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Characterization of volatile compounds nongrafted and pumpkin-grafted bitter gourd (*Momordica charantia* L.)

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Abstract: Bitter melon (*Momordica charantia* L.) is a unique vegetable-fruit possessing several multifarious health benefits. In this study, the plant extraction obtained from grafted bitter gourd onto pumpkin (*Cucurbita maxima*) and nongrafted bitter gourd cultivated under greenhouse conditions were evaluated for their constituents of volatile compounds. Gas chromatography-mass spectrometry (SPME-GC-MS) techniques were employed to determine these compounds in the leaves (young, middle, and old-age) and fruits (unripe and ripe) of bitter gourd. The volatile compounds in the leaves of pumpkin during the three stages mentioned above were also investigated. Overall, 72 in leaves and 56 in fruits of bitter gourd compounds belonging to eight major chemical groups were identified: acid, alcohol, aldehyde, alkane, ester, ketone, lactone, and terpene. A total of 52 compounds belonging to the eight major groups mentioned above were also identified from pumpkin leaves. Aldehyde, terpene, and ester were reactively the most prevalent groups found in bitter gourd and pumpkin. The grafted bitter gourds developed up to 23 new compounds in leaves and 15 in their fruits. The grafting of bitter gourd onto pumpkin also halted the production of up to 15 different compounds in fruits and seven in their leaves. Based on principal component analysis and cluster analysis results, the dissimilarity between grafted and nongrafted bitter gourd decreased over time from young to old leaves or unripped to ripe fruit. In conclusion, the grafting of bitter gourd onto pumpkin drastically reshaped the composition of volatile compounds in both leaves and fruits of bitter gourd.

Key words: *Cucurbitaceae,* gas chromatography, greenhouse, karela, principal component analysis

1. Introduction

Bitter gourd (*Momordica charantia* L.), also known as karela, balsam pear, or bitter melon, belongs to the family *Cucurbitaceae* native to tropical and subtropical parts of Africa and Asia, is known as 'the mainstream food of the 21st century' for several multifarious health benefits owing promising biological properties (Mukherjee et al., 2013; Fang and Ng, 2016). Consequently, it is used for both medicinal and food purposes (Morton, 1967; Gao et al., 2019). It is also known as vegetable insulin, containing an insulin-like compound called p-insulin or Polypeptide-p, effectively lowering the blood sugar (Joseph and Jini, 2013; Deshaware et al., 2018). Several studies suggest that the bitter gourd is beneficial for its antidiabetic (Wu and Ng, 2008), antiviral (Puri et al., 2009), antiosteoporosis (Yang et al., 2010), antioxidant, antiinflammatory (Shan et al., 2012), antiobesity (Bao et al., 2013), antihelminthic activity (Pereira et al., 2016), and anticancer (Raina et al., 2016). In traditional herbal medication, it has been used for the treatment of gout, dysmenorrhea, eczema, jaundice, piles, leprosy, psoriasis, scabies, rheumatism, and pneumonia (Bailey et al., 1986; Jia et al., 2017). Along with many good pharmacological activities, it was also potentially detrimental to induce abortion or even death in experimental animals and hypoglycemic coma in children (Grover and Yadav, 2004; Jia et al., 2017).

The pharmacological features of bitter gourd are well-documented (Grover and Yadav, 2004; Jia et al., 2017). However, the composition of their volatile compounds and contents, which have important ecological functions, received relatively little attention. The volatile compounds in plants are known to perform a variety of tasks such as allelopathic agents or as irritants as an indirect plant defense against insect (Mumm et al., 2003), pollinators attraction (Dudareva and Pichersky, 2000), defense from predators (War et al., 2012), environmental stress adaptation (Holopainen and Gershenzon, 2010) and plant-to-plant messengers (Baldwin et al., 2006). Grafting is generally known to improve plant resistance to diseases, tolerance to negative soil conditions, and increased plant yields (Lee et al., 2010; Colla et al., 2017).

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The present study aimed to investigate the presence and contents of different volatile compounds found in bitter gourd leaves and fruits during their young, middle, and old-age stages. This study also examined the impact of grafting on the quantity and quality of volatile compounds of bitter gourd grafted onto the pumpkin (*Cucurbita maxima*). The volatile compounds of grafted and nongrafted bitter gourd were then compared to determine the potential effects of grafting. The volatile compounds profile for pumpkin leaves during their young, middle, and old-age stages were also documented.

2. Materials and methods

2.1. Collection of plant material

Pumpkin (Cucurbita maxima) was used as rootstocks while bitter gourd (Momordica charantia L.) was used as scions. During June, seeds were sown in viols containing Peat + Perlite (2:1) mixtures under greenhouse conditions. The emergence of scions and rootstocks seedlings was completed in eight days. The grafting was carried out once the seedlings had 3–4 leaves that appeared in six days later from the emergence.

The procedures described in Tamilselvi and Pugalendhi (2017) and Akhila and George (2018) were used for grafting. One cotyledon method of grafting was employed. Grafted seedlings were transplanted into the greenhouse in July after keeping them in the shade for a duration of two weeks. They were placed at a distance of 100 × 50 cm. Data on the temperature and humidity of the greenhouse are provided in Figure 1.

