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Variation Patterns of the Volatile Compounds in Flowers of Chinese Native Citrus Species and Their Taxonomic Implications

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Abstract In this study, the volatile compounds in the flowers of nine Citrus species/varieties, seven of which are native to China, were analyzed using headspace-solid phase microextraction (HS-SPME) coupled with gas chromatography mass spectrometry (GC-MS). A total of 94 compounds were identified, including various terpenes, such as monoterpenes, sesquiterpenes, terpene alcohols and aldehydes, which together accounted for 80.4% to 92.4% of the total compounds analyzed. Limonene, linalool and γ-terpinene were the dominant terpenes. Different species/varieties were characterized by their volatile compounds. Papeda was characterized by a high level of β-ocimene, linalyl acetate, myrcene and neo-alloocimene; Citrophorum was characterized by a high level of limonene and caryophyllene, and Cephelocitrus by a high level of limonene, β-pinene and linolool. Sinocitrus had the highest amount of linolool. Sweet orange had the highest level of limonene, while sour orange was distinct from others with the highest level of γ-terpinene. The four basic types of the genus *Citrus* L., Papeda, Cephalocitrus, Citrophorum and Sinocitrus, can be clearly classified based on a cluster analysis of their volatile compounds. All of the presumed hybrid species, including Jinchengbeibei 447 (*C. sinensis* Osb.), Goutoucheng (*C. aurantium* L.), Ningmeng 4 (*C. limon* Burm.f.), and Changshanhuyou (*C. paradisi* cv. Changshanhuyou), were grouped closely together with a suggested parent species in the constructed dendrogram. Our study clearly demonstrates that Citrus flower volatile compounds and their variation patterns can be used for Citrus species identification and taxonomic study.

Keywords: *Citrus, volatile compounds, variation patterns, chemotaxonomy genus*

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1. Introduction

Citrus is one of the most important fruit crops in the world (Hwang et al. 2012). Citrus fruits are popular food items because of their beautiful colors, pleasant flavors, and rich nutritional components (Tripoli et al. 2007). Citrus fruits can be consumed fresh or be processed into desserts, juices and jams and are good resources of vitamin C, folic acid, flavonoids, dietary fiber and many other health-promoting substances (Miller et al. 2011). In the food industry, citrus processing produces an enormous amount of byproducts, such as pomace, which represents 50% of the raw fruits and is a good source of volatile compounds (Anwar et al. 2008; Omori et al. 2011). In addition, citrus essential oils, which are a mixture of volatile compounds consisting mainly of monoterpene hydrocarbons, have been widely used in food, pharmaceutical and cosmetic industries as flavoring agents, and as antibacterial, antifungal and antioxidant agents (Ferhat et al. 2006).

The volatile compounds of cultivated citrus species have been widely studied. Fisher and Phillips reported that the basic components of citrus volatiles were terpenes, aldehydes, ketones, alcohols, esters and other compounds (Fisher et al. 2008). Jabalpurwala et al. (2009) compared the blossom volatile compounds of grapefruit, sour orange, sweet orange, lemon, mandarin, pumelo and lime and found that the citrus volatile profiles are primarily quantitative. Flamini et al. (2007) compared the volatiles of the different organs of Citrus lemon and found that young leaves have higher amount of limonene than the old ones. In addition, the chemical polymorphisms in the blossom oils of *Citrus aurantium* var. amara cultivated in different provinces were analyzed (Boussaada et al. 2007; Liu et al. 2013). Chung (2012) compared the volatile compounds of liquid essential oils and volatiles of the

Hallabong blossom with two different fibers (CAR/PDMS and PAMS fibers). From the reports mentioned above, we found that many factors, such as genotype, province, season, developmental stage, extraction and analytical methods, influence the compositions of citrus volatiles (Liu et al. 2013; Cheong et al. 2011; González-Mas et al. 2011; Vekiari et al. 2001).

China is the most significant origin of the genus Citrus L. (Cheong et al. 2011). More than 4000 years of history of citrus cultivation have been recorded in China, and many important citrus species/varieties native to China have been described, such as Citrus ichangesis Swingle, *Citrus grandis* Osbeck, *Citrus reticulata* Blanco and *Citrus aurantium* L. (Al-Ababneh et al. 2002; Zhang et al. 2004; Spiegel et al. 1996). The bioactive components in Papeda, the texture and flavor of Guangxishatianyou, the color of Taiwanpenggan, and the resistance to stress of *Citrus aurantium* L. of these native Chinese germplasms have been investigated in previous studies (Spiegel et al. 1996). Citrus leaves, blossoms, fruit and the exocarp of some species, such as *Citrus aurantiu* L., *Citrus medica* L. and *Citrus grandis* (L.) Osbeck, have traditionally been used in Chinese medicines for their anti-inflammatory, cough relief and lung-purging properties (Lu et al. 2006). Despite the great economic and medicinal potential for citrus plants, information regarding the volatile components of various wild Citrus species, especially those native to China, is still limited (Jabalpurwala et al. 2009; González-Mas et al. 2011; Fanciullino et al. 2006; Hosni et al. 2010).

In this study, in an attempt to analyze the influence of genetic and/or evolutionary factors on the composition and contents of citrus volatile components, we analyze the volatile compounds in the flowers of basic Citrus types and their presumed hybrids, which are mostly represented by the genotypes of Chinese origin. We also explore the influence of hybridization on the variation of Citrus volatile compounds. The results provide useful information for future germplasm utilization and the taxonomy of the Citrus genus.

2. Materials and Methods

2.1. Plant Material

Blossoming flowers were collected from trees cultivated at the China National Citrus Germplasm Repository, Citrus Research Institute of Chinese Academy of Agricultural Sciences, Chongqing, China. For each species/variety, three trees were selected. All three of the basic species of the subgenus Eucitrus of Swingle's system, *C. medica* L., *C. grandis* Osbeck., *C. reticulata* Blanco. and Papeda were represented. The voucher specimens were returned to the Citrus Research Institute of Chinese Academy of Agricultural Sciences (CRIC), Chongqing, China. The information about the samples is given in detail in Table 1.

Table 1. The Citrus species analyzed in the present study

NO.[a]	Citrus species	Cutivars[b]	Abbreviation	Section name	National Unified number	Repository number[b]
1	*Citrus sinensis.* Osbeck	Jinchengbeibei 447	JCBB	Aurantium	LS0306	GPGJ0573
2	*C. aurantium* L.	Goutoucheng	GTC	Aurantium	LA0008	GPGJ0011
3	*C. ichangensis* Swingle	Yichangcheng	YCC	Papeda	LP0067	GPGJ0701
4	*C. reticulata* Blanco	Edanhongju	EDHJ	Sinocitrus	LR0040	GPGJ0059
5	*C. reticulata* Blanco	Taiwanpenggan	TWPG	Sinocitrus	LR0658	GPGJ1093
6	*C. grandis* Osbeck	Guangxishatianyou	GXSTY	Cephalocitrus	LG0016	GPGJ0100
7	*C. paradis* Macf.	Changshanhuyou	CSHY	Cephalocitrus	LG0181	GPGJ0645
8	*C. limon* (L). Burm.f.	Ningmeng 4	SHNM	Citrophorum	LM0183	GPGJ1001
9	*C. medica* L	Dannaxaingyuan	DNXY	Citrophorum	LM0026	GPGJ0539

[a] Number of sample

[b] Repository Number of the National Citrus Germplasm Repository, Chongqing, China.

