

## Sustainable cultivation of melon landraces: Effects of grafting on the accumulation of flavor-related compounds

A. Flores-León <sup>a</sup>, R. Martí <sup>b</sup>, M. Valcarcel <sup>b</sup>, S. Roselló <sup>c</sup>, J. Beltrán <sup>d</sup>, S. García-Martínez <sup>e</sup>, J.J. Ruiz <sup>e</sup>, C. Gisbert <sup>a</sup>, J. Cebolla-Cornejo <sup>b,\*<sup>1</sup></sup>, B. Picó <sup>a,1</sup>

<sup>a</sup> Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Cno de Vera s.n. 46022, Spain

<sup>b</sup> Joint Research Unit UJI-UPV Improvement of Agri-food Quality, COMAV, Universitat Politècnica de València, Cno de Vera s.n. 46022, Spain

<sup>c</sup> Joint Research Unit UJI-UPV Improvement of Agri-food Quality, Universitat Jaume I, Avda. Sos Baynat s/n, 12071, Castelló de la Plana, Spain

<sup>d</sup> Instituto Universitario de Plaguicidas y Aguas (IUPA), Universitat Jaume I, Campus de Riu Sec, Avda. Sos Baynat s/n, 12071, Castellón, Spain

<sup>e</sup> Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Universidad Miguel Hernández, Ctra. Beniel Km 3,2, 03312 Orihuela, Spain

### ARTICLE INFO

#### Keywords:

*Cucumis melo* L.  
Sugar  
Acid  
Volatile  
Soilborne diseases  
Landrace

### ABSTRACT

Melon landraces are highly appreciated by consumers who pay price premiums to compensate for lower yields, enabling on-farm conservation. However, they are highly susceptible to soilborne diseases. This study analyses the impact of Cucurbita and *Cucumis* rootstocks on the accumulation of flavor-related metabolites in Spanish landraces of the Ibericus melon group, as a strategy to promote their sustainable cultivation. Scion genotype was the main factor conditioning the accumulation of sugars and acids both under standard and saline organic farming conditions. The effects of grafting on organic acid accumulation were negligible, while the effects on sugar content were significant. The latter effects were dependent on specific scion-rootstock combinations, though wild *Cucumis* (e.g. Fian) rootstocks represent an alternative that should be further studied. The effect on the accumulation of volatiles was limited, and again depended on specific scion-rootstock combinations. The rootstock effect even differed between populations of the same landrace.

### 1. Introduction

Melon (*Cucumis melo* L), with an average consumption of 8 g per capita and day (data for 2020), is one of the top ten most consumed fruits in the World (<https://www.fao.org/faostat>). It is highly appreciated worldwide, and several countries from Europe, Africa, Asia, and Oceania have consumption levels higher than 15 g per capita and day. In order to satisfy the increasing demand, its production has experienced a steady increase during the last 20 years. In 2020, World production reached 27.5 million tonnes, a 50 % increase compared to two decades earlier.

Spain outstands by its particular diversity in this species. Romans already described the cultivation of *C. melo* in this area, although it seems that it was mainly restricted to the *Flexuosus* group. It would be the Arabs who probably introduced the sweet melon in Spanish agriculture (Lázaro et al., 2017). Centuries of cultivation would result in a great range of diversity in the Ibericus group of melon, represented in the subgroups *Piel de Sapo*, *Amarillo*, *Tendral*, *Rochet*, and *Blanco*

(Pitrat, 2016). These landraces are still highly appreciated in the area. In fact, they are still more valued than commercial varieties due to their specific sensorial attributes (Escribano & Lázaro, 2012).

The maintenance of this germplasm diversity, and the cultivation of melon in general, is jeopardized by the incidence of diseases, especially soilborne diseases. It would be the case of melon wilt and root rot, that affects melon cultivation in arid and semi-arid cucurbit-growing areas worldwide and where it compromises melon cultivation (Castro et al., 2020). Sources of resistance are available, but the main strategy to control these damages relies on the use of grafted plants and the development of new rootstocks (Picó, Thompson, Gisbert, Yetisir & Bebeli, 2017).

In cucurbits, the use of grafting has become commonplace ever since the initial use (*circa* 1930) of *Lagenaria* rootstocks to provide resistance against *Fusarium* in watermelon (Kawaide, 1985). This practice has been mainly used to provide tolerance against soilborne diseases and abiotic stress (Rouphael, Kyriacou, & Colla, 2018). In fact, cucurbits are increasingly cultivated under unfavorable conditions, including among

\* Corresponding author.

E-mail address: [jaicecor@upv.es](mailto:jaicecor@upv.es) (J. Cebolla-Cornejo).

<sup>1</sup> Equal contribution.

other soils with high salinity, and fertility problems. In the case of salinity, grafting arises a solution to minimize its impact in yield and quality. In the case of organic farming grafting is an essential approach, considering the limited availability of disease control alternatives and the high impact of soilborne diseases on *Cucumis* production (Flores-León et al., 2021).

Different types of rootstocks can be employed for melon production. Among them, the interspecific crosses of *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch. lead the use of rootstocks for watermelon and melon production, although other alternatives have been explored (Karaağaç & Balkaya, 2013; Cáceres, Perpiña, Ferriol, Picó, & Gisbert et al., 2017). Nonetheless, the use of intraspecific melon rootstocks is yet to be efficiently exploited and it may have an interesting alternative influence of the scion, as decreasing the high vigor typical of *Cucurbita* rootstocks and the negative impact on fruit quality (Picó et al., 2017). Indeed, quality can be affected by grafting. Although this effect is indeed highly dependent on the specific scion-rootstock combination, a majority of studies highlight a negative impact on soluble solids contents, dry matter content, and organoleptic perception (as reviewed by Németh et al. (2020).

Regarding the impact on specific compounds, Kolayli et al. (2010) found that grafted melons reduced total individual sugar contents, and citric acid contents, and increased the fructose to glucose ratio. This, added to a negative impact on aroma perception led to a negative impact on taste perception in sensory evaluations. As regards volatile compounds, a limited amount of literature is available in this species. Nevertheless, pumpkin hybrids have been found to induce a negative effect on the accumulation of key odorant esters. In muskmelon, they reduced the activity of alcohol dehydrogenases and alcohol acyl-transferases (Chuan-qiang, Yu-xue, & Lin, 2011). This impact can be considerably important, as it was reported in melons of the group reticulatus, in which high reductions in ethyl 2-methylbutanoate and ethyl butanoate contents, 20–55 % and 63–95 % respectively were induced (Condurso et al., 2012). Within the inodorus group Verzera et al., (2014) found in honeydew melons that the content of key aroma aldehydes, such as (Z)-3-nonen-1-ol and (Z)-6-nonenal were lower in grafted plants, with reductions of 20–60 % and 8–45 %, respectively. In snake melon (*Cucumis melo* var. *flexuosus*) *Cucumis* and *Cucurbita* rootstocks tended to increase the production of volatiles, especially the former, and the latter reduced the accumulation of hexoses and affected negatively flavour perception (Flores-León et al., 2021).

Despite the progress made, little is known regarding the impact of grafting on melon landraces and the effect of alternative rootstocks. In this context, the purpose of the present work is to analyze the impact of interspecific and intraspecific *Cucurbita* and *Cucumis* rootstocks on the accumulation of specific sugars, acids, and volatiles analyzing its effects in melons of the ibericus group. Melon landraces were selected as materials of study precisely to evaluate the impact on high quality materials and to prospect the use of grafting as an alternative for the production of these resources under constraining conditions such as the impact of soilborne-diseases and high salinity in sustainable organic farming cultivation.

## 2. Materials and methods

### 2.1. Plant materials

Eight accessions of five melon landrace types belonging to the *C. melo* ibericus group were used as the scion. One accession of the Amanillo landrace type, "Groc d'Ontinyent" 22AM-GO (BGV016451), one accession, 35TN (BGV004298) of the Tendral type, two accessions of Blanco type, 29BL (BGV015753) and 32BL (BGV016453), one accession of Rochet type, 02RC (BGV003718), and two accessions of Piel de Sapo type, 03PS (BGV016356) and 11PS (BGV013188). These accessions are available through the GeneBank of the Universitat Politècnica de Valencia, Spain. One commercial F1 hybrid was included as scion

control: Finura RZ F1 (Rijk Zwaan Ibérica S.A.R.L.), representing the Piel de Sapo type.

These scions were grafted onto five rootstocks. F1Pat81, an experimental interspecific cross between a *Cucumis melo* accession of the agrestis subspecies, resistant to *Monosporascus cannonballus* (Roig et al., 2012), and another *C. melo* accession of the melo subspecies, ibericus Piel de Sapo type, two hybrid rootstocks between wild species: *Cucumis ficifolius* A. Rich. × *Cucumis anguria* L. and *C. ficifolius* × *Cucumis myriocarpus* E. Mey. ex Naud. (Fian and Fimy, respectively), with resistance to different soilborne diseases (Cáceres et al., 2017), and one commercial *Cucurbita maxima* Duch. × *Cucurbita moschata* Duch. ex Poir. hybrid rootstock: Shintoza F1 (Intersemillas S.A.), with resistance to *Fusarium oxysporum* and *Verticillium albo-atrum*. The type of grafting method employed was the tongue-approach method, and plants were grafted approximately one month before transplantation. In the case of Fian, that number of seeds available was limited and seeds did not germinate uniformly. Consequently, not enough plantlets could be grafted appropriately due to differences in the development between the rootstock and the scions.