2.2. Collection of leaves and fruits

The leaves and fruits samples from grafted and nongrafted bitter gourd were collected from five plants each at the end of the second month. Leave maturity was categorized into three stages *viz.* young, middle, and old age leaves. Leaf with a length of 7–10 cm was considered as young, middle-age leave with a length of 15 cm while old-age leaves were of >15 cm in length with no freshness. Furthermore, fruits were also classified into unripe and ripe. The unripe bitter melon was described as when pericarp was green (approximately 3–4 weeks post flowering). While the ripe bitter melon was described as when the pericarp turned yellow/orange while the aril turned red (approximately 4–5 weeks after flowering) (Horax et al., 2010). In pumpkin, leaves with a 15–20 cm length were considered young, 20–25 cm middle-age leaves, while 25–30 cm leaf as old-age leaves. Samples were taken when all samples were in the plant at the same time. Samples were cut into small pieces, blended, and dried at 40 °C. After dried, samples were kept for cooling in a desiccator containing silica gel at room temperature. Dried samples were ground into powdered in a ceramic mortar. Then they stored at +4 °C in tightly closed plastic bags until analysis. The volatile compounds were analyzed using solid-phase microextraction–gas chromatography-mass spectrometry (SPME-GC-MS).

2.3. Volatile's extraction: headspace SPME

SPME was carried out using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibre, 50/30 µm film thickness (Supelco, Bellefonte, PA, USA). Dry sample which is weighed 0.2 g was placed into the microreaction vessel and heated on a hotplate at 50 °C for 15 min. For the samples placed over 40 mL, the aromatic used for homogeneous dispersion was mixed at 200 rpm. Before the SPME analysis, the fibers were conditioned for 5 min at 250 °C in the GC injector. The syringe injector of the SPME unit was inserted through the septum of the microreaction vessel cap into the headspace of the sample. After the sampling, the SPME fibre was introduced onto the split injector of a GC-MS system (Renda et al., 2017).

2.4.Gas chromatography-mass spectrometry analysis

GC–MS analysis was performed on gas chromatography-mass spectrometry GCMS (PQ2010 Ultra; Shimadzu Corp., Kyoto, Japan) equipped with a fused-silica capillary column Rxi-5Sil MS (30 m, 0.25 mmID/i.d., 0.25-µm film thickness; Restek, Bellefonte, PA, USA). The injector temperature was 250 °C; injection mode was split mode

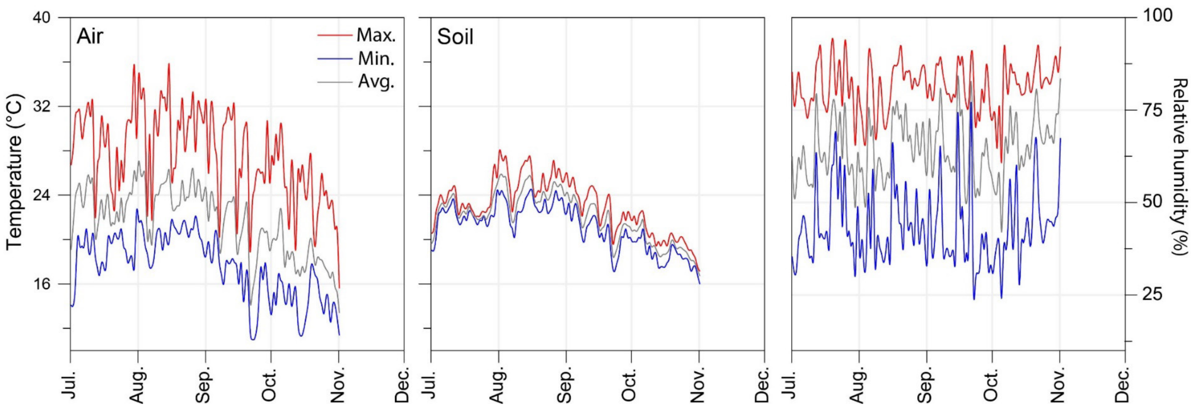

Figure 1. Temperature, °C and relative humidity, % conditions in the greenhouse.

(1:25). The MS ion source and interface temperatures were maintained at 210 °C and 250 °C, respectively. Column oven temperature program was 40 °C constantly. Helium with high purity (99.999%) was used as carrier gas at a flow rate of 1.00 mL min^{-1}. Detection was implemented in electronic impact mode (EI); ionization voltage was fixed at 70 eV, scan mode (40–450 m/z) was used for mass acquisition. All components were identified the NIST 1992 and Wiley5 mass spectral libraries, retention indices from the literature and retention indices of authentic standards.

2.5. Statistical analysis

The similarities or dissimilarities in the volatile compound profiles of grafted and nongrafted bitter gourd were depicted by SIMPER (analysis of similarity percentages) dendrogram. Principal component analysis (PCA) was used to determine major groups of volatile compounds found in bitter gourd, and their results are provided as vector and sample maps (only components 1 and 2). The data were transformed (log + 1) before these analyses (Alkan et al., 2019). The SIMPER, dendrogram, and PCA analyses also included the results obtained for pumpkin leaves. The PRIMER 6 software was used for SIMPER and dendrogram analyses while PCA was done with R v4.0 using the packages "FactoMineR" and "factoextra".

3. Results

3.1. Volatile compounds profile

Overall, 75 in leaves and 56 in fruits of bitter gourd volatile compounds belonging to eight major chemical groups were identified: acid, alcohol, aldehyde, alkane, ester, ketone, lactone, and terpene (Table 1). In the leaves of pumpkin, a total of 52 volatile compounds belonging to the eight major groups, as mentioned above, were also identified. The percentage share of each major chemical group for bitter gourds' leaves and fruits as well as for the leaves of pumpkin, are presented in Figure 2.