2.2. Isolation and Concentration of Volatiles

Freshly collected flowers were taken to the laboratory immediately, washed using deionized water and patted dry with gauze. The cleaned flowers were immediately ground into powder and stored at -80°C until use. The isolation and concentration of flower volatiles was carried out by headspace-solid phase microextraction (HS-SPME) method. For each extraction, 1.5 g of the powder, 3 ml of a saturated solution of sodium chloride and 2 μL of cyclohexanone (added as an internal standard) were added to a 20-mL capped vial. The sealed vials were analyzed by HS-SPME. A temperature of 50°C was selected for pre-incubation and headspace extraction based on a previous study (Liu et al. 2013). Pre-incubation and extraction times were 20 min and 50 min, respectively. Desorption was performed for 5 min at 250 °C in splitless mode. All experiments were performed in triplicate.

2.3. GC-MS Conditions

The GC-MS analyses were carried out using an Agilent 7890A gas chromatograph coupled to a flame ionization detector and a 5975C single quadrupole mass detector. A DB-5MS silica capillary column (30 m×0.25mm×0.25μm film thickness) was used. The initial temperature of column oven was held constant at 35°C for 5 min, followed by an increase to 180°C at 3°C min^{-1} ramp and was held constant at 250°C for 10 min. The temperature was then increased to 240°C at 5°C min^{-1} (held for 2 min). Helium was used as the carrier gas at 1 mL min^{-1} constant flow rate, and the mass spectrometer operated in the electron impact (EI) mode (ionization energy, 70 eV; source temperature 230°C). Full scanning mode was used for the data acquisition, and the mass range was m/z 35-400.

Table 2. The chemical compositions and their contents of in the flower volatiles of the Citrus species analyzed.

No.	RI[a]	CAS[b]	Compounds	Codes[c]	Genotypes[d, f, g]								
					JCBB	GTC	YCC	EDHJ	TWPG	GXSTY	CSHY	SHNM	DNXY
			Monoterpenes										
1	993	123-35-3	myrcene	M1	396.3±18.8	369.3±21.4	**542.9±32.1**	158.4±9.84	488.3±39.2	90.4±7.2	112.5±6.56	348.6±32.5	nd
2	925	2867-5-2	α-thujene	M2	nd	52.11±3.21	nd	20.03±1.56	118.9±8.23	3.56±0.31	nd	10.53±1.26	nd
3	929	7785-26-4	(-)-α-pinene	M3	22.3±1.06	nd	nd	nd	nd	nd	nd	nd	61.94±4.88
4	930	80-56-8	α-pinene	M4	64.39±2.98	256.6±17.5	nd	57.19±4.55	156.1±9.54	67.31±5.15	66.21±4.69	203.2±18.4	nd
5	932	99-83-2	α-phellandrene	M5	26.04±3.15	252.8±19.1	nd	nd	32.36±2.06	nd	10.47±0.84	nd	4.71±0.25
6	944	79-92-5	camphene	M6	2.78±0.39	3.79±0.18	nd	nd	nd	7.29±5.16	nd	13.74±2.15	nd
7	975	127-91-3	β-pinene	M7	3.79±0.26	322.1±18.2	nd	nd	nd	**954.2±69.8**	**325.7±26.5**	**1629±89.3**	nd
8	976	3387-41-5	sabinene	M8	nd	nd	nd	nd	nd	nd	nd	nd	nd
9	982	18172-67-3	(-)-β-pinene	M9[e]	nd	nd	nd	94.07±6.25	nd	nd	nd	nd	144.4±9.8
10	998	460-01-5	cosmene	M10[e]	nd	nd	13.81±1.23	nd	nd	10.83±1.65	nd	nd	nd
11	1016	99-86-5	α-terpinene	M11	93.67±6.91	53.37±4.91	2.44±0.25	14.13±2.12	147.3±9.84	6.87±0.58	nd	1.51±0.01	4.05±0.26
12	1021	99-87-6	p-cymene	M12	nd	469.2±23.3	nd	97.8±6.35	188.2±9.21	nd	154.9±9.2	5.29±0.38	nd
13	1029	138-86-3	limonene	M13	**1016±59.8**	436±25.3	60.91±4.53	268.8±18.9	382.8±35.2	**1073±65.4**	**268.6±19.4**	**5650±145**	**5224±89.1**
14	1039	3338-55-4	(z)-ocimene	M14	410.1±25.6	10.93±1.45	nd	6.24±0.42	nd	43.99±5.21	nd	171.2±13.2	210.9±19.5
15	1044	13877-91-3	β-ocimene	M15[e]	3.79±0.29	**924.3±56.6**	**1940±98.5**	831.9±75.6	1304±56.4	nd	346.5±26.8	nd	532.4±17.8
16	1055	3779-61-1	(e)-ocimene	M16	nd	nd	396.5±26.9	nd	nd	**1173±105.2**	nd	1173±96.4	nd
17	1060	13466-78-9	3-carene	M17	42.83±3.61	nd	19.33±3.11	nd	nd	nd	nd	23.47±2.19	nd
18	1064	99-85-4	γ-terpinene	M18	151.4±9.7	**1591±105**	1.44±0.21	428.6±32.5	847.5±85.3	16.66±0.85	**920.1±56.8**	735.5±14.2	258.8±14.5
19	1088	586-62-9	δ-terpinene	M19	52.14±3.71	nd	19.25±2.15	nd	nd	11.7±1.23	68.57±4.15	66.56±4.56	nd
20	1131	7216-56-0	neo-alloocimene	M20	29.49±2.94	45.31±3.15	**364.3±15.4**	47.35±4.11	56.83±3.25	57.72±6.35	14.15±2.11	171.1±8.22	66.99±4.56
			Total		3925±209	4787±196	3367±205	2024±147	5533±213	3516±236	2288±89	10203±369	6508±185
			Relatives (%)		61.33%	60.35%	53.05%	38.04%	51.52%	54.60%	53.89%	82.59%	57.00%
			Sesqueterpenes										
21	1336	20307-84-0	elemene	S1	nd	97.97±6.15	22.75±3.21	63.57±1.97	70.73±4.05	35.59±2.38	27.77±1.66	27.96±3.09	nd
22	1348	17699-14-8	α-cubebene	S2	2.78±0.36	10.97±1.56	77.18±5.61	3.41±0.48	4.05±0.51	1.88±0.24	3.94±0.28	1.15±0.24	22.1±1.46
23	1374	3856-25-5	copaene	S3	7.29±0.82	19.98±2.03	229.7±15.6	6.31±0.31	7.25±0.56	1.75±0.24	nd	1.07±0.13	15.16±1.22
24	1383	5208-59-3	β-bourbonene	S4[e]	nd	nd	26.6±0.31	nd	nd	nd	nd	nd	nd
25	1391	515-13-9	β-elemene	S5	103.4±8.2	30.77±2.96	41.85±3.03	27.44±1.47	34.98±2.65	9.85±1.07	160.9±10.6	2.48±0.25	nd
26	1406	17699-05-7	α-bergamotene	S6	10.9±1.06	9.58±1.23	2.14±0.15	nd	25.4±2.33	nd	11.93±0.84	152.3±9.14	535.7±25.5
27	1419	87-44-5	caryophyllene	S7	92.09±6.53	299.6±35.6	552.9±63.6	69.95±4.54	73.9±5.71	219.2±18.3	160.1±10.7	414.3±19.6	897.8±69.8
28	1427	13744-15-5	β-cubebene	S8	nd	9.65±1.24	9.82±1.02	2.88±0.18	5.78±0.41	2.71±0.36	5.33±0.29	nd	17.8±1.57
29	1433	339154-91-5	γ-elemene	S9	nd	72.63±8.62	55.71±4.76	62.13±8.27	68.32±4.36	8.51±1.07	53.8±0.67	nd	nd
30	1436	13474-59-4	trans-α-bergamotene	S10	nd	nd	40.95±	nd	nd	nd	nd	nd	nd

