited higher alcohol contents (10.16–11.55) than both NG (8.19) and other grafted fruits (4.44–8.73) (Fig. 3b). Research has indicated that wild watermelon rootstocks enhance fruit quality by augmenting sugar, organic acid, and total phenolic content[52]. Furthermore, pumpkin-grafted fruits displayed increased aldehyde levels and decreased alcohol, aromatic hydrocarbon, and ketone levels when compared to NG and other grafted fruits (Fig. 3c–f). The volatile compounds 6-methyl-5-hepten-2-one and (*E*,*Z*)-3,6-nonadienol were the most prevalent in NG, wild watermelon, and bottle gourd-grafted watermelons (Tables 3 & 6). In contrast, hexanal and (*E*,*Z*)-3,6-nonadienol were the first and second most abundant volatiles, respectively, in pumpkin-grafted samples.

Grafting significantly influenced the concentrations of hexanal, (*Z*)-6-nonenal, and 6-methyl-5-hepten-2-one[21], but here only the levels of (*Z*)-6-nonenal exhibited a notable difference, with a substantial increase in pumpkin-grafted samples compared to NG. Petropoulos et al.[22] observed that grafting onto the 'TZ148' rootstock (*C. moschata* × *C. maxima* hybrid) elevated the levels of seven volatile compounds, including (*Z*)-6-nonenal, nonanal, (*E*,*Z*)-2,6-nonadienal, (*Z*,*Z*)-3,6-nonadienol, and (*E*)-2-nonenal in seeded watermelons, a change considered detrimental to the fruit's volatile profile. Additionally, select volatile compounds such as propyl-cyclopentane, 2,4-dimethyl-1-heptene, RI1008, and (*E*,*Z*)-2,6-nonadienal were more concentrated in pumpkin-grafted samples, distinctly differentiating them from other watermelons (Tables 3 & 6). The study by Fredes et al.[20] found increased levels of (*Z*)-6-nonenal and (*E*,*Z*)-2,6-nonadienal, which impart melon-like and cucumber-like aromas, in *Cucurbita*-grafted watermelons, a finding corroborated by our research. Notably, these grafted fruits exhibited elevated levels of (*Z*)-6-nonenol, associated with a pumpkin-like odor and considered adverse to fruit quality[20]. However, the pumpkin rootstocks in the present study did not significantly raise the levels of (*Z*)-6-nonenol. In contrast, acetophenone, (*Z*)-3-nonenol, (*E*,*Z*)-3,6-nonadienol, and γ-nonalactone were markedly more abundant in wild

Table 4. Total number of volatile compounds in each chemical class in watermelon grafted onto different rootstocks.

Class	NG	Type										
		Wild watermelon			Bottle gourd		Pumpkin					
		NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
Acid	0	1	1	1	0	0	0	0	0	0	0	0
Alcohol	28	28	25	27	27	27	26	26	26	26	25	27
Aldehyde	35	34	34	34	35	34	35	35	35	35	35	35
Aromatic hydrocarbon	10	9	10	11	11	11	10	9	9	9	10	8
Epoxide	1	1	1	1	1	1	1	1	1	1	1	1
Ester	5	5	5	5	5	4	3	3	3	4	3	4
Furan	4	4	4	4	4	4	4	4	4	4	4	4
Ketone	22	22	22	23	22	22	22	22	23	22	22	21
Lactone	1	1	1	1	1	1	1	1	1	1	1	1
Monoterpene	2	2	2	2	2	2	2	2	2	1	1	1
Other	7	7	7	7	7	7	7	7	7	7	7	7
Phenol	1	1	1	0	0	0	0	1	0	0	0	1
Sulfide	3	3	3	3	3	3	3	3	3	3	3	3
Total	119	118	116	119	118	116	114	114	114	113	112	113

Table 5. Total relative content of volatile compounds in each chemical class in watermelon grafted onto different rootstocks.