### 2.2. Experimental design and cultivation

Cultivation was performed during the spring-summer crop cycle (from May to August) in two sites with different agroclimatic characteristics, both open-field, on the East coast of Spain. The field in La Punta (39°26'41.3" N, 0°21'14.9" W, Valencia, province of Valencia), had a long history of melon cultivation with a high incidence of soilborne diseases. The field of Carrizales (38°08'32.8" N, 0°42'44.7" W, Elche province of Alicante) was selected as representative cultivation of melon under high salinity conditions (irrigation water higher than 3dS m<sup>-1</sup>). The use of saline water irrigation in the area leads to the production of high-quality recognized melons. In each field, a randomized complete block design with four blocks and four plants per treatment and block was used. In the case of Fian a lower number of plants was available for some combinations due to the lack of sufficient seed. Not enough samples could be obtained for volatile analysis of 35TN and Finura in Carrizales, and these accessions were excluded from the MANOVA biplot analysis. In the case of 02RC in Carrizales several rootstock combinations could not be sampled due to disease effects and it was excluded from the general ANOVA analysis.

Plants were transplanted onto ridges with black mulch. In La Punta a separation of 2 m between ridges and 0.6 m between plants was used. In Carrizales the distance between plants was slightly higher (0.9 m). Flood irrigation every two weeks was used in the case of La Punta, and drip irrigation in Carrizales, covering Etc and following commercial practices in the area. In both cases, organic farming management was followed.

### 2.3. Analysis of sugars and acids

Fruits were collected when they reached commercial maturity, with one fruit per plant being analysed. A 5 cm wide cross-section of the equatorial area was obtained from each fruit, homogenized, and frozen at -80 °C until analysis. An aliquot was used to determine individual sugars (sucrose, glucose, and fructose) and organic acids (citric, malic, and glutamic) using an Agilent 7100 capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) following the procedure described by (Cebolla-Cornejo, Valcárcel, Herrero-Martínez, Roselló, & Nuez, 2012).

Thawed samples were centrifuged at 13200 rpm (F45-24-11 fixed angle rotor, Eppendorf, Hamburg, Germany) for 5 min. The resulting supernatant was diluted (1:20) with ultrapure water (Elix 3, Millipore, Billerica, MA, USA) and filtered using 0.22 µm centrifuge tube filters (Costar® Spin-X®, Corning, Amsterdam, The Netherlands). Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 µm id, 375 µm od, 67 cm total length, and 60 cm effective length were used for the separation. Before their first use, capillaries were prepared

flushing NaOH 1 mol/L at 50 °C for 5 min, NaOH 0.1 mol/L for 5 min at 20 °C, and water for 10 min. At the beginning of each sequence, the capillary was flushed at 20 °C with the running buffer for 30 min. The running buffer consisted of 20 mmol/L 2,6-pyridine dicarboxylic acid and 0.1 % w:v hexadimethrine bromide solution at pH 12.1. Between runs, the capillary was flushed with 58 mmol/L sodium dodecyl sulphate (2 min) and running buffer (5 min). Samples were hydrodynamically injected at 3400 Pa for 10 s, the separation was performed applying a voltage of -25 kV at 20 °C, and the absorbance was measured at 214 nm. Results were expressed in g kg<sup>-1</sup> fresh weight (fw). Total sugars, the ratio fructose to glucose and hexoses to sucrose were determined, as well as sucrose equivalents (SEq), which was calculated by multiplying sucrose, glucose, and fructose contents by their relative sweetening power, 1, 0.74, and 1.73, respectively, and adding them up (Koehler & Kays, 1991).

#### 2.4. Analysis of volatile compounds

The purge and trap followed by gas chromatography-mass spectrometry (GC-MS) analysis method described by Fredes et al., (2017) were used for the analysis of volatile compounds. Only the samples from Carrizales were analyzed, selecting one random sample per block. Solid Phase Extraction (SPE) cartridges were conditioned with 5 mL of diethyl ether, 5 mL of *n*-hexane, and air-dried for 10 min. For extraction, 30 g of thawed sample was weighed into a 150 mL stoppered Erlenmeyer flask. A 1.6 mL min<sup>-1</sup> nitrogen gas flow was used for the inlet tube of the purge and trap headspace system, and the SPE cartridge for the outlet tube. The samples were extracted for 49 min at 40 °C using magnetic agitation. Then, the cartridges were eluted using 5 mL of diethyl ether/*n*-hexane 1:1 (v:v) solution, and 5 mL of diethyl ether. Finally, the collected elution solvents were evaporated to 0.5 mL at 35 °C under a nitrogen gas flow. The resulting extracts were divided into two aliquots and frozen at -40 °C in sealed vials until analysis.

The quantification of volatile compounds was performed using a TQ-GC gas chromatography system from Waters (Milford, MA, USA). A Supelcowax 10 column of 30 m × 0.25 mm × 0.25 µm (Sigma-Aldrich, San Luis, MO, USA) and a 1 mL min<sup>-1</sup> helium gas flow were used. The samples were injected in splitless mode (1 µL) at 280 °C. The temperature program started at 40 °C during 5 min after the injection followed by a rise to 160 °C (40 °C min<sup>-1</sup>), and finally, a rise to 250 °C (30 °C min<sup>-1</sup>) which was maintained for 2 min. Electron ionization in positive mode was used at 250 °C and 230 °C for the interphase and the ion source respectively. The mass spectra were acquired in Selected Ion Monitoring (SIM) mode using the *m/z* relation for each compound.

#### 2.5. Statistical analysis

MANOVA tests were performed with the SPSS 22.0 software (NYSE: IBM, Armonk, NY, USA) to evaluate the effects of the site of cultivation, scion, and rootstock and their interactions. P-value was calculated using the Pillai trace test. ANOVA tests, Tukey and Dunnett's tests were performed to delve into the effect on individual variables. StatGraphics Centurion version 17.2.04 for Windows and IBM SPSS Statistics 25 for Windows were used for this purpose.

The effect of main effects and interaction in the accumulation of volatiles was studied with a graphical MANOVA Biplot representation (freeware licensed software by Vicente-Villardón, 2015). Bonferroni circles were plotted to represent the confidence intervals ( $\alpha = 0.05$ ). Non-overlapping projections of a couple of treatments on each variable indicate significant differences. In the MANOVA Biplot, dashed lines were used to indicate non-significant effects.

### 3. Results

#### 3.1. Accumulation of sugars and acids

The assays took place under organic farming conditions in order to verify the performance of the rootstocks in actual infestation contexts. In both fields, soilborne pathogens were detected during cultivation, as described in a previous study dealing with snake melon in the same fields (Flores-León et al., 2021), but La Punta presented a higher mortality and pathogen presence than Carrizales. In the case of La Punta *Macrophomina phaseolina*, *Fusarium* species and *Neocosmospora falci-formis* represented the main pathogens, while in Carrizales predominated *Macrophomina phaseolina* and *Fusarium* species.

Both cultivation sites differed in climatic conditions, management, and even disease incidence, but probably the main difference was related to salinity levels. In Carrizales, salinity of irrigation water (4.5 dS m<sup>-1</sup>) doubled that of La Punta. Soil salinity was also considerably higher in Carrizales (3.2 dS m<sup>-1</sup> vs. 0.67 dS m<sup>-1</sup>). Accordingly, location influenced the accumulation of soluble metabolites leading to significant effects in the levels of glutamic acid, fructose, glucose, sucrose, the ratio fructose to glucose, and hexoses to sucrose, as well as SSC (Table 1). The contents of hexoses were higher in La Punta, while sucrose content and SSC were higher in Carrizales. Regarding acids, higher contents of glutamic acid were found in La Punta. In the case of citric acid, the higher contents of La Punta were not significant but close to the threshold ( $p = 0.07$ ).

The scion had a significant effect on all the variables related to soluble solids (Table 1). Accession 03PS outstood for malic acid accumulation with levels that more than doubled the rest of accessions, even 11PS that also belonged to the same landrace of the Piel de Sapo type. In contrast, 03PS presented the lowest levels of citric acid. Glutamic acid was detected at very low levels, with 35TN having the highest accumulation. As sugars are concerned, Finura, 32BL, and 35TN had the highest values of fructose and 11PS the lowest. A similar trend was found for glucose accumulation. Sucrose levels were similar in all the accessions, but Finura offered lower levels. The differences in total sugars were limited, with significant differences between the 101.20 mg kg<sup>-1</sup> of Finura and 110.21 mg kg<sup>-1</sup> of 32BL. A similar trend was observed in the case of sucrose equivalents, which is weighed by the sweetening power of each sugar, and SSC. On the contrary, the profile in sugar accumulation was more variable. In this sense, the fructose to glucose ratio, ranged from 0.82 in 11PS to 1.04 in 32BL. Even higher variation was found for hexoses to sucrose ratio that varied from 0.53 in 11PS to 0.95 in Finura.