Aldehyde and terpene were the most prevalent groups found in the leaves of nongrafted bitter gourd (except middle age) and pumpkin with 48% to 69% contribution. The content of these two groups increased from a young age leave to old age leave in pumpkin. While in grafted bitter gourds' leaves, the two most prevalent groups were aldehyde and ester in young age leave (72%) while ester and terpene in the middle and old age leaves with 44% to 48%. In fruits, terpene was the most prevalent group recorded from nongrafted and grafted bitter gourds, except grafted ripe fruit, where aldehyde was the most prevalent group, followed by terpene.

3.1.1. Acid

A total of eight different acid compounds in bitter gourds and five in pumpkin were identified (Table 1). The 2-methyl butyric acid, 3(E)-hexenoic acid, and isobutyric acid were only associated to the pumpkin while sharing 2-methyl-1-ethylhexyl 2(E)-Butenoic acid, 2-methyl 2-pentenoic acid with bitter gourds. The highest contents of total acids were recorded in nongrafted bitter gourds than grafted bitter gourds (except grafted unripe fruit). The leaves of bitter gourds had the highest contents of total acids than their fruits (Table 1). The grafted bitter gourds had developed a new acid compound verdox identified in their middle and old age leaves.

3.1.2. Alcohol

The highest contents of total alcohol were recorded in nongrafted bitter gourds than in the grafted group. The grafting has resulted in the elimination of several compounds from the leaves and fruit of grafted bitter gourds such as in leaves: Heptadecyl alcohol, Pentadecanol, Tetrahydrofurfuryl alcohol and Tricyclo[6.3.1.02,5] dodecan-8-ol < 1,4,4-trimet while cis,cis-Farnesol in fruit. Furthermore, grafted has also resulted in some new compounds, e.g., α-Amylcinnamyl alcohol and Viridiflorol, in the leaves of grafted bitter gourds.

3.1.3. Aldehyde

A total of 16 different aldehyde compounds were recorded in bitter gourds, while a total of 7–8 different aldehyde compounds were identified in the leaves of pumpkin. All the six compounds of aldehyde were also recorded from both grafted and nongrafted bitter gourds, except 2(E)-Pentenal (Table 1). The grafted eliminated 2-methyl-3-ethyl acrolein, bergamal, myrtenal, and phellandral from the leaves of grafted bitter gourds while 2(E)-hexenal, Lauric aldehyde and 2-methyl-undecanal from the fruits of grafted bitter gourd. The fruits of grafted bitter gourd had developed three new aldehyde groups, viz. Acetaldehyde phenethyl propyl acetal, (E)-cinnamaldehyde and Enanthaldehyde. Furthermore, it also resulted in increased contents of Benzaldehyde, Capraldehyde, 2(E)-hexenal, Pelargonaldehyde, and 2-methyl-undecanal in the leaves of grafted bitter gourds than the control group.

3.1.4. Alkane

A total of 13 different alkane compounds were identified in bitter gourds. In comparison, a total of eight alkane compounds were recorded in pumpkin leaves, and all of these compounds were present in bitter gourds, especially in grafted (Figure 2). The contents of alkane in grafted leaves tend to increase through young to old. Alkane content in nongrafted bitter gourd also increased from young to middle age leaves that abruptly decrease in old age leaves. Compared to the young leaves of nongrafted, leaves of the grafted group at this stage had up to 122% higher content of total alkane (Figure 2). In contrast to the bitter gourd leaves, total alkane content decreases through unripe to ripe fruits in both grafted and nongrafted bitter gourd. Several new compounds of alkane were identified

Table 1. Concentration of free volatile compounds (in %) identified in leaves and fruits of nongrafted and grafted (grafting onto pumpkin, *Cucurbita maxima*) bitter gourd (*Momordica charantia* L.). The concentration of free volatile compounds (in %) are also provided for the leaves of pumpkin.

COMPOUNDS		*LRI	LEAVES									BITTER GOURDS' FRUIT			
			Nongrafted bitter gourd			Grafted bitter gourd			Pumpkin			Nongrafted		Grafted	
			Young	Middle	Old	Young	Middle	Old	Young	Middle	Old	Unripe	Ripe	Unripe	Ripe
	2-Methyl-1-ethylhexyl 2(E)-Butenoic acid	1039	4.32		2.58	2.62					2.23				
	2-Methylbutyric acid	850									1.76				
	Caproleic acid	1003	1.60				1.85						1.93		
	Enanthic acid	984			1.76								3.01		1.54
	3(E)Hexenoic acid	800								1.19	3.23				
	Isobutyric acid	752									2.04				
Acid	Palmitic acid	1901				2.76						4.03			
	2-Methyl 2-pentenoic acid	1004	4.51	8.58	5.24	1.48		2.76			3.21	4.62	11.48	5.88	6.76
	Valeric acid	986	2.45			0.68								5.69	3.99
	3-Methyl valeric acid	968											1.61		
	Verdox	1299					2.89	2.37							
	N		**4**	**1**	**3**	**4**	**2**	**2**		**1**	**5**	**2**	**4**	**2**	**3**
	ΣAcid		**12.88**	**8.58**	**9.58**	**7.54**	**4.74**	**5.13**		**1.19**	**12.47**	**8.65**	**18.03**	**11.57**	**12.29**
Alcohol	Cetyl alcohol	1662		1.94				2.04		0.93				1.55	1.97
	α-Amylcinnamyl alcohol	1843					6.15								
	cis,cis-Farnesol	2268										12.65			
	Heptadecyl alcohol	1498			1.82										
	3(Z)-Hexenol	859	15.82	7.02	13.40		3.90	5.87	2.39						
	Lauryl alcohol	1499													1.44
	3(Z)-Octenol	1002												1.55	
	Pentadecanol	1700		2.54					2.03					1.01	
	Tetrahydrofurfuryl alcohol	1606		3.14											
	Tricyclo[6.3.1.02,5]dodecan-8-ol <1,4,4-trimet	1537	2.91												
	Tridecyl alcohol	1620			1.86		1.87			1.61					
	Viridiflorol	1630						1.78		1.05				3.02	
	N		**2**	**4**	**3**		**3**	**3**	**2**	**3**		**1**		**4**	**2**
	ΣAlcohol		**18.73**	**14.64**	**17.08**		**11.92**	**9.69**	**4.42**	**3.59**		**12.65**		**7.13**	**3.41**
Aldehyde	Acetaldehyde phenethyl propyl acetal	780													4.69
	2-Methyl-3-ethyl acrolein	794	2.95												
	Benzaldehyde	962	7.76	2.16	2.19	1.41		2.71	2.77						
	Bergamal	861			3.92										
	Capraldehyde	1210	1.82	4.31	4.62	0.94	2.93	6.38	2.51	3.95	3.92	5.89	6.80	3.08	1.61
	Capronaldehyde	980			1.77	1.40			1.86	1.39	2.11		1.63	2.06	
	(E)-Cinnamaldehyde	790												0.94	18.72
	Enanthaldehyde	1654													1.88
	trans,trans-2,4-Heptandienal	1005				0.89		1.75			2.04				