Class	NG	Type										
		Wild watermelon			Bottle gourd		Pumpkin					
		NZ101	YS	YZ1	JX1	SZ111	JC	MX	QY1	ZS	JX2	NZ1
Acid	0	0.01	0	0	0	0	0	0	0	0	0	0
Alcohol	8.19^{abcd}	11.55^{a}	10.99^{ab}	10.16^{abc}	8.33^{abcd}	8.73^{abcd}	5.46^{bcd}	5.47^{bcd}	6.54^{abcd}	7.09^{abcd}	4.96^{cd}	4.44^{d}
Aldehyde	6.49	7.45	5.71	6.77	5.23	7.41	8.17	10.22	12.9	12.37	7.36	9.08
Aromatic hydrocarbon	0.77^{ab}	1.2^{a}	1.07^{ab}	1.12^{a}	0.79^{ab}	1.2^{a}	0.6^{ab}	0.62^{ab}	0.63^{ab}	0.85^{ab}	0.6^{ab}	0.51^{b}
Epoxide	0.02	0.02	0.02	0.03	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.02
Ester	0.03	0.04	0.04	0.1	0.02	0.04	0.03	0.02	0.02	0.03	0.01	0.02
Furan	0.67	0.97	0.71	1.03	0.46	1.19	0.64	0.69	1.09	0.97	0.68	0.66
Ketone	14.6	15.54	15.5	16.39	11.64	17	10.39	9.95	11.88	13.23	10.9	11.21
Lactone	0.03^{ab}	0.06^{ab}	0.07^{ab}	0.09^{a}	0.04^{ab}	0.04^{ab}	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{ab}	0.02^{b}	0.01^{b}
Monoterpene	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.04	0.02	0.01	0.01
Other	0.66	0.81	0.67	0.84	0.57	0.81	0.28	0.35	0.72	0.49	0.28	0.32
Phenol	0.08	0.05	0.05	0	0	0	0	0.02	0	0	0	0.01
Sulfide	0.03	0.11	0.06	0.04	0.33	0.6	0.04	0.06	0.04	0.02	0.3	0.01
Total	31.58	37.82	34.9	36.58	27.44	37.06	25.66	27.44	33.91	35.12	25.13	26.32

The relative content data are presented as the mean based on three biological replicates. In the same row, differing letters indicate a statistically significant difference as determined by Tukey's Honestly Significant Difference test ($p < 0.05$).

watermelon-grafted samples. Mendoza-Enano et al.[53] suggested that a higher concentration of acetophenone might lead to undesirable odors. In line with Gong et al.[40], who reported a positive correlation between TSS and (*Z*)-3-nonenol, the present study found that wild watermelon rootstocks significantly boosted both central soluble solids and (*Z*)-3-nonenol levels in grafted fruits ($r = 0.84$).

A Venn diagram was constructed to analyze the specific volatile compounds unique to NG and grafted watermelons. It revealed that wild watermelon, bottle gourd, and pumpkin grafts contributed three, one, and two exclusive volatile compounds, respectively (Fig. 2c). For instance, 3-octen-2-one was exclusively detected in wild watermelon and pumpkin grafted samples (Table 3). In contrast to the others, wild watermelon-grafted samples lacked (*E*)-2-nonenal, which may be attributed to their elevated levels of (*Z*)-3-nonenol; this compound is known to arise from the reduction and isomerization of (*E*)-2-nonenal[19]. Additionally, propyl-cyclopentane was present in all grafted variants but absent in NG samples. This compound is common in grafted plants and was also identified in *Populus deltoides* following methyl jasmonate treatment[54].

Pairwise comparisons were conducted to identify volatile compounds that differed significantly among the 12 samples (NG and 11 grafted), resulting in the detection of 36 differentially accumulated aroma volatiles in at least one comparison (Table 3). The impact of grafting on watermelon fruit aroma was assessed using PCA, which revealed that PC1 and PC2 accounted for 60.9% and 19% of the total variance, respectively (Fig. 2d). Specifically, PC1 effectively distinguished pumpkin-grafted watermelons based on their relative concentrations of alcohols and aromatic hydrocarbons, such as (*Z*)-3-nonenol, (*Z*)-3-hexenol, (*E*)-3-hexenol, (*Z*)-3-octenol, (*Z,Z*)-3,6-nonadienol, cumene, 1,3-bis(1,1-dimethylethyl)-benzene, propyl-cyclopentane, 2,4-dimethyl-1-heptene, and 2,4-dimethyl-heptane (Table 7). The volatile profiles of watermelon fruits grafted onto citron melon (*C. lanatus* var. *citroides*) showed similarity to those of NG and self-grafted samples[20]. Notably, the NG and the wild watermelon-, bottle gourd-grafted samples clustered closely along PC1, suggesting comparable volatile profiles.