The accumulation of acids, SSC and fructose was not significantly affected by the rootstock (Table 1). In fact, the rootstock effect was only significant for the accumulation of glucose, sucrose, the ratio fructose to glucose, total sugars, and sucrose equivalents. The non-grafted control had higher values of glucose and lower levels of sucrose compared to grafted plants. Among the rootstocks, the use of Fian (*F1 C. ficifolius* × *C. anguria*) led to higher sucrose contents. As opposed to the scion effect, the variation in the fructose to glucose ratio was low, with the lowest levels found in Shintoza and Fimy. Finally, the high accumulation of sucrose in Fian led to higher levels of total sugars and sucrose equivalents observed with this rootstock.

Important interactions between factors were detected (Table 1). The interaction location × scion was highly significant for almost all the variables. In the case of location × rootstock, it was only significant for glutamic acid contents and the ratio fructose to glucose. The scion × rootstock interaction was significant for all the variables except those regarding acid accumulation.

Considering the existence of such interactions a more thorough evaluation, extended to the volatile profile, was performed in Carrizales, a cultivation site with saline water, that maximizes melon quality, and milder soilborne pathogen stress. This time the focus was placed in the evaluation of a higher range of landraces.

**Table 1**

Effect of the location, scion, rootstock and their interaction on the accumulation of sugars and acids in sweet melon fruits from non-grafted (NG) plants and those grafted onto *Cucurbita* commercial rootstock Shintoza and experimental *Cucumis* rootstocks (F1Pat81, Fian, and Fimy) grown at La Punta and Carrizales. ANOVA p-values are indicated and for each effect, different letters indicate significant difference at  $p < 0.05$  (Tukey test).

		Malic acid g kg <sup>-1</sup>	Citric acid g kg <sup>-1</sup>	Glutamic acid g kg <sup>-1</sup>	Fructose g kg <sup>-1</sup>	Glucose g kg <sup>-1</sup>	Sucrose g kg <sup>-1</sup>	Fructose/Glucose ratio	Hexoses/Sucrose ratio	Total sugars g kg <sup>-1</sup>	Sucrose equivalents g kg <sup>-1</sup>	Soluble solids content (°Brix)
Location (L)	Carrizales	0.185 <sup>a</sup>	4.679 <sup>a</sup>	0.019 <sup>a</sup>	18.142 <sup>a</sup>	19.884 <sup>a</sup>	67.248 <sup>b</sup>	0.892 <sup>a</sup>	0.643 <sup>a</sup>	105.275 <sup>a</sup>	113.348 <sup>a</sup>	12.918 <sup>b</sup>
Scion (S)	La Punta	0.222 <sup>a</sup>	4.875 <sup>a</sup>	0.040 <sup>b</sup>	22.102 <sup>b</sup>	22.743 <sup>b</sup>	60.849 <sup>a</sup>	0.977 <sup>b</sup>	0.800 <sup>b</sup>	105.694 <sup>a</sup>	115.915 <sup>a</sup>	11.522 <sup>a</sup>
	<i>p</i> -value	0.41	0.07	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.82	0.16	<0.01
03PS	0.55 <sup>b</sup>	4.08 <sup>a</sup>	0.03 <sup>ab</sup>	17.70 <sup>b</sup>	19.04 <sup>a</sup>	66.27 <sup>b</sup>	0.93 <sup>bc</sup>	0.58 <sup>a</sup>	103.01 <sup>ab</sup>	110.98 <sup>a</sup>	12.4 <sup>b</sup>	
11PS	0.23 <sup>a</sup>	4.86 <sup>b</sup>	0.03 <sup>ab</sup>	14.70 <sup>a</sup>	17.55 <sup>a</sup>	71.82 <sup>b</sup>	0.81 <sup>a</sup>	0.53 <sup>a</sup>	104.06 <sup>ab</sup>	110.22 <sup>a</sup>	12.7 <sup>b</sup>	
32BL	0.06 <sup>a</sup>	4.76 <sup>b</sup>	0.02 <sup>a</sup>	22.84 <sup>c</sup>	21.86 <sup>b</sup>	65.52 <sup>b</sup>	1.04 <sup>d</sup>	0.73 <sup>b</sup>	110.21 <sup>b</sup>	121.20 <sup>b</sup>	12.5 <sup>b</sup>	
35TN	0.12 <sup>a</sup>	5.15 <sup>b</sup>	0.04 <sup>b</sup>	21.68 <sup>c</sup>	23.76 <sup>bc</sup>	63.51 <sup>b</sup>	0.91 <sup>b</sup>	0.82 <sup>bc</sup>	108.94 <sup>ab</sup>	118.59 <sup>ab</sup>	12.2 <sup>ab</sup>	
Finura	0.06 <sup>a</sup>	5.04 <sup>b</sup>	0.03 <sup>ab</sup>	23.70 <sup>c</sup>	24.36 <sup>c</sup>	53.14 <sup>a</sup>	0.97 <sup>c</sup>	0.95 <sup>c</sup>	101.20 <sup>a</sup>	112.17 <sup>a</sup>	11.4 <sup>a</sup>	
	<i>p</i> -value	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01
Rootstock (R)	NG	0.20	4.81	0.03	20.55	22.02 <sup>b</sup>	59.29 <sup>a</sup>	0.93 <sup>ab</sup>	0.79 <sup>a</sup>	101.86 <sup>a</sup>	111.14 <sup>a</sup>	12.0 <sup>a</sup>
F1Pat81	0.25 <sup>a</sup>	4.89 <sup>a</sup>	0.02 <sup>a</sup>	19.41 <sup>a</sup>	19.80 <sup>a</sup>	65.00 <sup>ab</sup>	0.97 <sup>b</sup>	0.68 <sup>a</sup>	104.21 <sup>ab</sup>	113.23 <sup>ab</sup>	12.0 <sup>a</sup>	
Shintoza	0.16 <sup>a</sup>	4.63 <sup>a</sup>	0.03 <sup>a</sup>	20.01 <sup>a</sup>	21.49 <sup>ab</sup>	63.42 <sup>ab</sup>	0.92 <sup>a</sup>	0.73 <sup>a</sup>	104.92 <sup>ab</sup>	113.94 <sup>ab</sup>	12.2 <sup>a</sup>	
Fian	0.25 <sup>a</sup>	4.69 <sup>a</sup>	0.03 <sup>a</sup>	20.46 <sup>a</sup>	21.67 <sup>ab</sup>	68.73 <sup>b</sup>	0.93 <sup>ab</sup>	0.68 <sup>a</sup>	110.86 <sup>b</sup>	120.16 <sup>b</sup>	12.5 <sup>a</sup>	
Fimy	0.16 <sup>a</sup>	4.87 <sup>a</sup>	0.03 <sup>a</sup>	20.18 <sup>a</sup>	21.59 <sup>ab</sup>	63.80 <sup>ab</sup>	0.92 <sup>a</sup>	0.74 <sup>a</sup>	105.57 <sup>ab</sup>	114.69 <sup>ab</sup>	12.4 <sup>a</sup>	
	<i>p</i> -value	0.54	0.47	0.63	0.54	0.01	0.05	0.02	0.20	0.05	0.04	0.22
LxS	<i>p</i> -value	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.13	<0.01
LxR	<i>p</i> -value	0.2635	0.18	0.02	0.54	0.33	0.23	0.05	0.09	0.34	0.31	0.10
SxR	<i>p</i> -value	0.75	0.23	0.23	0.05	0.05	0.04	<0.01	0.50	<0.01	<0.01	<0.01

Two independent MANOVAs confirmed highly significant effects of the scion genotype, rootstock, and their interaction in the global accumulation of sugars and acids and volatiles (Pillai trace *p*-values  $< 0.001$ ). It was confirmed then, the necessity to evaluate the impact of rootstocks on each specific scion genotype.

Indeed, in the case of the sugar and acid profile, the response of the plants to grafting depended on the specific scion-rootstock combination. Even more, within the same landrace type, the response varied depending on the precise accession being considered. In the Amarillo landrace accession 22AM-GO, the sugar and acid profile of F1Pat81 was quite similar to that of the non-grafted control (Fig. 1). In this case the Fian rootstock combination was not available. With this landrace, the use of the commercial *Cucurbita* hybrid Shintoza mainly had a small effect increasing the amounts of glutamic acid over the quantification limit, while Fimy tended to offer higher sugar accumulation, though this difference was not significant (Supp. Table 1).

Limited differences were found when the Rochet landrace accession 02RC was used as scion. Although grafted plants tended to reach lower citric acid contents than NG, this difference was not significant.