Table 1. (Continued).

COMPOUNDS		*LRI	LEAVES									BITTER GOURDS' FRUIT			
			Nongrafted bitter gourd			Grafted bitter gourd			Pumpkin			Nongrafted		Grafted	
			Young	Middle	Old	Young	Middle	Old	Young	Middle	Old	Unripe	Ripe	Unripe	Ripe
Aldehyde	2(E)-Hexenal	854	10.40	4.33	2.62	10.69	4.45	4.10	9.79	22.46	32.60		3.41		
	Lauric aldehyde	1663											2.62		
	Myrtenal	1200	2.18												
	Pelargonaldehyde	1109	2.39	4.08	5.54	1.14	4.66	4.83	3.22	4.10	5.99	3.68	2.62	1.47	
	2(E)-Pentenal	755								1.10	3.17				
	Phellandral	1225	2.05												
	Phenylacetaldehyde	1046						1.38	1.85		1.78	3.24	3.54	3.98	3.71
	2-Methyl undecanal	1823	1.50			27.89						3.50			
	N		**8**	**4**	**6**	**6**	**3**	**5**	**6**	**5**	**6**	**4**	**6**	**5**	**5**
	ΣAldehyde		**31.05**	**14.88**	**20.66**	**43.47**	**12.04**	**19.40**	**22.00**	**33.00**	**49.57**	**16.31**	**20.62**	**11.53**	**30.61**
Alkane	Eicosane	1503												4.00	
	Hendecane	1094		2.31						1.29		4.40			1.73
	Heneicosane	2054				1.40									
	Heptadecane	1660					1.92								
	Hexadecane	1607		3.71	4.01		1.59	7.08		2.80		6.02		3.37	3.08
	Nonadecane	1906				0.79			3.00						
	Octadecane	1552				0.80	1.83			2.69					
	Pentacosane	1430		3.68	2.16	1.91									1.85
	Pentadecane	1506		2.83			2.82	3.18	5.03	2.18					
	Tetracosane	1937				1.62								2.17	
	Tetradecane	1406	1.97	5.21	5.18	0.72	2.28	5.12	3.87	3.73	2.74	4.82	4.22	4.17	3.32
	Tridecane	1306		2.89	3.22		2.40	3.02	3.11	2.36	2.70	3.43	2.95	3.72	2.00
	N		**1**	**6**	**4**	**7**	**6**	**5**	**4**	**6**	**3**	**4**	**3**	**5**	**5**
	ΣAlkane		**1.97**	**20.63**	**14.57**	**8.13**	**12.84**	**20.15**	**15.01**	**15.05**	**7.48**	**18.67**	**8.50**	**17.43**	**11.98**
Alkene	Neodene	1700											1.33		
Ester	Isobutyl angelate	2293				28.93									
	Isoamyl benzoate	1719		2.89			4.38	2.98		1.05					1.37
	Isopropyl 2-methylbutyrate	1098		2.89	5.43		10.39	6.97		2.77			5.69	2.54	4.16
	Octyl butyrate	1642						4.03				2.92			
	Allyl heptanoate	1141							1.58						
	3-Methyl- 3(E)Hexnoate	999						1.37							
	Ethyl 3-hydroxyhexanoate	1091											1.36		
	Isobornyl acetate	1292					3.90	3.63					2.64		
	Isopulegyl acetate	1460								2.35				2.67	2.09
	Neomenthol acetate	1075	3.25								2.60				
	Allyl octanoate	1609							1.82						
	Pentylallyl butyrate	1111	3.30				1.73			4.00	5.29				
	Isopropyl phenylacetate	1043													1.80

Table 1. (Continued).