HCA based on the relative concentrations of differentially accumulated volatiles categorized the 12 watermelon samples into two primary clusters (Fig. 2e). All pumpkin-grafted

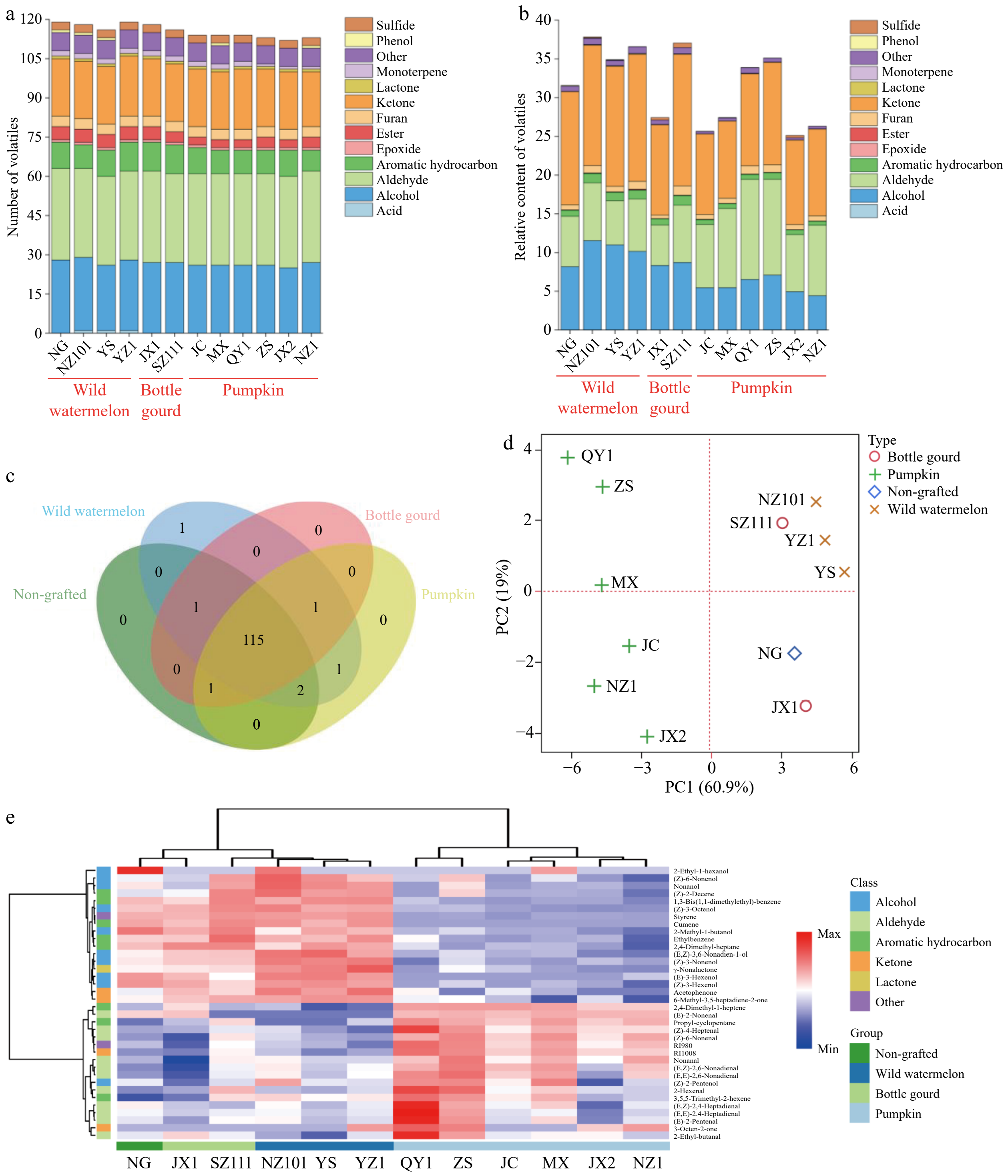

Fig. 2 Multivariate statistical analysis of volatile profiles for 'Ningnongke huadai' watermelon grafted onto different rootstocks. (a) Number, and (b) relative content of volatile compounds in each chemical class. (c) Venn diagram analysis of volatile compounds. (d) PCA score plot of volatile compounds that were identified as differentially accumulated among watermelon samples. (e) HCA of volatile compounds that were identified as differentially accumulated among watermelon samples. Each column in the figure corresponds to a sample, and each row to a volatile compound. The color coding indicates the relative content of volatile compounds in the respective sample groups. On the left, the dendrogram illustrates the clustering of volatile compounds, whereas the top dendrogram shows the clustering of samples, with sample names listed below.

Table 6. Differentially accumulated volatile compounds identified in watermelon grafted onto each rootstock type compared to NG.

Code	Compound	Class	NG	Wild watermelon	Bottle gourd	Pumpkin
3	2-methyl-1-butanol	alcohol	0.14a	0.1a	0.12a	0.05b
43	propyl-cyclopentane	aromatic hydrocarbon	nd b	tr b	tr b	0.01a
48	2,4-dimethyl-1-heptene	aromatic hydrocarbon	tr b	tr b	tr b	0.01a