In the Blanco landrace two accessions were studied, and a different response was observed in each one (Fig. 1). The differences between grafted and non-grafted plants were higher in 29BL. In this accession, NG plants tended to show higher hexoses and lower sucrose accumulation leading to higher hexoses to sucrose ratio. Although grafted plants tended to offer higher sucrose contents, total sugars and SSC, especially in the case of Shintoza, but a high level of variability restricted the significance of this effect on most rootstocks. In the accession 32BL the differences were attenuated, and only Shintoza tended to offer a less acidic profile (Supp table 1).

A difference in the response was also found between the two accessions evaluated of Piel de Sapo. In the 11PS accession, the differences between the NG control and grafted plants were limited, though grafted plants tended to reach higher sugar accumulation and SSC (Fig. 1). But this effect was only significant for Fian and Fimy (Supp table 1). This also applied in the case of 03PS accession with Fian. For this accession Shintoza tended to show a less acidic profile, as it happened with the Blanco accession, but this effect was not significant (Supp Table 1). Finally, F1Pat81 presented a high fructose to glucose ratio.

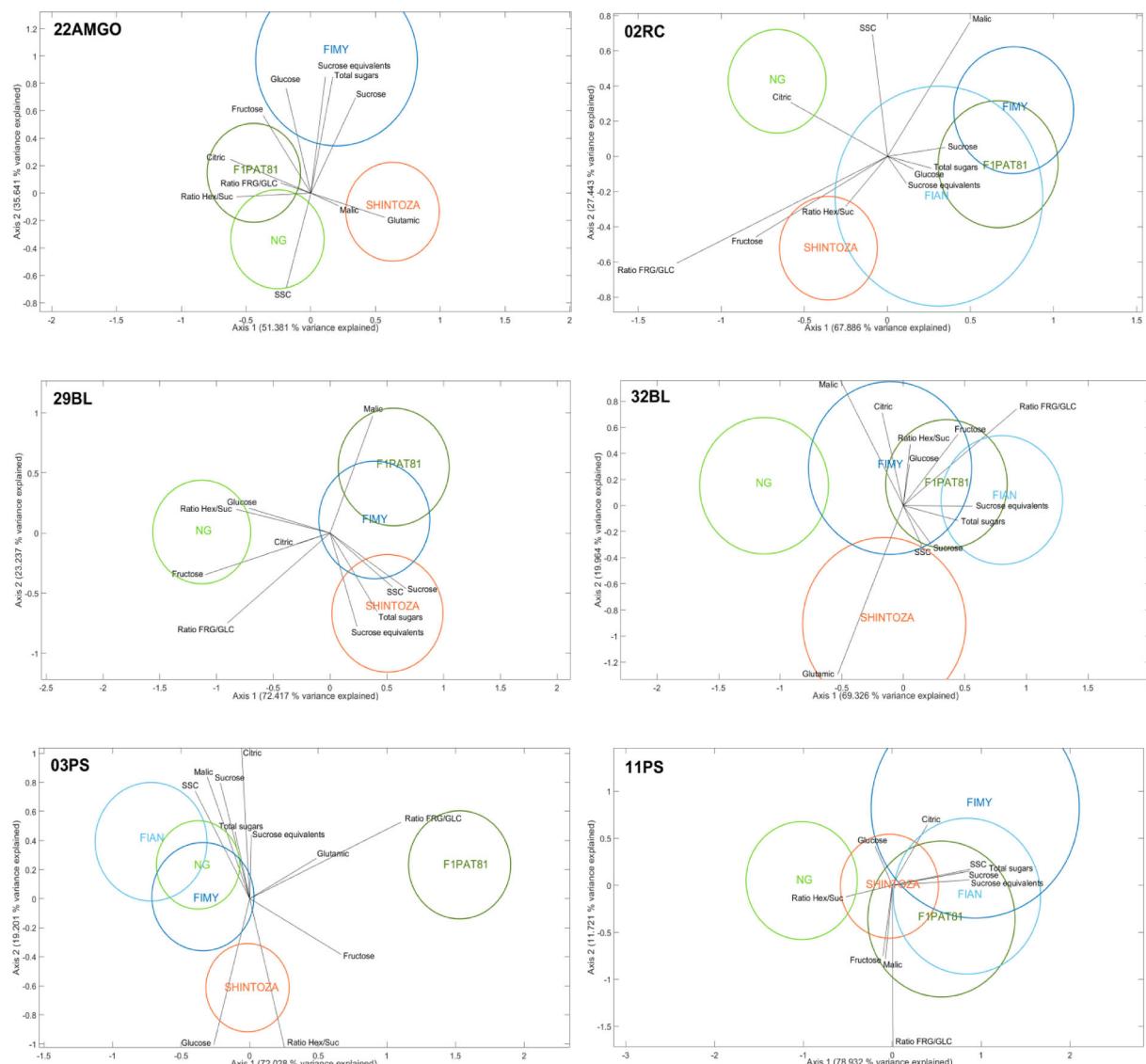
### 3.2. Volatile organic compound profiles

Important differences were found among accessions in the volatile profile of non-grafted (NG) controls, even between those belonging to the same landrace (Table 2). Accession 02RC of Rochet and 03PS from Piel de Sapo showed the lowest total volatiles contents, mainly due to a low accumulation of aldehydes. On the other hand, 29BL from Blanco reached the highest total volatiles and total aldehydes contents, while 02RC and 32BL had high total esters content. The effect of grafting on fruits volatile profile regarding total volatiles and groups of volatiles was limited. Nonetheless, a more detailed analysis of this effect was studied with independent MANOVA biplots for each accession.

A significant effect of rootstocks on the total amount of specific groups of volatiles was restricted to the accessions 22AG-GO of Amarillo, 29BL of Blanco and 3PS of Piel de Sapo. In Amarillo, grafting tended to reduce the amount of total volatiles, but this effect was only significant when the Fimy rootstock was used (Table 2). A similar effect was detected in 29BL of Blanco, with a trend to reduce total volatiles and total aldehydes in fruits from grafted plants, but this time this effect was only significant in the case of F1Pat81. In the case of 03PS differences in the amount of apocarotenoids were found between Fimy and Fian, but not between grafted plants and the non-grafted control.

Nonetheless, the specific scion-rootstock combination also seemed determinant in the case of the volatile profile and it affected specific compounds. Consequently, it was further reviewed in a case-by-case basis. In the Amarillo accession 22AM-GO the use of *Cucumis* rootstocks had a reduced effect on the volatile profile compared to the non-grafted control (Fig. 2, Supp. Table 2). The highest differences were found between the non-grafted control and Shintoza, the *Cucurbita* F1 Hybrid, which tended to show higher accumulation levels of certain esters (e.g. ethyl butanoate, 1,3 and methyl-2-methyl butyrate, 1,1), and a much lower aldehyde content (e.g. nonanal, 2,6, and (Z)-6-nonenal, 2,7).

In the case of the Rochet accession 02RC the differences between rootstock combinations and the NG control were, in general, negligible. Nonetheless, *Cucumis* rootstocks increased the content of (Z)-3-Nonen-1-ol, 3,6 in the figure (Fig. 2, Supp. Table 2). The use of the Fian rootstock led to a different volatile profile, plotting in MANOVA biplot far from the NG. This rootstock tended to increase the levels of certain esters (ethyl butanoate, 1,3 and ethyl-2-methyl butyrate, 1,4) and alcohols, though



**Fig. 1.** MANOVA Biplot for the Sugars and Acids analyzed in different melon landrace and rootstock combinations grown in Carrizales. Circles represent Bonferroni confidence intervals. NG: non-grafted control, F1Pat81 (*C. melo* subsp *melo* group ibericus,  $\times$  *C. melo* subsp *agrestis* group chinensis, Pat 81), Shintoza (*C. maxima*  $\times$  *C. moschata*), Fimy (*C. ficifolius*  $\times$  *C. myriocarpus*), Fian (*C. ficifolius*  $\times$  *C. anguria*).

these changes were not significant when compared in case-by-case basis (Supp. Table 2).

The two accessions of Blanco had certain resemblances in their response (Fig. 2, Supp. Table 2). In both cases, the differences in the volatile profile between grafted combinations were limited, but they seemed to differ from the NG control. Indeed, in both cases the control plotted at some distance from the rest of the treatments and the projections of the Bonferroni confidence circles did not overlap for several volatile vectors. The response of both accessions was different, though in some aspects. Fruits from 29BL grafted plants presented lower aldehyde content (e.g. (E,Z)-2,6-nonadienal, 2.11 and nonanal, 2.6), while those from 32BL grafted plants had higher content of specific compounds in certain combinations. It was the case of 2-methyl propyl acetate (1.2) in Fian, (Z)-6-nonenal (2.7) in Fimy, or geranylacetone (4.1) in F1Pat81.

Differences between accessions of the same landrace were also observed in the case of Piel de sapo (Fig. 2). In the accession 03PS, melons from plants grafted onto Fian, and F1Pat81 clearly differed from the rest of the treatments and the NG control. They displayed higher content of esters (e.g. heptyl acetate and butyl butyrate in Fian) and alcohols (e.g. 1-pentanol, 3.1, 1 decanol, 3.8 and phenol, 3.12, in Pat81).