							2.17						
31	1439	3691-12-1	α-guaiene	S11^{e}	nd	nd	12.7±1.08	nd	nd	nd	2.77±0.32	nd	nd
32	1441	25246-27-9	l-alloaromadendrene	S12^{e}	nd	14.17±2.13	nd	15.92±1.36	nd	nd	nd	0.92±0.13	nd
33	1451	6753-98-6	α-caryophyllene	S13^{e}	15.59±1.36	32.53±2.98	74.32±8.16	14.11±1.29	14.41±0.97	20.96±1.56	34.55±3.05	**30.9±2.38**	**71.53±6.28**
34	1458	18794-84-8	β-farnesene	S14	146.2±10.2	nd	nd	nd	nd	9.31±1.12	193.2±15.7	9.32±1.03	19.82±3.21
35	1460	28973-97-9	(z)-β-farnesene	S15^{e}	nd	253.3±19.1	46.59±6.31	1.54±0.18	356.5±26.3	nd	nd	6.33±0.54	19.51±1.45
36	1475	30021-74-0	γ-muurolene	S16^{e}	nd	nd	41.91±3.16	5.33±0.41	4.76±0.39	2.39±0.27	6.89±0.81	1.53±0.22	11.01±0.96
37	1480	37839-63-7	germacrene d	S17	nd	59.46±3.69	27.24±1.87	18.59±2.34	25.34±2.99	12.62±1.97	34.81±4.56	nd	77.75±8.22
38	1493	473-13-2	(-)-α-selinene	S18^{e}	1.87±0.25	nd	18.05±2.13	nd	nd	nd	17.72±0.54	nd	nd
39	1495	3242-8-8	elixene	S19^{e}	nd	nd	nd	40.35±3.62	53.69±4.58	19.43±2.55	nd	29.94±1.67	29.66±3.11
40	1506	3691-11-0	δ-guaiene	S20^{e}	nd	nd	92.62±7.54	nd	nd	nd	nd	nd	nd
41	1511	495-61-4	l-β-bisabolene	S21	nd	nd	193.3±23.8	48.97±6.57	nd	nd	nd	149.4±6.5	586.9±37.7
42	1512	26560-14-5	α-farnesene	S22	32.99±4.12	nd	nd	nd	110.2±9.85	26.12±3.24	4.81±0.57	nd	nd
43	1522	483-76-1	δ-cadinene	S23	15.63±2.14	41.25±2.98	nd	13.47±1.07	nd	4.28±0.31	21.62±3.88	0.31±0.05	31.48±4.15
44	1523	20307-83-9	β-sesquiphellandrene	S24	nd	nd	nd	nd	37.45±4.15	nd	nd	nd	nd
45	1525	54324-03-7	(+)-epi-bicyclosesquiphellandrene	S25^{e}	nd	nd	nd	nd	nd	nd	nd	nd	nd
46	1537	4630-07-3	(+)-valencene	S26^{e}	nd	nd	nd	nd	nd	nd	nd	nd	15.35±1.47
47	1742	10486-19-8	valencene	S27	nd	nd	nd	nd	nd	nd	nd	nd	17.8±0.82
			Total		428.7±56.4	1003±84.2	1677±205	395.2±78.7	892.8±54.1	375.8±29.8	740.2±58.6	827.7±54.1	2369±145
			Relatives (%)		6.70%	12.65%	26.42%	7.43%	8.31%	5.84%	17.43%	6.70%	20.75%
Terpene Alcohols													
48	1068	7299-41-4	cis-β-terpineol	TA1^{e}	22.31±3.27	1.74±0.19	nd	nd	45.49±3.67	1.26	nd	5.03±0.37	nd
49	1102	78-70-6	linalool	TA2	**1097±127**	**1198±105**	34.65±4.31	**2099±175**	**2793±308**	1160±159	489.3±24.3	129.8±15.8	64.97±5.14
50	1124	1960-12-8	phenethyl alcohol	TA3^{e}	10.27±2.15	1.33±0.23	nd	10.41±2.15	nd	nd	17.43±2.65	nd	nd
51	1176	20126-76-5	(-)-4-terpineol	TA4^{e}	nd	11.07±1.45	nd	nd	82.01±6.24	nd	4.16±0.37	13.47±1.65	nd
52	1188	562-74-3	4-terpineol	TA5	46.79±2.15	nd	nd	5.19±0.41	nd	nd	nd	nd	12.13±1.57
53	1189	10482-56-1	α-terpineol	TA6	61.61±2.39	57.88±4.71	2.66±0.29	11.18±1.67	132.9±13.8	6.19±0.34	7.69±0.64	55.78±6.71	57.5±3.66
54	1218	1197-06-4	cis-carveol	TA7	nd	1.39±0.31	nd	nd	nd	4.12±0.19	nd	16.14±2.14	44.38±3.25
55	1295	499-75-2	carvacrol	TA8^{e}	nd	83.02±6.34	nd	165.9±12.3	168.1±21.4	0.25±0.04	nd	nd	nd
56	1229	106-25-2	nerol	TA9	6.65±0.57	2.61±0.32	nd	nd	nd	148.4±16.7	11.89±13.4	17.75±2.11	450.4±38.9
57	1256	106-24-1	geraniol	TA10	1.55±0.17	6.85±0.57	nd	1.79±0.24	12.31±1.95	102.5±9.45	14.08±2.07	32.66±6.17	345.3±28.3
58	1565	7212-44-4	nerolidol	TA11	71.89±6.35	nd	nd	7.57±0.87	15.5±1.21	195.2±23.6	nd	55.38±4.36	nd
59	1584	142-50-7	d-nerolidol	TA12	nd	nd	nd	nd	nd	nd	85.51±7.88	nd	49.41±5.16
60	1690	20576-54-9	2,3-dihydro-6-trans-farnesol	TA13^{e}	nd	nd	nd	nd	nd	nd	nd	14.86±1.27	28.29±3.34
61	1731	106-28-5	(e,e)-farnesol	TA14	nd	nd	nd	nd	nd	nd	nd	17.1±1.69	nd
62	1735	4602-84-0	farnesol	TA15	6.33±	nd	nd	nd	1.55±	30.7±	18.83±	8.84±	27.61±