COMPOUNDS		*LRI	LEAVES									BITTER GOURDS' FRUIT			
			Nongrafted bitter gourd			Grafted bitter gourd			Pumpkin			Nongrafted		Grafted	
			Young	Middle	Old	Young	Middle	Old	Young	Middle	Old	Unripe	Ripe	Unripe	Ripe
Ester	Diethyl phthalate	1602					1.61						2.12	1.31	
	Decyl propanoate	1595						1.30							
	Tetrahydrofurfuryl propionate	1606	2.55				7.10						4.58		
	(Z)-3-Tiglate hexenyl	1558										4.25			
	Hexyl tiglate	1642								5.33					
	N		**3**	**2**	**1**	**1**	**6**	**6**	**2**	**5**	**2**	**2**	**5**	**3**	**4**
	ΣEster		**9.10**	**5.78**	**5.43**	**28.93**	**29.11**	**20.28**	**3.40**	**15.50**	**7.89**	**7.17**	**16.39**	**6.52**	**9.42**
Ketone	Acetylbutyryl	1042												1.14	
	Benzylideneacetone	790												6.82	
	Diphenylketone	1637		2.42			2.76								
	6-Methyl-5-hepten-2-one	1459	1.39				2.35	3.12	2.66		2.04				
	Hexyl methyl ketone	1107								1.78					
	Tridecyl methyl ketone	1112							2.58						
	N		**1**	**1**			**2**	**1**	**2**	**1**	**1**			**2**	
	ΣKetone		**1.39**	**2.42**			**5.11**	**3.12**	**5.24**	**1.78**	**2.04**			**7.96**	
Lactone	γ-Butyrolactone	917	2.05						2.66						
	Ethylene brassylate	1643		11.80	5.13	0.86			9.00		3.43		7.47	7.80	7.00
	δ-Undecalactone	1643	1.60				5.19					7.47			
	N		**2**	**1**	**1**	**1**	**1**		**2**		**1**	**1**	**1**	**1**	**1**
	ΣLactone		**3.65**	**11.80**	**5.13**	**0.86**	**5.19**		**11.66**		**3.43**	**7.47**	**7.47**	**7.80**	**7.00**
Terpene	Alloaromadendrene	1428			3.04				2.92						2.43
	Aromadendrene	1406	2.05												
	10-β(H)-Cadina-1(6),4-diene	1532				2.45			8.73					1.36	
	δ-Cadinene	1533	2.26	4.48	4.40			3.43		4.67		6.05	7.46	4.75	5.37
	γ-Cadinene	1490	6.13	3.14	4.29	1.04		1.85	2.60	3.08	5.91	3.85	4.45	7.13	2.98
	Carotol	1630		2.00			4.33								
	β-Caryophyllene	1428						4.26							
	Caryophyllene oxide	1428					2.43								
	β-Cedrene									1.06					
	α-Copaene		1.39					1.28						1.47	
	α-Cubebene									1.27					
	p-Cymene					4.47	2.04								
	Dihydrocitronellol								1.59						
	Dihydromyrcenol				2.72		1.93								
	β-Elemene		4.29	5.54	5.94	1.16		3.08	6.95	6.80	3.19	8.95	7.45	8.33	7.58
	Geranyl acetone	1450										2.87			
	Germacrene D	1383							2.91						

Table 1. (Continued).

COMPOUNDS		*LRI	LEAVES									BITTER GOURDS' FRUIT			
			Nongrafted bitter gourd			Grafted bitter gourd			Pumpkin			Nongrafted		Grafted	
			Young	Middle	Old	Young	Middle	Old	Young	Middle	Old	Unripe	Ripe	Unripe	Ripe
Terpene	α-Himachalene	1428								3.27		3.55	3.23		
	α-Humulene									1.00					
	(E)-β-Ionone		5.11	2.92	4.03	0.83	1.81		5.62	4.74	4.46			3.63	1.40
	cis- Limonene oxide			3.19	3.13			3.78	5.26	4.00	3.82	3.83	4.53		3.18
	Linalool						1.61								
	α-Pinene					1.14									
	α-Terpineol						4.91	4.53					1.88		2.33
	δ-Undecalactone														
	Valencene													3.35	
	Viridiflorene								1.67						
	N		**6**	**6**	**7**	**6**	**7**	**7**	**9**	**9**	**4**	**6**	**6**	**7**	**7**
	ΣTerpene		**21.23**	**21.27**	**27.55**	**11.09**	**19.06**	**22.21**	**38.25**	**29.89**	**17.38**	**29.10**	**29.00**	**30.02**	**25.27**
Total no. of recorded compounds			**27**	**25**	**25**	**25**	**30**	**29**	**27**	**30**	**22**	**20**	**25**	**29**	**27**

* LRI, linear retention index

in grafted bitter gourds such as Heneicosane, Heptadecane, Nonadecane and Octadecane in leaves while Eicosane, Pentadecane, and Tetracosane in their fruits (Table 1).

3.1.5. Ester

There were 16 different ester compounds identified in bitter gourds, while the pumpkin leaves had eight ester compounds. Except Allyl heptanoate and Allyl octanoate, all identified ester compounds were recorded in bitter gourds (Table 1). A wide variety of different ester compounds were recorded in the leaves of grafted bitter gourds of middle and old aged. The grafting resulted in six new ester compounds in the leaves of bitter gourd viz. Isobutyl angelate, Octyl butyrate, 3-methyl-3(E)-hexenoate, Isobornyl acetate, Diethyl phthalate and Decyl propanoate. Furthermore, Isoamyl benzoate, Isopulegyl acetate, and Isopropyl phenylacetate were new ester compounds identified in the fruits grafted bitter gourds. The grafting also eliminated some ester compounds such as Neomenthol acetate from leaves while Ethyl 3-hydroxyhexanoate and Isobornyl acetate in fruit.