On the other hand, melons from plants grafted on Shintoza and Fimy had a volatile profile very similar to the NG control. This time those grafted on F1Pat81 tended to show higher levels of esters and certain aldehydes and alcohols (e.g. (E)-2-nonenal, 2.10, (E,Z)-2,6-nonadienal, 2.11), and 1-pentanol, 3.1). In the case of 11PS the differences between the different rootstock/scion combinations and the NG control were rather limited. A higher level of variation was observed though, which led to higher values for the Bonferroni circles of confidence in the MANOVA biplot. Nonetheless, some common responses were identified. In this sense F1Pat81 again tended to show higher levels of certain alcohols, and lower levels of certain aldehydes, though these differences in a case-by-case analysis were not significant (Supp. Table 2).

#### 4. Discussion

Organic farming is becoming increasingly important in areas such as Europe, where an important effort has been made to increase its adoption as a means to contribute to sustainable development. It is true that under organic management crops tend to offer lower yields, but they can also be more profitable, environmentally friendly, and deliver equally or

Table 2

Accumulation of volatiles in fruits grown in Carrizales with different landrace and rootstock combinations. Totals of each volatile group obtained as the sum of individual compound concentrations within that group. ANOVA p-values are indicated. Different letters indicate significant difference at  $p < 0.05$  (Tukey test).

Scion	Rootstock	Total volatiles (ng/g)	Total esters (ng/g)	Total aldehydes (ng/g)	Total alcohols (ng/g)	Total apocarotenoids (ng/g)
22AM-GO	F1Pat81	319.92 $\pm$ 36.17 <sup>ab</sup>	2.17 $\pm$ 0.32 <sup>a</sup>	207.37 $\pm$ 30.87 <sup>a</sup>	108.53 $\pm$ 11.28 <sup>a</sup>	1.85 $\pm$ 0.22 <sup>a</sup>
	Fimy	223.19 $\pm$ 11.89 <sup>a</sup>	2.83 $\pm$ 0.86 <sup>a</sup>	128.58 $\pm$ 36.64 <sup>a</sup>	90.41 $\pm$ 24.08 <sup>a</sup>	1.39 $\pm$ 0.19 <sup>a</sup>
	Shintoza	265.29 $\pm$ 28.7 <sup>ab</sup>	7.92 $\pm$ 2.29 <sup>a</sup>	123.54 $\pm$ 36.2 <sup>a</sup>	132.22 $\pm$ 8.19 <sup>a</sup>	1.61 $\pm$ 0.14 <sup>a</sup>
	NG	387.68 $\pm$ 34.17 <sup>b</sup>	2.09 $\pm$ 0.2 <sup>a</sup>	277.24 $\pm$ 28.6 <sup>a</sup>	106.91 $\pm$ 16.78 <sup>a</sup>	1.44 $\pm$ 0.15 <sup>a</sup>
02RC	<i>p</i> -value	0.0450	0.0404	0.0383	0.3426	0.3269
	F1Pat81	282.02 $\pm$ 31.45 <sup>a</sup>	6.47 $\pm$ 2.02 <sup>a</sup>	103.84 $\pm$ 21.51 <sup>a</sup>	170.05 $\pm$ 12.29 <sup>a</sup>	1.66 $\pm$ 0.08 <sup>a</sup>
	Fimy	234.89 $\pm$ 37.69 <sup>a</sup>	10.35 $\pm$ 5.99 <sup>a</sup>	35.97 $\pm$ 6.62 <sup>a</sup>	186.94 $\pm$ 38.21 <sup>a</sup>	1.63 $\pm$ 0.11 <sup>a</sup>
	Shintoza	218.86 $\pm$ 28.55 <sup>a</sup>	2.93 $\pm$ 0.15 <sup>a</sup>	77.53 $\pm$ 7.44 <sup>a</sup>	136.39 $\pm$ 29.64 <sup>a</sup>	2.01 $\pm$ 0.29 <sup>a</sup>
29BL	NG	201.28 $\pm$ 33.91 <sup>a</sup>	4.47 $\pm$ 0.93 <sup>a</sup>	100.11 $\pm$ 20.52 <sup>a</sup>	95.21 $\pm$ 17.92 <sup>a</sup>	1.49 $\pm$ 0.25 <sup>a</sup>
	<i>p</i> -value	0.3688	0.2599	0.1237	0.1214	0.4161
	F1Pat81	328.31 $\pm$ 44.31 <sup>a</sup>	2.84 $\pm$ 1.09 <sup>a</sup>	216.48 $\pm$ 51.8 <sup>a</sup>	107.19 $\pm$ 8.62 <sup>a</sup>	1.8 $\pm$ 0.4 <sup>a</sup>
	Fimy	452.18 $\pm$ 54.59 <sup>ab</sup>	46.78 $\pm$ 44.43 <sup>a</sup>	264.2 $\pm$ 67.7 <sup>a</sup>	139.91 $\pm$ 15.4 <sup>a</sup>	1.28 $\pm$ 0.23 <sup>a</sup>
32BL	Shintoza	460.52 $\pm$ 91.35 <sup>ab</sup>	13.72 $\pm$ 7.85 <sup>a</sup>	287 $\pm$ 42.65 <sup>a</sup>	158.32 $\pm$ 45.56 <sup>a</sup>	1.48 $\pm$ 0.13 <sup>a</sup>
	NG	678.92 $\pm$ 71.99 <sup>b</sup>	3.89 $\pm$ 0.33 <sup>a</sup>	568.4 $\pm$ 44.35 <sup>b</sup>	105.02 $\pm$ 28.19 <sup>a</sup>	1.6 $\pm$ 0.49 <sup>a</sup>
	<i>p</i> -value	0.0375	0.5131	0.0058	0.5058	0.7513
	F1Pat81	601.02 $\pm$ 63.31 <sup>a</sup>	7.75 $\pm$ 1.52 <sup>a</sup>	422.06 $\pm$ 85.58 <sup>a</sup>	168.53 $\pm$ 37.02 <sup>a</sup>	2.69 $\pm$ 0.07 <sup>a</sup>
11PS	Fian	473.22 $\pm$ 26.24 <sup>a</sup>	10.51 $\pm$ 2.34 <sup>a</sup>	318.64 $\pm$ 23.24 <sup>a</sup>	142.27 $\pm$ 34.5 <sup>a</sup>	1.8 $\pm$ 0.21 <sup>a</sup>
	Fimy	1094.38 $\pm$ 350.4 <sup>a</sup>	7.99 $\pm$ 0.76 <sup>a</sup>	587.08 $\pm$ 69.24 <sup>a</sup>	148.29 $\pm$ 21.44 <sup>a</sup>	1.74 $\pm$ 0.16 <sup>a</sup>
	Shintoza	571.51 $\pm$ 131.18 <sup>a</sup>	6.68 $\pm$ 0.28 <sup>a</sup>	420.3 $\pm$ 117.23 <sup>a</sup>	142.56 $\pm$ 23.33 <sup>a</sup>	1.97 $\pm$ 0.06 <sup>a</sup>
	NG	441.74 $\pm$ 63.88 <sup>a</sup>	4.73 $\pm$ 1.26 <sup>a</sup>	329.46 $\pm$ 44.93 <sup>a</sup>	106.08 $\pm$ 19.41 <sup>a</sup>	1.47 $\pm$ 0.4 <sup>a</sup>
03PS	<i>p</i> -value	0.1245	0.1361	0.1491	0.6392	0.1610
	F1Pat81	407.12 $\pm$ 66.57 <sup>a</sup>	3.45 $\pm$ 0.42 <sup>a</sup>	276.62 $\pm$ 53.28 <sup>a</sup>	104.98 $\pm$ 56.45 <sup>a</sup>	2.26 $\pm$ 0.08 <sup>a</sup>
	Fian	439.46 $\pm$ 126.3 <sup>a</sup>	3.46 $\pm$ 0.35 <sup>a</sup>	164.79 $\pm$ 52.35 <sup>a</sup>	270.01 $\pm$ 179.5 <sup>a</sup>	1.2 $\pm$ 0.51 <sup>a</sup>
	Fimy	217.55 $\pm$ 45.47 <sup>a</sup>	3.65 $\pm$ 0.48 <sup>a</sup>	95.11 $\pm$ 31.34 <sup>a</sup>	116.84 $\pm$ 13.71 <sup>a</sup>	1.94 $\pm$ 0.9 <sup>a</sup>
	Shintoza	351.58 $\pm$ 65.81 <sup>a</sup>	3.11 $\pm$ 0.15 <sup>a</sup>	222.14 $\pm$ 54.14 <sup>a</sup>	124.34 $\pm$ 25.93 <sup>a</sup>	2 $\pm$ 0.15 <sup>a</sup>
	NG	404.36 $\pm$ 126.39 <sup>a</sup>	2.13 $\pm$ 0.68 <sup>a</sup>	292.53 $\pm$ 95.55 <sup>a</sup>	108.17 $\pm$ 30.1 <sup>a</sup>	1.53 $\pm$ 0.2 <sup>a</sup>
	<i>p</i> -value	0.6116	0.5224	0.5136	0.6197	0.4800
	F1Pat81	289.53 $\pm$ 57.42 <sup>a</sup>	7.74 $\pm$ 4.72 <sup>a</sup>	133.92 $\pm$ 14.08 <sup>a</sup>	146.32 $\pm$ 61.71 <sup>a</sup>	1.54 $\pm$ 0.13 <sup>ab</sup>
	Fian	246.22 $\pm$ 18.7 <sup>a</sup>	3.79 $\pm$ 0.08 <sup>a</sup>	118.55 $\pm$ 6.95 <sup>a</sup>	122.04 $\pm$ 25.14 <sup>a</sup>	1.85 $\pm$ 0.44 <sup>b</sup>
	Fimy	216.41 $\pm$ 57.92 <sup>a</sup>	3.25 $\pm$ 0.62 <sup>a</sup>	90.51 $\pm$ 81.77 <sup>a</sup>	121.78 $\pm$ 26.13 <sup>a</sup>	0.86 $\pm$ 0.1 <sup>a</sup>
	Shintoza	98.45 $\pm$ 31.43 <sup>a</sup>	1.53 $\pm$ 0.04 <sup>a</sup>	27.68 $\pm$ 4.46 <sup>a</sup>	68.26 $\pm$ 27.93 <sup>a</sup>	0.98 $\pm$ 0.23 <sup>ab</sup>
	NG	176.73 $\pm$ 2.78 <sup>a</sup>	2.29 $\pm$ 0.29 <sup>a</sup>	96.37 $\pm$ 5.67 <sup>a</sup>	76.81 $\pm$ 7.89 <sup>a</sup>	1.26 $\pm$ 0.13 <sup>ab</sup>
	<i>p</i> -value	0.0804	0.3927	0.4412	0.5142	0.0458