51	(*E*)-3-hexenol	alcohol	0.01ab	0.01a	tr bc	tr c
55	(*Z*)-3-hexenol	alcohol	0.22ab	0.21a	0.16b	0.05c
65	hexanol	alcohol	1.12a	0.83ab	0.86ab	0.55b
70	styrene	other	0.33a	0.46a	0.41a	0.02b
92	cumene	aromatic hydrocarbon	tr a	0.01a	tr a	nd b
117	2-methyl-1-hepten-6-one	ketone	0.02a	0.01ab	0.01ab	0.01b
118	(*Z*)-3-heptenol	alcohol	0.02a	0.01ab	0.01ab	tr b
128	phenol	phenol	0.08a	0.03ab	nd b	0.01b
154	RI1008	ketone	tr b	tr b	0.01b	0.03a
161	(*Z*)-2-decene	aromatic hydrocarbon	0.57bc	0.89a	0.76ab	0.5c
163	2-ethyl-1-hexanol	alcohol	0.01a	tr b	nd b	tr b
191	acetophenone	ketone	tr b	tr a	tr b	tr b
192	6-methyl-3,5-heptadiene-2-one	ketone	tr bc	tr a	tr ab	tr c
227	(*Z*)-6-nonenal	aldehyde	0.24b	0.33b	0.43b	1.2a
254	(*E*,*Z*)-2,6-nonadienal	aldehyde	0.54b	0.86ab	0.61b	1.24a
256	(*Z*)-3-nonenol	alcohol	0.29bc	0.47a	0.34b	0.18c
258	(*E*,*Z*)-3,6-nonadienol	alcohol	3.77bc	5.74a	4.39b	2.67c
358	γ-nonalactone	lactone	0.03b	0.07a	0.04b	0.02b

The relative volatile content was quantified by normalizing the peak area of each compound to the peak area of the internal standard. The values represent the mean of three biological replicates per type. The notation 'tr' indicates a detected peak with a value below 0.0095, while 'nd' signifies not detected. Variations in letters within the same rows denote statistically significant differences as determined by Tukey's Honestly Significant Difference test ($p < 0.05$).