					0.58				0.37	2.19	3.12	0.97	4.22
			Total		1324± 54.3	1364± 78.1	37.31± 2.68	2301± 125	3251± 189	1649± 65.4	649.3± 56.4	367.2± 24.1	1080± 84.8
			Relatives (%)		20.70%	17.19%	0.59%	43.25%	30.27%	25.60%	15.28%	2.97%	9.46%
Terpene Aldehydes													
63	1154	106-23-0	citronellal	TD1	4.46± 0.57	nd	nd	1.29± 0.32	17.17± 2.15	24.82± 3.61	8.55± 0.95	23.1± 2.67	58.71± 4.94
64	1221	18031-40-8	l-perillaldehyde	TD2[e]	2.91± 0.27	nd	nd	nd	14.56± 1.22	nd	nd	nd	nd
65	1243	106-26-3	neral	TD3	5.34± 0.62	1.33± 0.07	nd	nd	4.24± 0.58	173.4± 21.5	13.46± 0.41	164.3± 16.7	349.9± 45.4
66	1273	141-27-5	geranial	TD4	nd	nd	1.57± 0.28	nd	5.67± 0.36	nd	nd	nd	nd
67	1697	4955-32-2	1,3,6,10-farnesatetraen-12-al	TD5[e]	7.15± 0.67	nd	nd	nd	10.35± 1.23	nd	nd	nd	nd
			Total		19.86± 2.19	1.33± 0.18	1.57± 0.16	1.29± 0.08	51.99± 6.7	418.4± 32.5	39.25± 4.7	421.5± 26.4	408.6± 33.8
			Relatives (%)		0.31%	0.02%	0.02%	0.02%	0.48%	6.50%	0.92%	3.41%	3.58%
Aliphatic Adehydes And Alcohols													
68	798	66-25-1	hexanal	P1	85.52± 5.69	60.45± 4.94	42.27± 3.61	97.57± 5.99	96.75± 6.37	26.77± 4.04	101.5± 9.31	58.01± 6.62	91.41± 8.08
69	849	6728-26-3	trans-2-hexenal	P2[e]	230.2± 17.5	140.9± 12.7	114.9± 10.5	91.57± 5.27	121.3± 11.8	144.2± 15.9	188.4± 13.5	171.1± 21.4	45.22± 6.36
70	957	100-52-7	benzaldehyde	P3	18.77± 2.55	7.87± 1.21	nd	19.84± 1.19	36.15± 2.08	nd	29.33± 3.02	nd	4.22± 0.26
71	1042	122-78-1	benzeneacetaldehyde	P4	112.8± 9.57	13.03± 2.12	nd	51.34± 4.17	201.6± 16.2	nd	117.9± 19.8	nd	nd
72	1115	124-19-6	nonanal	P5[e]	nd	nd	nd	nd	0	nd	nd	nd	25.95± 3.65
73	1720	2765-11-9	pentadecanal	P6[e]	nd	nd	nd	nd	4.06± 0.54	19.02± 2.11	10.14± 0.84	26.77± 3.24	nd
74	1307	112-44-7	undecanal	P7	1.99± 0.21	1.93± 0.17	nd	nd	nd	nd	nd	nd	26.82± 3.96
75	1262	5392-40-5	3,7-dimethyl-2,6-octadienal	P8[e]	6.16± 0.81	1.64± 0.28	nd	nd	nd	nd	nd	nd	557.4± 69.7
76	1833	629-80-1	hexadecanal	P9[e]	nd	nd	nd	nd	nd	nd	nd	nd	16.02± 2.13
77	870	111-27-3	1-hexanol	P10	nd	nd	nd	nd	11.57± 1.26	nd	nd	nd	31.91± 4.17
78	872	928-95-0	trans-2-hexenol	P11	15.75± 1.36	nd	20.73± 1.87	nd	nd	38.91± 4.56	nd	22.89± 3.23	nd
			Total		471.1± 27.5	225.8 ± 32	177.9 ± 16	260.3± 18.3	471.4 ± 34	231.4 ± 15.4	447.3 ± 29.6	281.5± 16.5	799.2± 55.3
			Relatives (%)		7.36%	2.85%	2.80%	4.89%	4.39%	3.59%	10.53%	2.28%	7.00%
Esters													
79	1263	115-95-7	linalyl acetate	E1	nd	nd	**931.2± 86.5**	nd	nd	nd	nd	nd	nd
80	1341	134-20-3	methyl anthranilate	E2	98.18± 6.37	nd	22.97± 3.18	4.76± 0.57	12.12± 1.68	157.5± 13.4	5.48± 0.39	49.47± 3.38	nd
81	1366	141-12-8	neryl acetate	E3	nd	nd	24.81± 3.25	nd	nd	nd	nd	8.69± 0.64	nd
82	1385	105-87-3	geraniol acetate	E4	nd	nd	47.43± 5.11	nd	nd	1.02± 0.05	nd	5.23± 0.32	nd
			Total		98.2± 5.64	nd	1026± 92.1	4.8±0.27	12.1± 1.36	158.5± 13.6	5.5±0.29	63.4± 0.46	nd
			Relatives (%)		1.53%	0.00%	16.17%	0.09%	0.11%	2.46%	0.13%	0.51%	0.00%
Others													
83	1398	488-10-8	cis-jasmone	O1	8.85± 0.65	12.64± 1.33	2.44± 0.27	9.35± 0.86	27.22± 3.45	nd	nd	0.49± 0.05	nd
84	1081	100-64-1	cyclohexanone oxime	O2[e]	5.54± 0.58	nd	nd	3.96± 0.53	11.94± 1.98	nd	3.45± 0.42	nd	nd
85	1089	1195-32-0	p-dimethylstyrene	O3	nd	518.6± 29.6	nd	170.3± 15.3	348.6± 45.8	nd	nd	nd	nd
86	1138	6909-30-4	trans- limonene oxide	O4[e]	nd	nd	nd	nd	nd	33.25± 37.6	nd	118.4± 22.1	22.52± 3.67
87	1140	140-29-4	benzyl nitrile	O5	32.06± 4.54	nd	nd	48.99± 3.65	71.15± 3.97	nd	29.83± 4.12	nd	nd

88	1141	57396-75-5	3,4-dimethyl-2,4,6-octatriene	O6[e]	7.89± 1.05	11.37± 1.56	31.59± 4.37	13.03± 2.08	13.38± 1.56	18.69± 2.03	4.27± 0.34	12.89± 0.85	nd
89	1292	120-72-9	indole	O7	42.83± 6.37	nd	1.33± 0.31	nd	26.45± 1.54	38.83± 3.24	25.02± 1.59	53.74± 3.88	nd
90	1500	629-62-9	pentadecane	O8	nd	nd	nd	14.26± 1.28	nd	nd	nd	5.44± 0.61	95.76± 5.65
91	1581	1139-30-6	caryophyllene oxide	O9	nd	nd	15.25± 0.56	nd	nd	nd	nd	nd	9.68± 1.05
92	1677	2579-04-6	8-heptadecene	O10[e]	34.05± 5.23	nd	9.47± 1.21	64.43± 2.37	nd	nd	14.14± 2.18	nd	124.7± 15.8
93	1678	6765-39-5	1-heptadecene	O11[e]	nd	8.29± 0.67	nd	nd	24.42± 3.35	nd	nd	nd	nd
94	1703	629-78-7	heptadecane	O12	1.04± 0.02	nd	nd	10.05± 1.27	4.22± 0.53	nd	nd	nd	nd
			Total		132.3± 9.65	550.9± 39.4	60.08± 3.87	334.1± 19.7	527.4± 54.2	90.77± 6.38	76.71± 5.32	190.5± 15.6	252.7± 32.1
			Relatives(%)		2.07%	6.95%	0.95%	6.28%	4.91%	1.41%	1.81%	1.54%	2.21%
			Terpene		5698± 319	7155± 299	5083± 286	4721± 321	9729± 452	5959± 225	3716± 187	11818± 658	10366± 489
			Telatives(%)		89.04%	90.21%	80.08%	88.74%	90.59%	92.54%	87.53%	95.67%	90.79%
			Total		6400 ± 201	7932 ± 285	6347± 336	5321± 568	10740± 568	6440 ± 325	4246 ± 187	12354 ± 526	11417± 389
			Sorts		54	50	46	50	56	51	48	55	47

[a] RI: retention indices were determined using a series of alkanes C5-C30 as external references.

[b] CAS: Chemical abstracts service.