more nutritious foods that contain fewer pesticide traces, compared with conventional farming (Reganold & Wachter, 2016). Consequently, it represents the perfect scenario to promote the active cultivation of melon landraces, contributing to their *in situ* on-farm conservation. Nonetheless, for that purpose it is necessary to assure a price premium that compensates for lower productivity. This is usually achieved targeting the production to high quality markets that value organoleptic and functional quality, as it is the case for example in tomato landraces or high pigment varieties (Cebolla-Cornejo, Soler, & Nuez, 2007).

Spain represents the perfect scenario for this approach. This area represents a secondary center of melon diversity, characterized by a wide variety of landraces of the Ibericus group, which have been retained in the domestic markets thanks to taste attributes (López-Sesé, Staub, & Gómez-Guillamón, 2003). But its cultivation is highly jeopardized by the incidence of soilborne diseases. Accordingly, the use of grafting may represent a reliable alternative to promote their cultivation in these conditions, as long as it does not affect their typical quality standards.

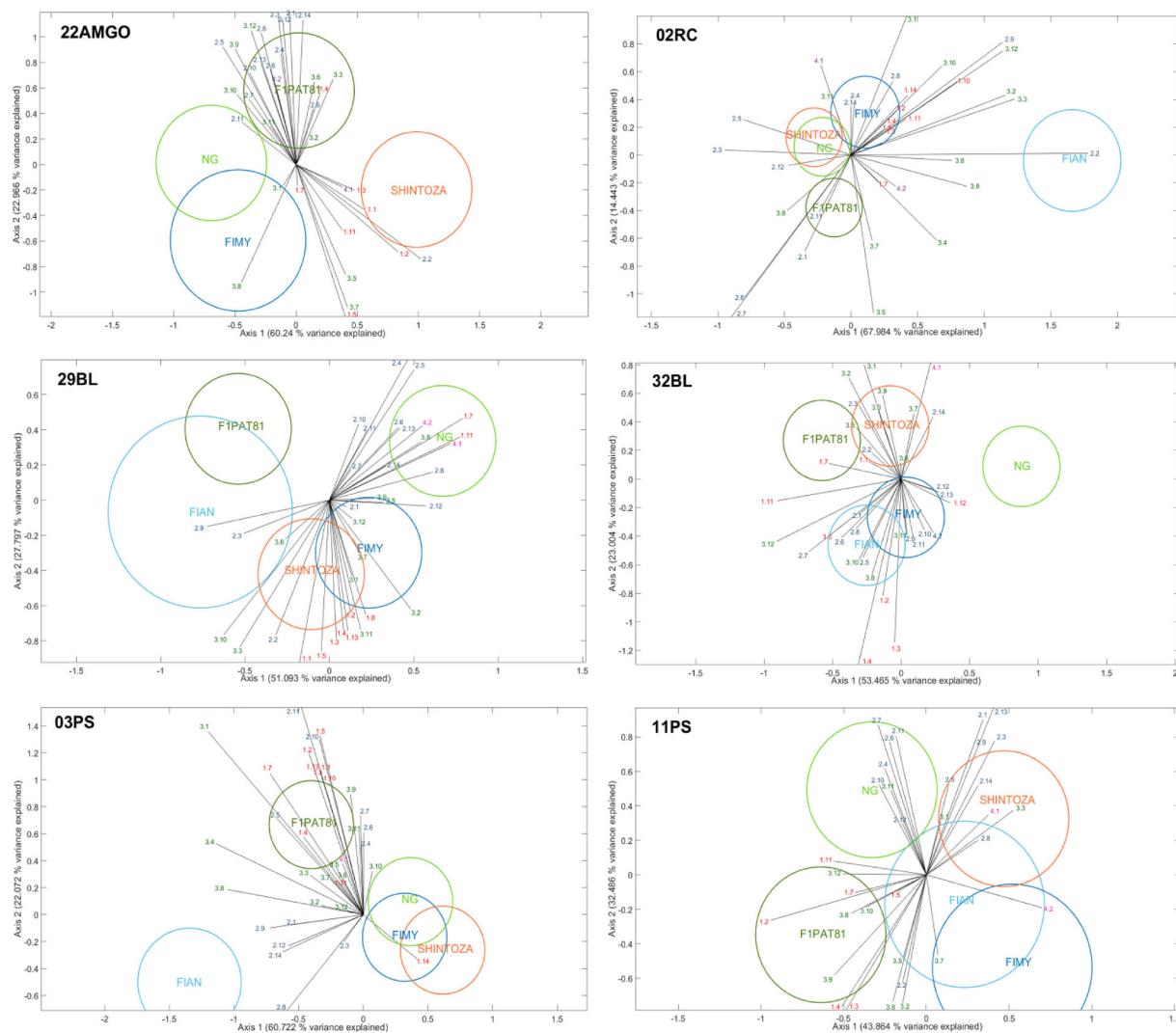
#### 4.1. Effect on soluble solids

The use of grafting also offers an alternative strategy to promote cultivation under saline conditions. Indeed, salinity is a growing concern worldwide, especially in arid and semi-arid areas, and the development of new cultivars tolerant to salinity stress is becoming increasingly important (Akrami & Arzani, 2019). In the case of melon, it has been proved that the use of certain *Cucurbita* rootstocks can help to minimize salinity negative effects (Colla et al., 2006). From the point of view of fruit quality, growing melons in saline conditions usually results in

increased SSC. The effect, though, is dependent on local conditions and the varieties being considered. For example, Huang et al. (2012) reported that increasing water conductivity from 1 to 2.665 and 7.03 dS m<sup>-1</sup> led to increases in SSC from 9.03° to 11.03° and 11.61°Brix in Northwest China using Huanghemi melon. On the other hand, Tedeschi, Lavini, Riccardi, Pulvento, & d'Andria, (2011) observed a more limited increase with Tendral melon grown in Italy. In these conditions salinity (8.7 dS m<sup>-1</sup>) resulted in 10.5°Brix SSC, only slightly higher than the control (10.1°Brix at 0.9 dS m<sup>-1</sup>) and a higher rise (11.3°Brix) was only achieved with extreme levels (28.2 dS m<sup>-1</sup>). Colla et al., (2006) also found an increase in SSC under saline conditions with Cyrano melons in Italy (e.g. 0.6 to 0.7°Brix increase between 2.0 and 4.0 dS m<sup>-1</sup>), but this effect was reduced in the case of grafted plants.

In our case, the saline waters used in Carrizales probably explained the 1.4°Brix increase in SSC compared to La Punta. Interestingly, this increase would be mainly motivated by a high increase in sucrose content, being hexoses content higher in La Punta. This result agrees with those of (Burger, Shen, Petreikov, & Schaffer, 2000), which also found that differences in SSC were rather conditioned by sucrose contents than hexoses. Nevertheless, It couldn't be ruled out an advance in the ripening process in Carrizales, as the hexoses to sucrose ratio was clearly lower in this location. In this sense, sucrose contents increase in melon during the last steps of ripening, while hexoses keep constant or tend to decrease (Burger et al., 2006; Perpiñá, Cebolla-Cornejo, Esteras, Montforte, & Picó, 2017).