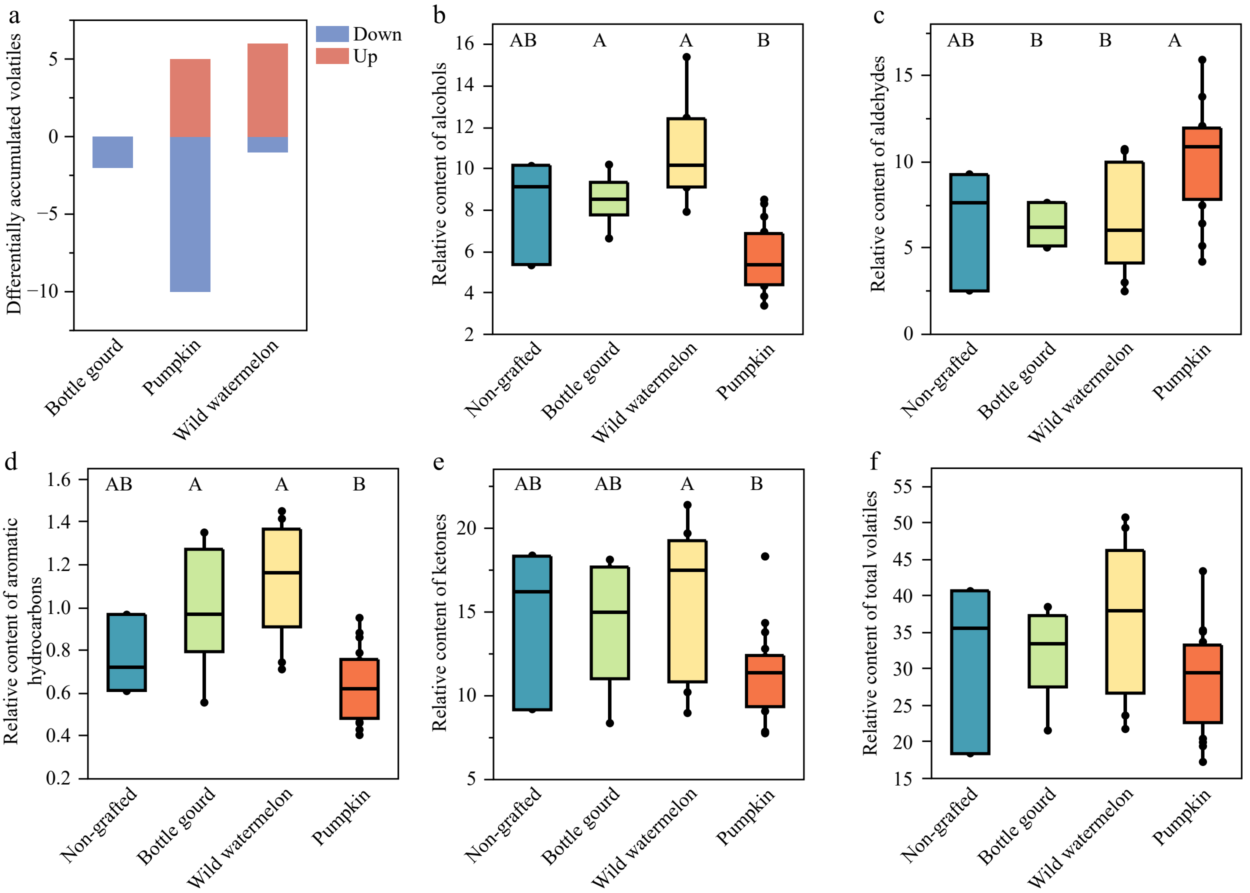

Fig. 3 Comparative analysis of volatile compounds in 'Ningnongke huadai' watermelon grafted onto different rootstock types. (a) Number of differentially accumulated volatile compounds identified in watermelons grafted onto each rootstock type compared to the non-grafted watermelons ($p < 0.05$). Red bars represent volatile compounds that were more abundant, while blue bars indicate those that were less abundant in the grafted watermelons compared to the non-grafted. The boxplots show the relative contents of volatiles in each chemical class among watermelon samples including (b) alcohols, (c) aldehydes, (d) aromatic hydrocarbons, (e) ketones, and (f) total volatiles. Groups denoted by the same letter are not significantly different as determined by Tukey's honestly significant difference test at $p < 0.05$.

Table 7. Volatile compounds with high contribution to the PC1 and PC2 in Fig. 1d.