[c] Code :M, monoterpenes; S, sesqueterpenes; H, alcohols; P, aliphatic adehydes; A, aldehydes; E, esters; O, others.

[d] The content of blossom volatiles (μg.g^{-1} FW equivalent of cyclohexanone).

[e] Compound reported in the present study for the first time.

[f] Data are expressed as means ± standard deviation of triplicate samples.

[g] Numbers in bold present the dominant compounds for each species.

Identification of volatile compounds was first based on their retention indices (RI) relative to (C5–C20) n-alkane with those of the authentic compounds in the database. They were further confirmed by comparing mass spectral fragmentation patterns with data libraries (NIST-98 and FLAVOUR 2.0) of GC-MS data systems and other published mass spectra. The relative content of each compound was calculated by using the peak areas of all of the compounds relative to an internal standard (cyclohexanone).

2.4. Data Analysis

All data are expressed as means ± standard deviation of three replicates. The mean values were used for further analysis. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using SPSS v19.0 software (SPSS Inc., Chicago, IL, USA). OriginPro 7.5 G (Microcal Software, Inc., Northampton, MA, USA) was used to determine the standard deviation and the variation patterns.

3. Results and Discussion

3.1. Yields and Citrus Flower Volatile Components

The yield of flower volatile components in the Citrus species/varieties studied varied from 4249 to 12354 μg g-1 FW. The highest yield was found in Ningmeng 4, and the lowest in Changshanhuyou (Table 2 and Figure 1). Significantly higher total volatile contents were found in Ningmeng 4, Dannaxiangyuan and Taiwanpenggan than in other genotypes. From the citrus flowers analyzed in this study, a total of 149 compounds were detected, 94 of which had volatile contents over 10 μg g^{-1} FW, while others had trace amounts. The 94 compounds included 20 monoterpenes, 27 sesquiterpenes, 12 terpene alcohols, 5 terpene aldehydes, 11 aliphatic aldehydes and alcohols, 4 esters and 12 others; 31 of these compounds are reported for the first time in this study (Table 2). Monoterpenes (38.04-82.59%), sesquiterpenes (6.70-26.42%), terpene alcohols (0.59-43.25%) and terpene aldehydes (0.02-6.50%) were the major components of citrus flower volatiles (Figure 1, Table 2) and accounted for up to 92.4% of the total volatiles.

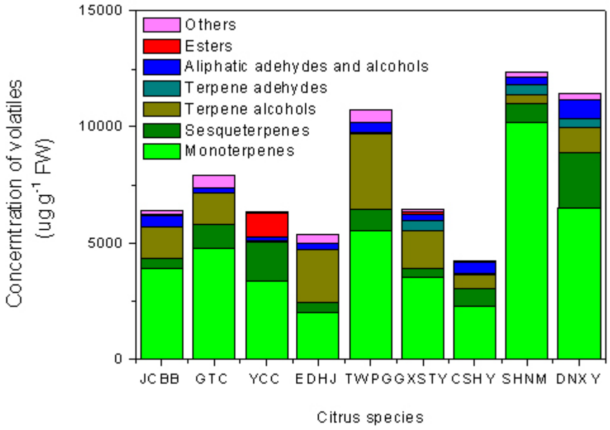

Figure 1. The main volatile components identified in the flowers of nine citrus genotypes studied in the present study and their contents variation

Monoterpenes were the most predominant compounds in all of the species/varieties analyzed. The highest level of monoterpenes was found in Ningmeng 4 (10203 μg g^{-1} FW), and the lowest in Edanhongju (2024 μg g^{-1} FW). Limonene was the most abundant monoterpene (60.91-5650 μg g^{-1} FW), followed by γ-terpinene. The highest level of limonene was found in Ningmeng 4, and the lowest was found in Yichangcheng. Limonene contributed to almost 45.7% and 45.8% of the total monoterpenes in Ningmeng 4 and Dannaxiangyuan, respectively, and making the two varieties distinct from all of the other varieties studied. Limonene, α-terpinene, γ-terpinene and neo-alloocimene were detected in all of the species/varieties tested. (-)-α-pinene was only detected in Jinchengbeibei 447 and Dannaxaingyuan, and (-)-β-pinene was only in Edanhongju and Dannaxaingyuan. Cosmene was only in Yichangcheng and Guangxishatianyou. High levels of γ-terpinene were detected in Goutoucheng, Taiwanpenggan and Changshanhuyou (> 848 μg g^{-1} FW). A high level of (e)-ocimene was detected in Guangxishatianyou (1172.61 μg g^{-1} FW) and Ningmeng 4 (1173 μg g^{-1} FW). High levels of β-pinene were detected in Guangxishatianyou (954.2μg g^{-1} FW) and Ningmeng 4 (1629.3 μg g^{-1} FW). High levels of sabinene were detected in Jinchengbeibei 447 (1610 μg g^{-1} FW) and Taiwanpenggan (1811 μg g^{-1} FW) (Table 2).

Sesquiterpenes were the second most abundant components (375.8-2369 μg g^{-1} FW) of the volatile compounds analyzed. The highest levels of sesquiterpenes were found in Dannaxaingyuan, and the lowest in Guangxishatianyou. Caryophyllene (69.95-897.8 μg g^{-1} FW) was the main component of the sesquiterpenes tested, followed by β-elemene and α-caryophyllene. The highest level of caryophyllene was found in Dannaxaingyuan, and the lowest in Edanhongju. Caryophyllene, α-cubebene and α-caryophyllene were present in all of the species/varieties examined. β-bourbonene, trans-α-bergamotene and δ-guaiene were only detected in Yichangcheng; β-sesquiphellandrene was only in Taiwanpenggan; α-guaiene was only in Yichangcheng and Changshanhuyou; and (+)-epi-bicyclosesquiphellandrene was only in Yichangcheng and Guangxishatianyou. High levels of copaene (229.7 μg g^{-1} FW) and L-β-bisabolene (193.3 μg g^{-1} FW) were detected in Yichangcheng. High levels of (z)-β-farnesene were detected in Goutoucheng (253.3 μg g^{-1} FW) and Taiwanpenggan (356.5 μg g^{-1} FW).

Terpene alcohols were the third most abundant components (58.8-3267 μg g^{-1} FW) in the volatile samples analyzed. The highest level of total terpene alcohol was found in Taiwanpenggan, and the lowest in Yichangcheng. Linalool (34.65-2099 μg g^{-1} FW) was the main terpene alcohol of the tested citrus flowers, followed by α-terpineol and geraniol. The highest level of linalool was found in Taiwanpenggan, composing 85.5% of the total alcohol; the lowest level was found in Yichangcheng. Linalool and α-terpineol were identified in all of the species/varieties tested. D-nerolidol was only detected in Changshanhuyou and Dannaxaingyuan, and 2,3-dihydro-6-trans-farnesol was only in Ningmeng 4 and Dannaxaingyuan; (e,e)-farnesol was only in Ningmeng 4. High levels of nerol (450.4 μg g^{-1} FW) and geraniol (345.3 μg g^{-1} FW) were detected in Dannaxaingyuan. A high level of nerolidol was detected in Guangxishatianyou (195.2 μg g^{-1} FW). In this study, only 5 terpene aldehydes were detected in the varieties studied, and citronellal and neral were the major terpene aldehydes. l-perillaldehyde and 1,3,6,10-farnesatetraen-12-al were only detected in Jingchengbeibei 447 and Taiwanpenggan, while geranial was only detected in Yichangcheng and Taiwanpenggan.