3.1.6. Ketone

The ketone was the least diverse group with a total of two 2 different compounds in bitter gourd leaves, viz. Diphenylketone, and 6-Methyl-5-hepten-2-one, whereas two compounds of ketone Benzylideneacetone and Acetylbutyryl were identified in unripe grafted bitter fruits (Table 1). The Hexyl methyl ketone and Tridecyl methyl ketone were only identified in the leaves of pumpkin.

3.1.7. Lactone

Similar to the ketone, lactone was the least diverse group with a total of three different compounds: γ-Butyrolactone, ethylene brassylate, and δ-Undecalactone identified in bitter gourds (Table 1). The γ-butyrolactone and ethylene brassylate were also identified in the leaves of pumpkin.

3.1.8. Terpene

A total of 20 different terpene compounds in bitter gourd and 14 in the pumpkin leaves were identified. Except β-Cedrene, α-Cubebene, dihydrocitronellol, germacrene D, α-Humulene, and viridiflorene, all identified compounds in pumpkin leaves were also recorded in bitter gourd (Table 1). The total terpene contents tend to increase through young to old age leaves of both grafted and nongrafted bitter gourds. While an inverse trend was observed in the leaves of the pumpkin where the contents terpene decreased through young to old age leaves (Figure 2). Also, the content of total terpene decreased in the fruits of bitter gourds from unripe to ripe. The highest content of terpene was recorded in the leaves of nongrafted bitter gourd than grafted group. Several new compounds of terpene such as 10-β(H)-Cadina-1(6),4-diene, β-Caryophyllene, Caryophyllene oxide, p-Cymene, Linalool, α-Pinene, and α-Terpineol were observed in the

Figure 2. The ratio of different compounds identified in leaves and fruits of nongrafted and grafted (grafting onto pumpkin) bitter gourd (*Momordica charantia* L.) and in leaves of pumpkin.

leaves of grafted bitter gourds. Also, the fruits of grafted bitter gourd develop some new terpene compounds such as Alloaromadendrene, 10-β(H)-Cadina-1(6),4-diene, α-Copaene, (E)-β-Ionone, and Valencene.

3.2. Cluster analysis

3.2.1. Principal component analysis

The PCA of the leave explained 41.9% and 23.0% of the data variance on the first and second axis, respectively, while they explained 60.3% and 31.0% of the data variance on the first and second axis, respectively for fruit (Figure 3). There was a positive correlation between acid and alcohol in leaves with a Pearson correlation of 0.34, whereas they showed a negative correlation in fruit with –0.93. Alkane had a positive correlation with alcohol in fruit with a Pearson correlation coefficient of 0.95, and it had a negative correlation with aldehyde with a Pearson correlation coefficient of –0.61. On the contrary, alkane negatively correlated with alcohol with a Pearson correlation of –0.37 while having a negligible positive correlation with the aldehyde (Figure 3). In fruit, ester and acid positively correlated with a Pearson correlation coefficient of 0.93 while negatively correlated in leaves. The correlation of ketone with alcohol and ester in fruits was also the opposite of that in leaves, but their correlations were weak in both cases. Furthermore, terpene in fruits positively correlated with a Pearson correlation coefficient of 0.40, whereas they negatively correlated with a Pearson correlation coefficient of –0.14.

3.2.2. Dendrogram

Dendrogram, based on data from Table 1, provided three main clusters assigned to leaves and fruits of bitter gourds and pumpkin leave (Figure 4). The young leave of grafted bitter gourds showed the highest dissimilarity with other groups. The highest similarity it shares was with unripe fruits from the grafted bitter gourds. The highest similarity (~69%) was observed between the leaves of nongrafted middle and old age separated from grafted-old with ~40% dissimilarity. The leaves of pumpkin from old and middle age shared up to 53% similarity while they shared up to 42% similarity with their young leaves. The old age leaves of grafted bitter gourd shared similarities with pumpkin leaves (of all stages), ranging from 42% to 47%. The unripe and ripe fruits from the grafted group shared the highest similarity, up to 57%, while from the nongrafted group, they shared up to 50% similarity. Likewise, the SIMPER analysis also provided similar results of similarities and dissimilarities among different groups. The compounds most contributing to the differences between different groups are different for each group, e.g., isobutyl angelate for nongrafted young versus grafted young, and (E)-cinnamaldehyde and Capraldehyde were the compounds that contributed the most in discrimination between nongrafted ripe versus grafted ripe groups (Table 2).

4. Discussion

This study evidenced that the grafting of bitter gourd onto pumpkin drastically reshaped the composition of volatile compounds in both leaves and fruits of bitter gourd. The grafted bitter gourds developed up to 23 new volatile compounds belonging to terpene (n = 9), ester (n = 6), alkane (n = 5), acid (n = 2), and alcohol (n = 1) in leaves. The fruits of grafted bitter gourd also developed up to 15 new volatile compounds composed of ester (n = 5), terpene (n = 3), aldehyde (n = 2), acid (n = 2), alcohol (n = 1), alkene (n = 1), and lactone (n = 1). Furthermore, grafting