Code	Compound	Class	PC1	PC2
70	styrene	other	0.94	
92	cumene	aromatic hydrocarbon	0.91	
256	(*Z*)-3-nonenol	alcohol	0.90	
55	(*Z*)-3-hexenol	alcohol	0.90	
51	(*E*)-3-hexenol	alcohol	0.89	
316	1,3-bis(1,1-dimethylethyl)-benzene	aromatic hydrocarbon	0.87	
183	(*Z*)-3-octenol	alcohol	0.87	
154	RI1008	ketone	0.86	
139	RI980	other	0.81	
258	(*Z*,*Z*)-3,6-nonadienol	alcohol	0.81	
43	propylcyclopentane	aromatic hydrocarbon	0.80	
80	(*Z*)-4-heptenal	aldehyde	0.80	
48	2,4-dimethyl-1-heptene	aromatic hydrocarbon	0.78	
38	2,4-dimethyl-heptane	aromatic hydrocarbon	0.78	
358	γ-nonalactone	lactone	0.77	
122	3,5,5-trimethyl-2-hexene	aromatic hydrocarbon		0.66
20	(*Z*)-2-pentenol	alcohol		0.61
13	(*E*)-2-pentenal	aldehyde		0.60
149	(*E*,*E*)-2,4-heptadienal	aldehyde		0.52
263	(*Z*)-6-nonenol	alcohol		0.45
52	2-hexenal	aldehyde		0.43
247	(*E*,*E*)-2,6-nonadienal	aldehyde		0.43
138	(*E*,*Z*)-2,4-heptadienal	aldehyde		0.42

watermelons grouped in one cluster, while the remaining samples formed the second cluster. The volatile profiles of pumpkin-grafted watermelons differed markedly from the others, characterized by higher levels of aldehydes and lower levels of alcohols and aromatic hydrocarbons. C6 and C9 aldehydes and alcohols are mainly regulated by genes such as *LOX* and *ADH* in the lipoxygenase pathway[55]. Due to the possible remarkable deterioration of fruit shape and taste, the *Cucurbita* spp. was rarely used as a rootstock for melon[15]. The HCA results were consistent with previous PCA findings, which indicated that the bottle gourd- and wild watermelon-grafted samples showed no clear differentiation from NG samples, whereas pumpkin-grafted watermelons had unique volatile profiles, resulting in a distinct watermelon fruit aroma. Similarly, pumpkin rootstocks significantly reduced the odor intensity and odor preference scores of the grafted melons decreased the concentration or even caused the absence of main aroma components, and down-regulated ADH and AAT activity and the expression levels of *CmADH* and *CmAAT* homologs, while muskmelon rootstocks displayed no significant grafting effect[56].

Grafting effect of different rootstocks on fruit volatile profiles

To elucidate the influence of 11 different rootstocks on watermelon fruit aroma, a comparative analysis of volatile compound profiles were performed. The total volatile content ranged from 25.31 in JX2 to 37.82 in NZ101. Relative to NG samples, six grafted watermelons exhibited higher total volatile concentrations. Notably, 'Sizhuang 111' (SZ111, 15.05) and 'Mingxiu' (MX, 8.68) demonstrated the highest and lowest concentrations of 6-methyl-5-hepten-2-one, respectively. The abundance of aldehydes varied significantly, with QY1 registering the highest (12.9) and 'Jingxinzhen 1' (JX1, 5.23) the lowest levels, predominantly due to differences in hexanal, (*E*,*Z*)-2,6-nonadienal, nonanal, and (*Z*)-6-nonenal accumulation. NZ101 contained the most alcohols (11.55), particularly (*E*,*Z*)-3,6-nonadienol and nonanol, while the least was found in NZ1 (4.44). Together, alcohols and aldehydes comprised over half of the 36 distinct volatiles identified among the 12 watermelon samples. Specifically, C_6 and C_9 alcohols and aldehydes, such as (*E*,*Z*)-3,6-nonadienol, nonanol, hexanal, and nonanal, are recognized as characteristic watermelon aroma compounds[4,43,57]. Grafting notably affected the relative concentrations of several C_6 and C_9 alcohols and aldehydes, including (*E*)-3-hexenol, (*Z*)-3-hexenol, (*E*,*Z*)-3,6-nonadienol, (*Z*)-3-nonenol, (*Z*)-6-nonenol, nonanol, (*E*)-2-nonenal, (*E*,*E*)-2,6-nonadienal, nonanal, (*E*,*Z*)-2,6-nonadienal, 2-hexenal, and (*Z*)-6-nonenal (Table 3). According to prior research, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal (fatty, green and waxy odors), and (*Z*)-3-nonenol (fresh, waxy and green odors) dominate the aroma profile of watermelon flesh[20]. Volatile compounds such as (*E*)-2-nonenal, (*Z*)-6-nonenal, and (*Z*)-6-nonenol correlated positively with watermelon fruit preference[53] and were found in higher levels in pumpkin rootstocks ZS and MX.