Only 4 terpene aldehydes were identified in the citrus flowers of all of the species/varieties analyzed. Except for Jinchengbeibei 447 and Goutoucheng, citronellal was detected in the flowers of all tested species/varieties. l-perillaldehyde and 1,3,6,10-farnesatetraen-12-al was only detected in Jinchengbeibei 447 and Taiwanpenggan. High levels of neral (349.9 μg g^{-1} FW) were found in Dannaxiangyuan, and high levels of geranial in Guangxishatianyou (220.2 μg g^{-1} FW) and Ningmeng 4 (233.6 μg g^{-1} FW).

In this study, 11 aliphatic aldehydes and alcohols were detected in the species/varieties studied. Aliphatic aldehydes, such as hexanal and trans-2-hexenal, were the main aldehydes in citrus flowers and were detected in all of the citrus species/variety tested. High levels of 3,7-dimethyl-2,6-octadienal were detected in Dannaxiangyuan (557.4 μg g^{-1} FW). Only two alcohols were detected in these tested cultivars. 1-hexanol was only detected in Taiwanpenggan and Dannaxaingyuan, and trans-2-hexenol was only detected in Jinchengbeibei 447, Yichangcheng, Guangxishatianyou and Ningmeng 4.

Esters were minor components in the volatile samples analyzed and varied significantly depending on the species/varieties (nd to 1026 μg g^{-1} FW). The highest level of esters was found in Yichangcheng. Methyl anthranilate was the predominant ester in all of the flowers tested. The highest level was found in Taiwanpenggan, and a relatively high level was also found in Yichangcheng (16.17%). All four esters, and an especially high level of linalyl acetate (931.2μg g^{-1} FW), were detected in Yichangcheng, but no esters were found in Dannaxiangyuan. In addition, alkenes, indoles, and terpene oxides were found in the citrus volatile samples. Especially high levels of p-dimethylstyrene were detected in Goutoucheng (518.6 μg g^{-1} FW) and Taiwanpenggan (348.6 μg g^{-1} FW).

In general, different genotypes were found to have different volatile components. In this study, (e)-ocimene, linalool and β-pinene were found to be the major volatile compounds in the Guangxishatianyou flower, and γ-terpinene and linolool were found to be the major volatile compounds in the Changshanhuyou flower. These results suggest that *C. grandis* flowers are abundant in linolool, but other characterized monoterpenes may depend on cultivars. The Yichangcheng flower was characterized by B-ocimene, linalyl acetate, caryophyllene and myrcene. Ningmeng 4 was rich in limonene, β-pinene and (e)-ocimene, Dannaxiangyuan was found rich in limonene, caryophyllene and L-β-bisabolene.

It has also been well documented that different citrus species have their own characteristic volatiles. Attaway et al. (1966) reported *C. sinensis* was dominated by β-myrcene, linalool and α-myrcene, but β-myrcene was the richest compound in *C. reticulata*. In addition, rich sabinene, myrcene, (+)-limonene, β-ocimene and linalool were found in petal and pistil oils of *C. sinensis* and *C. reticulate*. In the present study, we found that Jingchengbeibei 447 had high levels of sabinene (25.14%), linalool (17.13%) and limonene (15.86%). Goutoucheng was abundant in γ-terpinene (20.03%), linolool (15.07%) and p-cymene (5.90%). Edanhongju was abundant in linalool (38.21%) and o-methylthymol (2.93%). Taiwanpenggan was abundant in linalool (25.97%) and β-ocimene (12.12%). These results indicate that *C. sinensis* and *C. reticulate* flowers have similar major volatiles, but the volatile profiles are different from that of *C. aurantium*.

Jabalpurwala et al. found that *C. grandis* flower volatiles were predominantly linalool, p-cymene and methyl anthranilate (Jabalpurwala et al. 2009), while Cheong et al. found that limonene, linalool, ethanol and cis-β-ocimene were the major volatiles for Malaysian pumelo flowers (*C. grandis* Osbeck) (Cheong et al. 2011). Recently, Chung reported the volatiles of the Hallabong blossom (*C. kiyomi* × *C. ponkan*) (Chung 2012). They found that the most abundant volatile compounds of Hallabong were linalool, limonene, β-myrcene and sabinene. Numerous studies have shown that the flower oils of *C. aurantium* L. from different provinces primarily consist of linalool and limonene, although their percentages are different (Ramadan et al. 1996; Sarrou et al. 2013; Wei et al. 2010). These studies indicate that the flower volatiles might primarily be related to their genetic background (Figure 1).

3.2. Flower Volatile Compositions and Variation Patterns between Hybrid Citrus Species and Their Presumed Parents

The secondary metabolites of plants might be good complementary evidence for plant taxonomic study (Sumner et al. 2007). In the present study, the flower volatile components of nine citrus genotypes cultivated under the same climatic and horticultural conditions were examined, giving us opportunity to better evaluate the influence of genetic and/or province factors on the components and content of citrus volatile compounds.

It is known that Citrus volatile components are closely related to the genetic origin of the species/variety studied (Liu et al. 2013; González-Mas et al. 2011; Vekiari et al. 2001). To analyze the influence of genetic factors on the composition and content of citrus volatiles, the variation patterns of flower volatile components between the basic Citrus types in Swingle's system, i.e., Papeda (*C. ichangensis* Swingle), Cephalocitrus (*C. grandis* (L.) Osbeck), Citrophorum (*C. medica* L.) and Sinocitrus (*C. reticulata* Blanco), and their presumed hybrids, including *Citrus sinensis* (L) Osbeck, *C. aurantium* L., *C. paradis* Macf., and *C. limon* (L) Burm.f. were investigated. There were clear differences in volatile components between four basic Citrus types (Figure 2). As shown in Figure 2A and Table 1, Papeda contained the highest amounts of β-ocimene, linalyl acetate and myrcene. Sinocitrus had the highest levels of linalool and cavocrol. No characteristic volatile compounds were detected in Cephalocitrus, but this Citrus type has more β-pinene and limonene than Papeda and Sinocitrus. Citrophorum had the highest level of limonene, and high levels of β-pinene, caryophyllene and L-β-bisabolene were also detected in Citrophorum. The presence of 3,7-dimethyl-2,6-octadienal was unique to Citrophorum. These results are consistent with previous reports (Flamini et al. 2007; Hosni et al. 2010), indicating that different Citrus types are characterized by unique variation patterns in the composition and content of volatile compounds. In addition, our data suggests that Papeda, Citrophorum and Sinocitrus may be excellent sources of linalyl acetate, limonene and linalool, respectively.

In the Citrus genus, sweet orange, sour orange, grapefruit and lemon are well-accepted hybrids. To analyze the influence of hybridization on the flower volatile components, in this study we perform a detailed comparison of the variation patterns of the volatile compounds of these hybrids with those of their presumed parents.

It is well known that sweet orange is a hybrid between pumelo (*C. grandis* L.) and mandarin (*C. reticulate*) (Li et al. 2010). The differences in the composition and content of 94 volatile compounds identified in this study from *Citrus sinensis* Osbeck cv. Jinchengbeibei 447, *C. grandis* Osbeck cv. Guangxishatianyou and *C. reticulata* Blanco cv. Edanhongju are shown in Figure 2B. There were 24 volatiles widely distributed in sweet orange and its presumed parents. Unlike its presumed parents, the sweet orange contained significantly higher levels of myrcene, (z)-ocimene, β-elemene, α-terpineol and trans-2-hexenal. Additionally, sabinene, (-)-α-pinene, α-phellandrene, 3-carene, and (-)-α-selinene were detected only in sweet orange.