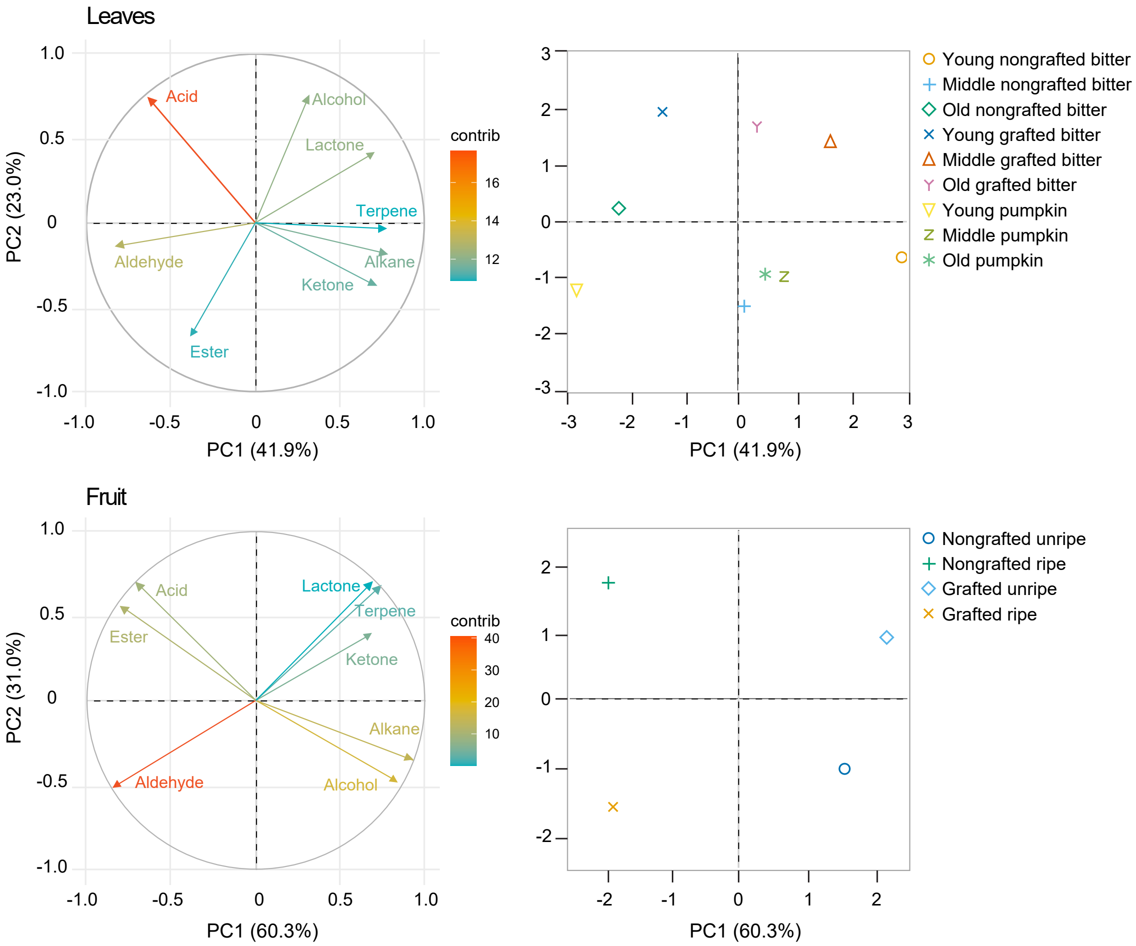

Figure 3. The vector and scatter plots of principal component analysis for leaves and fruits of nongrafted and grafted (grafting onto pumpkin) bitter gourd (*Momordica charantia* L.) as well for the leaves of pumpkin.

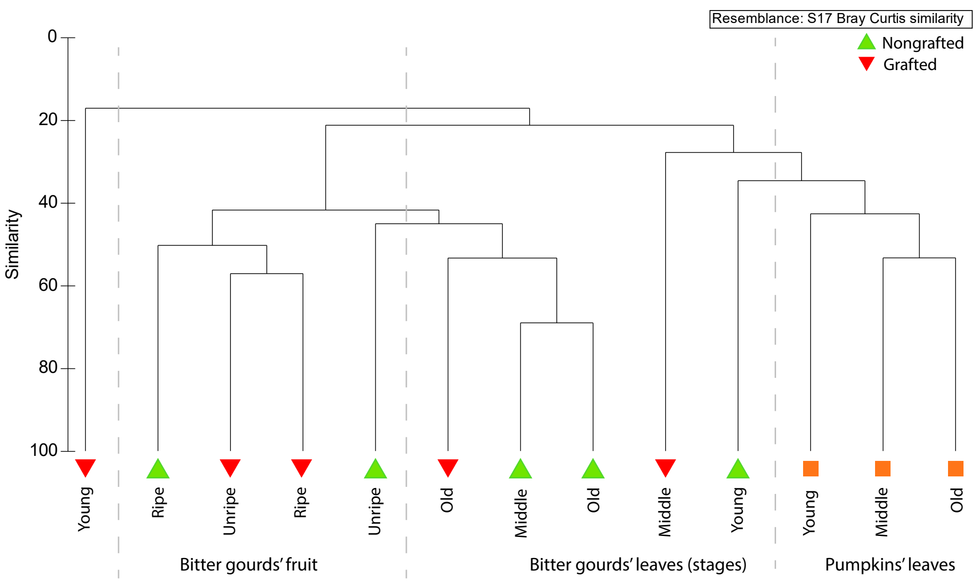

Figure 4. Dendrogram depicting the similarities between nongrafted and grafted (grafting onto pumpkin, *Cucurbita maxima*) bitter gourd (*Momordica charantia* L.) and *C. maxima* based on their volatile compounds profiles.

Table 2. Results of SIMPER comparison based on Bray Curtis dissimilarity matrix showed the average dissimilarity (%) between nongrafted and grafted (grafting onto pumpkin, *Cucurbita maxima*) bitter gourd (*Momordica charantia* L.).