Fewer information is available on the impact of salinity on acid content, as it has received less attention. Del Amor, Martínez, & Cerdá (1999) observed that not only SSC but also acidity increased with increasing salinity levels in Galia melons grown in Spain. Nonetheless,



**Fig. 2.** MANOVA Biplot of organic volatile compounds analyzed in different melon landrace and rootstock combinations grown in Carrizales. Circles represent Bonferroni confidence intervals. NG: non-grafted control, F1Pat81 (*C. melo* subsp *melo* group ibericus, x *C. melo* subsp *agrestis* group chinensis, Pat 81), Shintoza (*C. maxima* x *C. moschata*), Fimy (*C. ficifolius* x *C. myriocarpus*), Fian (*C. ficifolius* x *C. anguria*). Volatile compounds: 1.1 = Methyl-2-methyl butyrate, 1.2 = 2-Methyl propyl acetate, 1.3 = Ethyl butanoate, 1.4 = Ethyl-2-methyl butyrate, 1.5 = Butyl acetate, 1.6 = Diethyl carbonate, 1.7 = Butyl butyrate, 1.8 = Ethyl hexanoate, 1.9 = Hexyl acetate, 1.10 = (Z)-3-Hexen-1-ol, acetate, 1.11 = Heptyl acetate, 1.12 = (E,E)-2,4-Hexadienoic acid, ethyl ester, 1.13 = Ethyl-3-(Methylthio)propanoate, 1.14 = Benzyl acetate, 2.1 = Hexanal, 2.2 = (E)-2-methyl-2-butenal, 2.3 = Heptanal, 2.4 = Octanal, 2.5 = (E)-2-Heptenal, 2.6 = Nonanal, 2.7 = (Z)-6-Nonenal, 2.8 = (E,E)-2,4-Heptadienal, 2.9 = Benzaldehyde, 2.10 = (E)-2-Nonenal, 2.11 = (E,Z)-2,6-Nonadienal, 2.12 = Phenylacetaldehyde, 2.13 = (E,E)-2,4-Nonadienal, 2.14 = (E,E)-2,4-Decadienal, 3.1 = 1-Pentanol, 3.2 = 1-Hexanol, 3.3 = (Z)-3-Hexen-1-ol, 3.4 = 1-Octanol, 3.5 = 1-Nonanol, 3.6 = (Z)-3-Nonen-1-ol, 3.7 = (Z)-6-Nonen-1-ol, 3.8 = 1-Decanol; 3.9 = (E,Z)-2,6-Nonadien-1-ol, 3.10 = Benzyl Alcohol, 3.11 = 2-Phenylethanol, 3.12 = Phenol, 4.1 = Geranylacetone, 4.2 = Beta-Ionone.

the increase was limited to a 12 % in 2 dS m<sup>-1</sup> increases. A similar response was also observed by Colla et al., (2006), again with limited differences. In our case, though, the differences were not significant for malic and citric acids. Even more, lower contents of glutamic acid were found in Carrizales, although the contribution of this amino acid to acidity would be negligible.

Spanish landraces represent a rather particular subgroup in the wide range of global melon variability characterized by big fruits with high sugar content and usually non-climacteric behavior (Leida et al., 2015). The results obtained in this study confirmed this trend, as SSC were on average higher than 11°Brix. The variation in SSC and sucrose, the main sugar in melon fruits was negligible, with differences only detected with the commercial control Finura of the Piel de Sapo type. Nonetheless, variability was observed in the ratios fructose to glucose and hexoses to sucrose, evidencing different profiles of sugar accumulation. Wider variability, though, was detected in the accumulation of acids. In Piel de Sapo landraces the accumulation of malic acid in 03PS doubled that of

11PS and was higher than the rest of the landraces. In the case of 32BL of Blanco malic contents were really low, though significant differences with other landraces were limited. The accumulation of citric acid followed an opposite trend, with the lowest contents being found in 03PS. The accumulation of glutamic acid was insignificant. In fact in most samples it remained under the quantification limits. Only 35TN of Tendral outstood for its contents of glutamic acid, which doubled those found in other landraces. The contents of acids and the acidic profile were in accordance with those observed generally in melons, as citric acid tended to predominate (Burger et al., 2010). Flores-León et al., (2022) also obtained similar results for these landraces, although 03PS did achieve a higher content of citric acid. Flores-León et al., (2022) reported lower sugar content for Tendral melons, which was not observed in the accession included in present study. In any case, the accumulation of malic acid was for example considerably lower than that observed in the Cantalupensis Charentaiss group, as in that the accumulation levels were higher than 1 g kg<sup>-1</sup> (Perpiñá et al., 2017).

The effect of grafting on melon quality has been thoroughly studied in the last decades, spurred by the publication of inconsistent results. Most of these studies have been focused on the effect on SSC or acidity while only a few analysed specific effects on individual sugars and acids. It seems clear, though, that this inconsistency is due to the dependence of the response upon the specific rootstock-scion combinations being considered (Rouphael et al., 2018). For example, Colla et al., (2006) found that Cyrano Charentais melons grafted on *Cucurbita* hybrid tended to reduce SSC under saline conditions. This effect might not be exclusive of *Cucurbita* rootstocks, as (Fita, Picó, Roig, & Nuez, 2007) found a decrease of SSC of Piel de Sapo melons grafted on Pat81 *C. melo* agrestis rootstock. On the other hand, other authors have found negligible effects on basic quality parameters. In this sense, Crinò et al. (2007) in South Italy tested different *Cucurbita* hybrids and *C. melo* rootstocks on the quality of the winter melon Incas (inodorus group) and found no significant differences in CSS with the non-grafted control. Similarly, Park et al. (2013) in Korea did not find differences between the muskmelon Earls' elite (*reticulatus* type melon) grafted on selected *C. melo* rootstocks and the non-grafted control on SSC. Verzera et al., (2014) also found that most combinations did not affect SSC, but it was increased in one of the 5 rootstocks evaluated.

As stated before, few studies have analysed the impact of grafting on the accumulation of specific sugars. Soteriou, Papayiannis, & Kyriacou (2016) found differences in the profile of sugar accumulation in Galia and Ananas melons grafted on different *Cucurbita* rootstocks and grown in Cyprus. In that work, the authors studied sugar accumulation in scion rootstock combinations with different levels of incompatibility. It became clear that, at least in these cases total and individual sugar content, as well as sweetness index considering each sugar sweetening power would be dependent on specific scion rootstock combinations. In one of the cultivars tested (Elario) grafting tended to increase sucrose and decrease hexoses contents with some of the rootstocks, but without effects on total sugars. Other rootstocks did not affect the sugar profile while in one of them total sugars and sweetening index was higher due to higher sucrose accumulation.

In our case, sugar contents and sugar profile was affected by grafting. In general, the non-grafted control had higher values of glucose and lower levels of sucrose compared to grafted plants, an effect that might be related with differences in the ripening process. Among the rootstocks Fian could be explored as an alternative to increase sugar levels, though this possibility should be further explored as fewer plants were available in this combination. Nonetheless, the effect of rootstock varied with the specific scion/rootstock combination being considered, even between populations of the same landrace.

In watermelon, lower accumulation of hexoses at the onset of fruit development and a reduction in sucrose accumulation during ripening has been involved in the moderate reduction of fruit SSC from plants grafted on *Cucurbita* and *Lagenaria* rootstocks, though this effect is not consistent (Kyriacou, Rouphael, Colla, Zrenner, & Schwarz, 2017). It seems possible that grafting could be affecting flowering and ripening timing. It also seems that these rootstocks would tend to increase acidity.

The effect of grafting on melon fruit acidity is not clear, as few studies are available. Colla et al., (2006) found a reduction while Crinò et al. (2007) failed to find significant effects of the rootstock in acidity, and a recent review pointed out that the rootstock effect on acidity would be much lower than that exerted on sugar accumulation Kyriacou et al., (2017). Our results also confirm the negligible effect of rootstocks on the accumulation of organic acids in general. In some cases, such as all grafted Rochet combinations or 32BL grafted on Shintoza tended to show a less acidic profile, but the variation was high and in most cases there was no statistical significance. Other cucurbits, such as watermelon, are more prone to changes in acidity, as reviewed by Kyriacou et al., (2017), with a trend to increase acid levels and specifically malic acid contents (Fredes et al., 2017).

#### 4.2. Effect on the volatile profile

The volatile profile of Ibericus melons has recently been reviewed (Flores-León et al., 2022). Our results confirm that in these landraces the main volatile compounds are aldehydes followed by alcohols and esters. In general, the volatile profile of both studies is similar, though differences are found for certain compounds, which would be explained by environmental factors, as the importance of this effect in the volatile profile of non-climacteric melons has been previously reported (Zarid, Bueso, & Fernández-Trujillo, 2020).