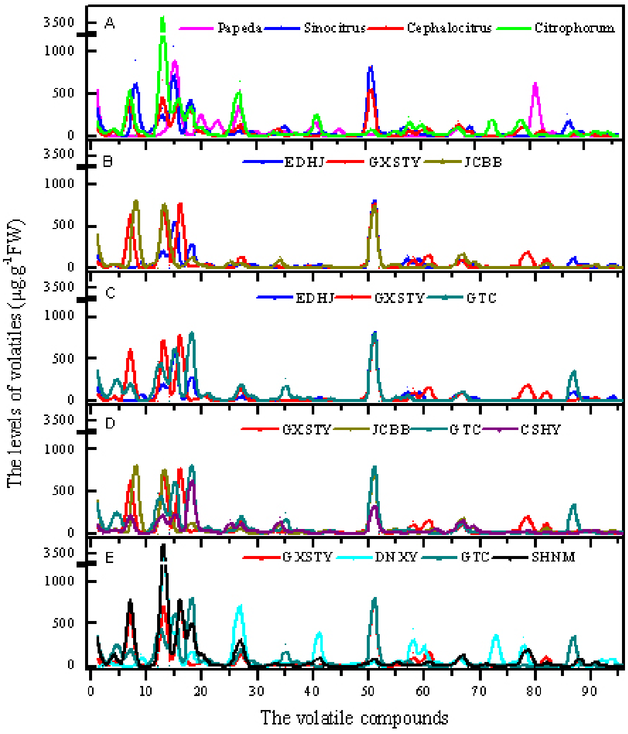

Figure 2. Variation patterns of the flower volatile compounds in basic Citrus species and their presumed hybrids

(A) variation patterns of four basic species-types; (B, C, D, E) variation patterns of the four hybrids (JCBB, GTC, CSHY, SHNM) and their presumed parents, respectively.

Most researchers suggest that sour orange is a hybrid of pumelo and mandarin (Li et al. 2010; Nicolosi et al. 2000). Figure 2C shows differences on the composition between *C. aurantium* L. cv. Goutoucheng and *C. grandis* Osbeck cv. Guangxishatianyou and *C. reticulata* Blanco cv. Edanhongju and the content of the 94 volatile compounds identified in this study. Twenty-six common volatiles were found in sour orange and its presumed parents pumelo and mandarin. Of the components detected above, the level of 13 volatiles, including γ-terpinene, elemene, α-cubebene, β-elemene, caryophyllene, β-cubebene, γ-elemene, α-caryophyllene, germacrene D, elixene, δ-cadinene, α-terpineol and copaene were significantly higher in sour orange than the presumed parents, especially α-terpinene and caryophyllene. The levels of α-caryophyllene, δ-cadinene, p-cymene, p-dimethylstyrene, (z)-β-farnesene and p-dimethylstyrene in sour orange were significantly higher than in their presumed parents. α-phellandrene, α-bergamotene, L-alloaromadendrene, phenethyl alcohol, (-)-4-terpineol, cis-jasmone and 1-heptadecene were detected only in the hybrids. The sour orange also had a higher content of common volatiles than its presumed parents. Notably, the sour orange was abundant in many unique volatiles, which showed that sour orange has remarkable heterosis and great potential for future utilization. These results suggest that the variation pattern in Goutoucheng is more similar to Edanhongju than Guangxishatianyou.

Changshanhuyou is thought to be a hybrid of *C. sinensis* and *C. grandis* (Nicolosi et al. 2000). Figure 2D shows the composition and content differences of the 94 volatile compounds identified in this study between Changshanhuyou (*C. paradisi* cv. Changshanhuyou), *Citrus sinensis* Osbeck cv. Jinchengbeibei 447, and *C. aurantium* L. cv. Goutoucheng. There were 21 common volatiles in the hybrids and their presumed parents. The levels of β-pinene, γ-terpinene, β-elemene and hexanal in the hybrids were significantly higher than those in their presumed parents. D-nerolidol was found only in Changshanhuyou. These results suggest that the variation pattern of Changshanhuyou is very similar to its presumed parents, especially that of Goutoucheng. The contents of volatiles identified in Changshanhuyou were all lower than in its presumed parents.

Lemon is widely thought to be a hybrid of citron and sour orange (Nicolosi et al. 2000; Barrett et al. 1976; Lu et al. 2011; Malik et al. 1974) Figure 2E shows the composition and content differences in the 94 volatile compounds identified in this study between *C. limon* (L). Burm.f cv. Ningmeng 4, *C. grandis* Osbeck cv. Guangxishatianyou, *C. medica* L cv. Dannaxaingyuan and *C. aurantium* L. cv. Goutoucheng. The compounds α-terpinene, limonene, neo-alloocimene, α-cubebene, caryophyllene, α-caryophyllene, γ-muurolene, linalool, α-terpineol, hexanal and trans-2-hexenal were all detected in lemon and its presumed parents. Significantly higher levels of limonene, β-pinene, (e)-ocimene, δ-terpinene and pentadecanal were detected in lemon than in its presumed parents. The origin of lemon was thought to be divergent for a long time. Our research of the flower volatile variation patterns of lemon shows its proposed parents are closely related (Figure 2E); the variation pattern of the hybrid is exactly the same as its parents.

3.3. The Taxonomic Implications of the Compositional Variation of Citrus Flower Volatile Compounds

To test the taxonomic value of citrus flower volatiles, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were carried out using the 94 compounds identified in this study. PCA analysis (Figure 3A, 3B) showed that the first two principal components accounted for 65.11% of the total variance and were high enough to represent all of the variables. Figure 3A showed the scatter plot of scores of principal components 1 and 2. Figure 3B showed the corresponding loading plot. The Citrus species/varieties studied could be divided into two major groups (Groups I and II), with group II being further divided into three subgroups (Subgroups IIa, IIb and IIc) (Figure 3A). The four basic Citrus types were clearly separated in two groups and three subgroups based on the PCA results. Citrophorum includes Dannaxiangyuan and Ningmeng 4 and is clustered in group I, group I, which was characterized by a high terpene content. Sinocitrus included Edanhongju and Taiwanpenggan and was clustered in subgroup IIa, and Goutoucheng was grouped with Edanhongju. Cephalocitrus included Guangxishatianyou and Changshanhuyou and was in subgroup IIb. Papeda, which was characterized by esters, was in subgroup IIc. Regarding the hybrids, Goutoucheng and Jinchengbeibei 447 were grouped with Guangxishatianyou and Edanhongju. Sihaonignmeng was grouped with Dannaxiangyuan, Guangxishatianyou and Goutoucheng. So the four basic types of the genus Citrus L., Papeda, Cephalocitrus, Citrophorum and Sinocitrus, can be clearly separated based on the cluster analysis of their volatile compounds in the PCA plot. All of the presumed hybrid species, including Jinchengbeibei 447 (*C. sinensis* Osb.), Goutoucheng (*C. aurantium* L.), Ningmeng 4 (*C. limon* Burm.f.), and Changshanhuyou (*C. paradisi* cv. Changshanhuyou), grouped closely together with one of their suggested parent species in the constructed dendrogram. These results revealed that the components of citrus flower volatiles were variable among different species/varieties types, although underlying intraspecific similarities and interspecific chemical varieties were found in flower volatiles. Similar findings have been reported for the chemical polymorphisms of citrus essential oils and leaf volatiles (Azam et al. 2013; Lota et al. 2000).