	COMPARISON	Average dissimilarity (%)	Compounds	Contribution (%)
LEAVES	Nongrafted young & Grafted young	76.08	Isobutyl angelate	19.01
			2-Methyl undecanal	17.34
			3(Z)-Hexenol	10.4
			Benzaldehyde	4.17
			γ-Cadinene	3.34
	Nongrafted middle & Grafted middle	63.66	Ethylene brassylate	9.27
			2-Methyl 2-pentenoic acid	6.74
			Isopropyl 2-methylbutyrate	5.89
			Tetrahydrofurfuryl propionate	5.58
			α-Amylcinnamyl alcohol	4.83
	Nongrafted old & Grafted old	48.03	3(Z)-Hexenol	7.84
			Ethylene brassylate	5.34
			α-Terpineol	4.72
			β-Caryophyllene	4.43
			Octyl Butyrate	4.2
FRUITS	Nongrafted unripe & Grafted unripe	59.69	cis,cis-Farnesol	10.6
			Ethylene brassylate	6.53
			δ-Undecalactone	6.26
			Benzylideneacetone	5.71
			Valeric acid	4.77
	Nongrafted ripe & Grafted ripe	49.21	(E)-Cinnamaldehyde	19.02
			Capraldehyde	5.27
			2-Methyl 2-pentenoic acid	4.8
			Acetaldehyde phenethyl propyl acetal	4.77
			Tetrahydrofurfuryl propionate	4.65

of bitter gourd onto pumpkin also halted the production of up to 15 different volatile compounds in fruits belonging to ester (n = 5), terpene (n = 3), aldehyde (n = 2), acid (n = 2), alcohol (n = 1), alkene (n = 1), and lactone (n = 1). Also, up to 7 different volatile compounds in leaves belonging aldehyde (n = 4), alkane (n = 1), ester (n = 1), and lactone (n = 1) were halted to produce in grafted bitter gourd. Hence, grafting of bitter gourd onto pumpkin proved to affect both leaves and fruits' aroma profile concerning enhancing or impairing volatile compounds production. These results are in line with previous research results that reported both advantageous or deleterious grafting effects (Rouphael et al., 2010). Several studies also reported considerable impact of grafting on firmness and texture (Huitrón-Ramírez et al., 2009), fruit appearances such as size, color, shape, and absence of defects and decay (Qi et al., 2006), and flavor (Mattheis and Fellman, 1999). Furthermore, grafting has been used to raise the levels of different health-related compounds such as Lycopene, β-carotene, vitamin C, and minerals such as potassium, magnesium, phosphorus, iron, and calcium (Rouphael et al., 2010).

Generally, acidity contents tend to decrease as fruits develop (Rouphael et al., 2010; Aslam et al., 2020). In contrast, the contents of acidity of nongrafted bitter gourd's fruit were found to increase as the fruit developed. The acidity of grafted bitter gourd's fruit was found to decrease as its fruit developed, which was in line with the findings of the studies mentioned above.

Binder et al. (1989) reported a total of 50 volatile compounds from the fruit and vines of bitter gourd that included are 27 alcohols, 15 aldehydes, 3 esters, and 2 ketones, 2 hydrocarbons, and 1 acid. Among these, diacetone alcohol and 4-methylpent-3-en-2-one were the most prevalent group. Along with these compounds, the present study reported three more major groups, viz. alkane and lactone, and terpene. Furthermore, the terpene was the most prevalent group recorded from the fruits of both nongrafted and grafted bitter gourds that is inconsistent with Binder et al. (1989)'s results. Similar to Binder et al. (1989), who reported myrtenol's presence, the present study reported the existence of myrtenal in the control group. These compounds have a major interest as they have a low potential for environmental pollution with high efficiency to control pests (Zhao et al., 2020). The results from Moronkola et al. (2009) and Gao et al. (2019) reported a larger number of different volatile compounds (63 and 74, respectively) from the oil or fresh juice of bitter gourds. The most prevalent group reported by Moronkola et al. (2009) and Gao et al. (2019) were alcohols.

Several studies have investigated the varieties and contents of volatile compounds present in pumpkins. Most of the previous studies found alcohol as the primary major group present with high content (Li et al., 2019), which is inconsistent with the result of this study that showed aldehyde as the most prevalent group found in the studied pumpkin. Li et al. (2019) identified 28 volatile compounds in the leaves of Xiangyu pumpkin, smaller than the present study, which reported 52 volatile compounds in the leaves of *C. maxima*. Zhou et al. (2017) reported up to 40 different volatile compounds for *C. maxima* pulp and found alcohols, aldehydes, and ketones are the most prevalent groups.

5. Conclusion

The results of this study evinced that grafting of bitter gourd onto the pumpkin strongly regulated the quantity and quality of volatile compounds in bitter gourd. Furthermore, the statistical analyses of the data proved that the dissimilarity between grafted and nongrafted bitter gourd decreased over time from young to old leaves or unripped to ripe fruit. Like the fermentation of bitter gourd juice with *Lactobacillus plantarum* (Gao et al., 2019), the grafted of bitter gourd onto pumpkin also seems to be a promising method to reshape and possibly improve the volatile profile of bitter gourd. The results of this study should provide important information on the utilization of strategies (e.g., grafting) to reshape as well as increase the contents of different volatile compounds found in bitter gourd. Future studies should further assess the potential of different grafting methods for a bitter gourd to improve its volatile profile to meet the market requirements.

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