PCA more effectively assessed the relationships among various watermelon samples (Fig. 2d). The close clustering of JX1 and YS with NG indicated that grafting watermelons onto them did not significantly alter their fruit volatile profiles. Distinctly, QY1 and ZS were separated from other pumpkin-grafted samples on PC2, primarily due to their elevated levels of volatile compounds such as 3,5,5-trimethyl-2-hexene, (*E*)-2-pentenal, (*E*,*E*)-2,4-heptadienal, 2-hexenal, and (*E*,*Z*)-2,4-heptadienal. The compounds acetophenone, dimethyl trisulfide, and (*E*,*E*)-2,4-heptadienal have been identified as key contributors to off-odor, substantially impacting the overall flavor preference of stored watermelon samples[53]. HCA grouped NG and bottle gourd-, wild watermelon-grafted samples together, reflecting their similar volatile profiles (Fig. 2e). The effects of different rootstocks on the volatile components and overall flavor quality of grafted watermelons are complex due to varietal and environmental differences. Investigating the underlying mechanisms for the alterations of volatile compounds in grafted watermelons is essential for future research. For example, studies on small RNAs or mobile proteins within the phloem will help reveal the interaction mechanisms between rootstock and scion and explore the influence of grafting on flavor quality in watermelon.

Conclusions

In summary, this study provides a comprehensive analysis of the volatile composition and their abundance in watermelon fruits, alongside fruit quality attribute assessments, to elucidate the impact of different rootstocks on grafted watermelons. The findings indicate that the choice of rootstock significantly influences the flavor quality of grafted fruit. Notably, wild watermelon rootstocks increased central soluble solids compared to NG, whereas pumpkin rootstocks enhanced fruit weight and flesh firmness but reduced central soluble solids. Watermelons grafted onto wild rootstocks YS and NZ101 exhibited higher central and edge soluble solids than NG. Using HS-SPME-GC-MS, the study identified 122 volatile compounds, with ketones as the predominant chemical group, followed by alcohols and aldehydes. Remarkably, 6-methyl-5-hepten-2-one was the most abundant volatile, along with (*E*,*Z*)-3,6-nonadienol and hexanal.

The influence of grafting on volatile profiles varied among rootstocks. Wild watermelon grafts had the highest levels of total volatile compounds, while pumpkin grafts had the least. Bottle gourd and wild watermelon grafts exhibited volatile compositions closely resembling those of NG. Grafting watermelons onto JX1, YS, YZ1, SZ111, and NZ101 did not significantly alter their fruit volatile profiles. In contrast, pumpkin grafts were particularly rich in aldehydes but deficient in alcohols and aromatic hydrocarbons, indicating significant differences in fruit volatile profiles. Watermelons grafted onto QY1 and ZS exhibited a highly distinct volatile composition from NG, primarily due to their elevated aldehydes. The wild watermelon rootstocks increased soluble solids and demonstrated a minimal impact on fruit volatile profiles. Consequently, wild watermelon rootstocks, such as YS and NZ101, with robust growth potential and strong disease resistance, were recommended to effectively mitigate the negative grafting effects on flavor quality in 'Ningnongke huadai' watermelon production. This research lays the groundwork for understanding how different rootstocks affect watermelon fruit quality and underscores the importance of considering fruit aroma in watermelon rootstock breeding programs. Future research should explore the molecular mechanisms driving aroma formation in various watermelon grafting combinations.

Author contributions

The authors confirm contribution to the paper as follows: study design and coordinating: Yu Y, Zhang Y, Li H; sample collecting and experiment performing: Yang W, Zhou J, Yu R; data analysis: Du H, Tian M, Guo S; writing manuscript with input from all authors: Yu Y, Zhang Y. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

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Conflict of interest

The authors declare that they have no conflict of interest.

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