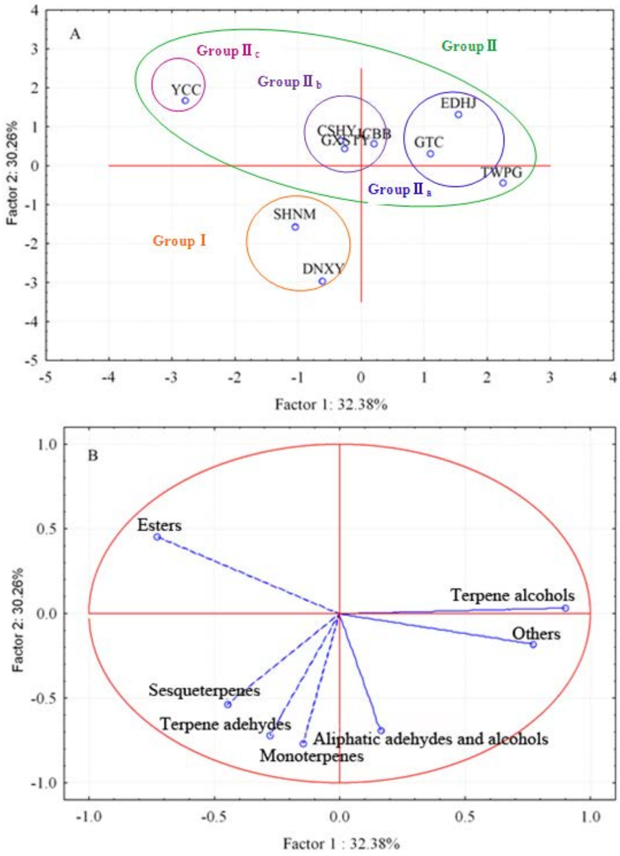

Figure 3. Principal component analysis (PCA) of Citrus flower volatiles:(A) score plot for the first and second principal components; and (B) loading plot of for the first and second principal components. Volatile variables explained according to the blossom volatile categories of the studied Citrus species

HCA was also carried out to explore the classification value of the citrus volatile compounds (Figure 3B). The results are shown in Figure 4. Nine of the species/varieties studied were grouped into two main clusters. Cluster I consists of *C. limon* (L) Burm.f. and *C. medica* L. and was characterized by a high level of limonene. This was inconsistent with the results from PCA analysis. Cluster II included three subgroups (IIa, IIb, IIc). Cluster IIa consisted of *C. aurantium*, *C. reticulata* (Edanhongju) and *C. paradisi* cv. Changshanhuyou. In Cluster IIa, Goutoucheng was rich in γ-terpinene, and Edanhongju had a higher content of o-methylthymol, and Changshanhuyou was rich in γ-terpinene. Cluster IIb included C. sinensis and *C. grandis*, which were distinct from others because of their high methyl anthranilate content. Cluster IIc was composed of *C. ichangensis* and *C. reticulata* (Taiwanpenggan) and was characterized by high 3,7-dimethyl-1,3,6-octatriene and myrcene content.

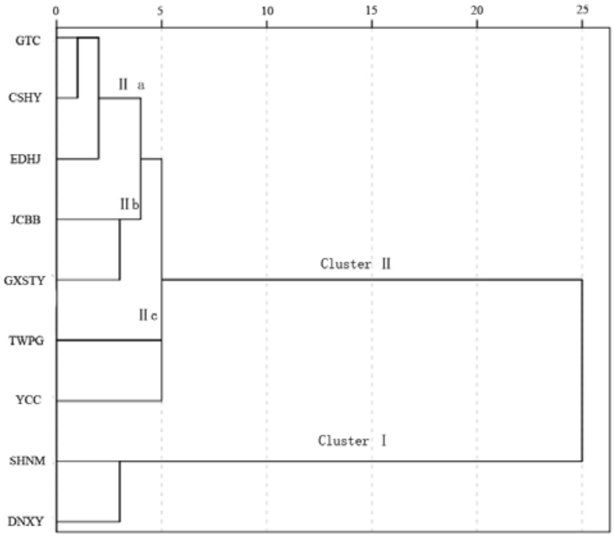

Figure 4. The dendrogram of the Citrus species studied based on the 94 flower volatile compounds (Table 1)

These results are in agreement with other molecular evidence from previous studies. Our previous studies demonstrated that sweet orange and sour orange were hybrids of mandarin (*C. reticulata*) and pumelo (*C. grandis*) by using internal transcribed spacer (ITS) and chloroplast DNA sequences and amplified fragment length polymorphism (AFLP) fingerprints. In this study, though similar volatiles pattern were not observed in Jinchengbeibei 447 or in their presumed parents, the majority of volatiles identified were common. The PCA and HCA model showed that the hybrid and its presumed parents share the same flower volatile profile, which provides the chemotaxonomic evidence that is in agreement with previous results based on enzyme and molecular evidence (Li et al. 2011; Barkley et al. 2006). Similar results were observed in Changshanhuyou. In this study, a very close variation pattern of flower volatiles was found in Ningmeng 4 and its proposed parents, which further confirmed the hypothesis that rough lemon is a cross between citron and mandarin (Miller et al. 2011; Rossi et al. 2013).

Li et al. analyzed the phylogenetic relationship of the four basic biotypes of Citrus, namely, Papeda, pumelo, citron and tangerine, using chloroplast DNA regions (cpDNA), amplified fragment length polymorphisms (AFLP) fingerprints and nuclear internal transcribed spacer (ITS). The molecular data of their study indicated that the Citrus genus was of monophyletic origin (Li et al. 2010), which is consistent with our current PCA and HCA results (Figure 3A). Their cpDNA results showed that *C. grandis* is closely related to the Papeda species. In our PCA plot, Changshanhuyou and Guangxishatianyou (in IIb) are close to Taiwanpenggan and Edanhongju (in IIc). The AFLP dendrogram shows that Ichang papeda, C. Reticulata and *C. grandis* are more closely related to each other than they are to other types in the genus and that papeda is distant from *C reticulate* (Li et al. 2010), which was consistent with our PCA and HCA results.

4. Conclusion

The composition and content variation of flower volatiles in seven native Chinese citrus species/varieties, namely, Jinchengbeibei 447, Goutoucheng, Yichangcheng, Edanhongju, Taiwanpenggan, Guangxishatianyou and Sihaoningmeng, are reported for the first time in this study. Monoterpenes (36.85-82.59%), sesquiterpenes (5.83-26.42%) and terpene alcohols (0.91-44.82%) were the major citrus flower volatile components. Monoterpenes were the most predominant compounds in all of the species/varieties analyzed, and limonene was the most abundant monoterpene followed by γ-terpinene. Different species/varieties flower had characteristic volatiles. Papeda was characterized by high levels of β-ocimene, linalyl acetate, myrcene and neo-alloocimene. Citrophorum (Ningmengsihao 4 and Dannaxiangyuan) had the highest levels of limonene, and α-caryophyllene. Cephelocitrus had the highest level of limonene, β-pinene and linolool. Sinocitrus had the highest level of linolool. Linolool was also the characteristic terpene in *C. sinensis* (L.) Osbeck and *C. aurantium* L. *C. sinensis* (L.) Osbeck (Jinchengbeibei 447) had a high level of limonene, while *C. aurantium* L. (Goutoucheng) had the highest level of γ-terpinene. The compositional variation patterns of the basic citrus species indicated strong genetic influence on the volatile compounds. The hybrid species/variety shared largely similar patterns to their presumed parents, which may be useful for chemotaxonomy. Overall, our study provides useful information for future citrus germplasm evaluation and utilization.

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