Few studies are available regarding the effects of grafting on melon aroma. Condurso et al., (2012) evaluated the effect of different *C. maxima* × *C. moschata* and *C. melo* rootstocks on the volatile profile of Proteo melo (var. *reticulatus*). In their study they found mainly an increase of alcohols and aldehydes responsible for green and fresh notes in fruits from grafted plants. Interestingly, Z-3-nonenol levels representative of melon notes were decreased in all grafted combinations. Regarding esters, pumpkin hybrids tended, in general, to decrease key odorant esters while the opposite happened when the rootstock Sting, from *C. melo*, was used. This effect had previously been reported by Chuan-qiang et al., (2011) in muskmelons grafted on pumpkin rootstocks and it was related to lower alcohol dehydrogenases (ADHs) and, especially, alcohol acyltransferases (AATs) activities. Nonetheless, the alteration of the volatile profile is highly depended on the specific rootstock/scion combination and it was possible to identify both *Cucurbita* and *C. melo* rootstocks with a minimum impact on volatile profile as compared to non-grafted control. In fact, Lecholocholo et al., (2022) described a raise in volatile esters in cantaloupe and honeydew melons grafted on *Cucurbita* hybrids. The effect on ester reduction was not found in our case, probably because Ibericus melons, which are non-climacteric, do not accumulate high levels of esters as compared to muskmelons, which are climacteric. Low AATs activities are expected *per se* in this group of melons as these enzymes convert aldehydes generated from alcohols via ADHs into esters (Gonda et al., 2016).

In any case, it seems clear that the effect of grafting on the volatile profile is limited. Some specific trends in some specific rootstock/scion combinations seem evident. For example, the *Cucurbita* rootstock Shintoza decreases aldehyde content in the Amarillo accession 22AM-GO, as in the 29BL F1Pat81 combination. Nonetheless, a high level of variability generated by uncontrolled factors minimize the significance of most of the specific trends detected. It should also be considered that even within the same landrace the effect of rootstock varies depending on the specific accession being considered. It seems clear then, that it would be difficult to select a grafting solution maximizing the volatile profile, but at the same time it seems that most rootstocks would have a minimum impact on it.

#### 5. Conclusions

Scion and salinity exert a higher effect than grafting on the accumulation of soluble and volatile compounds affecting flavour of melon landraces. The effect of experimental rootstocks of *Cucumis* seem to represent a valuable alternative to *Cucurbita* classic rootstocks, as the effect on the accumulation of sugars and acids and volatiles is limited. Among them, further studies should analyse the performance of Fian as it seems to improve the accumulation of sugars compared to the non-grafted control. Despite the limited effects being observed, a high influence of scion × rootstock interaction has been observed, implying the necessity to determine their ideal rootstock for each population, as the response may differ between populations of the same landrace.

#### Funding

This work was supported by grants PROMETEO/2017/078 and PROMETEO/2021/072 (to promote excellence groups) funded by Conselleria d'Educació, Investigació, Cultura i Esports and Conselleria d'Educació, Universitats i Ocupació (Generalitat Valenciana, Spain), and grants PID2020-116055RB-C21 and AGL2017-85563-C2-1-R-AR

funded by AEI <https://doi.org/10.13039/501100011033> and by “ERDF A way of making Europe”. AFL is a recipient of a predoctoral fellowship from Generalitat Valenciana. RM is a recipient of a postdoctoral grant (PAID-1020) from Universitat Politècnica de València. CRUE - Universitat Politècnica de València provided funding for the open access publication charges.

### CRediT authorship contribution statement

**A. Flores-León:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis. **R. Martí:** Visualization, Methodology, Investigation, Formal analysis. **M. Valcarcel:** Investigation. **S. Roselló:** Writing – review & editing, Methodology, Investigation. **J. Beltrán:** Methodology, Investigation. **S. García-Martínez:** Writing – review & editing, Resources, Methodology, Investigation. **J.J. Ruiz:** Writing – review & editing, Investigation, Resources, Funding acquisition. **C. Gisbert:** Resources, Methodology, Investigation. **J. Cebolla-Cornejo:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis. **B. Picó:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

### Acknowledgements

The authors thank Banco de Germoplasma de la Universitat Politècnica de València for the kind provision of germplasm for this study.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.138709>.

### References

Akrami, M., & Arzani, A. (2019). Inheritance of fruit yield and quality in melon (*Cucumis melo* L.) grown under field salinity stress. *Scientific Reports*, 9(1), 1–13. <https://doi.org/10.1038/s41598-019-43616-6>

Burger, Y., Paris, H. S., Cohen, R., Katzir, N., Tadmor, Y., Lewinsohn, E., & Schaffer, A. A. (2010). 3 Genetic Diversity of *Cucumis Melo*. *Horticultural Reviews*, 36(1), 165–198.

Burger, Y., Sa'ar, U., Paris, H. S., Lewinsohn, E., Katzir, N., Tadmor, Y., & Schaffer, A. A. (2006). Genetic variability for valuable fruit quality traits in *Cucumis melo*. *Israel Journal of Plant Sciences*, 54(3), 233–242. <https://doi.org/10.1560/IJPS.54.3.233>

Burger, Y., Shen, S., Petreikov, M., & Schaffer, A. A. (2000). The contribution of sucrose to total sugar content in melons. *Acta Horticulturae*, 510, 479–485. <https://doi.org/10.17660/actahortic.2000.510.75>.

Cáceres, A., Perpiñá, G., Ferriol, M., Picó, B., & Gisbert, C. (2017). New *Cucumis* rootstocks for melon: 'UPV-FA' and 'UPV-FMY'. *HortScience*, 52(5), 792–797. <https://doi.org/10.21273/HORTSCI11791-17>.

Castro, G., Perpiñá, G., Esteras, C., Armengol, J., Picó, B., & Pérez-de-Castro, A. (2020). Resistance in melon to *Monosporascus cannonballus* and *M. eutypoides*: Fungal pathogens associated with *Monosporascus* root rot and vine decline. *Annals of Applied Biology*, 177(1), 101–111. <https://doi.org/10.1111/aab.12590>

Cebolla-Cornejo, J., Soler, S., & Nuez, F. (2007). Genetic erosion of traditional varieties of vegetable crops in Europe: Tomato cultivation in Valencia (Spain) as a case study. *International Journal of Plant Production*, 1(2), 113–128. <https://doi.org/10.22069/IJPP.2012.531>.

Cebolla-Cornejo, J., Valcàrcel, M., Herrero-Martínez, J. M., Roselló, S., & Nuez, F. (2012). High efficiency joint CZE determination of sugars and acids in vegetables and fruits. *Electrophoresis*, 33(15), 2416–2423. <https://doi.org/10.1002/elps.201100640>

Chuan-qiang, X., Yu-xue, H., & Lin, Y. (2011). Effects of grafting on four characteristic ester aromas contents and related enzyme activities in muskmelon fruit. *North Hortic*, 16, 41–44.

Colla, G., Rouphael, Y., Cardarelli, M., Massa, D., Salerno, A., & Rea, E. (2006). Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. *Journal of Horticultural Science and Biotechnology*, 81(1), 146–152. <https://doi.org/10.1080/14620316.2006.11512041>

Condurso, C., Verzera, A., Dima, G., Tripodi, G., Crino, P., Paratore, A., & Romano, D. (2012). Effects of different rootstocks on aroma volatile compounds and carotenoid content of melon fruits. *Scientia Horticulturae*, 148, 9–16. <https://doi.org/10.1016/j.scienta.2012.09.015>

Crinò, P., Lo Bianco, C., Rouphael, Y., Colla, G., Saccardo, F., & Paratore, A. (2007). Evaluation of rootstock resistance to fusarium wilt and gummy stem blight and effect on yield and quality of a grafted "indodorus" melon. *HortScience*, 42(3), 521–525. <https://doi.org/10.21273/hortsci.42.3.521>.

Del Amor, F. M., Martínez, V., & Cerdá, A. (1999). Salinity duration and concentration affect fruit yield and quality, and growth and mineral composition of melon plants grown in perlite. *HortScience*, 34(7), 1234–1237. <https://doi.org/10.21273/hortsci.34.7.1234>.

Escribano, S., & Lázaro, A. (2012). Sensorial characteristics of Spanish traditional melon genotypes: Has the flavor of melon changed in the last century? *European Food Research and Technology*, 234(4), 581–592. <https://doi.org/10.1007/s00217-012-1661-7>

Fita, A., Picó, B., Roig, C., & Nuez, F. (2007). Performance of *Cucumis melo* ssp. *agrestis* as a rootstock for melon. *Journal of Horticultural Science and Biotechnology*, 82(2), 184–190. <https://doi.org/10.1080/14620316.2007.11512218>

Flores-León, A., García-Martínez, S., González, V., Garcés-Claver, A., Martí, R., Julián, C., & Picó, B. (2021). Grafting Snake Melon [*Cucumis melo* L. subsp. *melo* Var. *flexuoso* (L.) Naudin] in Organic Farming: Effects on Agronomic Performance; Resistance to Pathogens; Sugar, Acid, and VOC Profiles; and Consumer Acceptance. *Frontiers in Plant Science*, 12, 113. <https://doi.org/10.3389/fpls.2021.613845>

Flores-León, A., Peréz Moro, C., Martí, R., Beltrán, J., Roselló, S., Cebolla-Cornejo, J., & Picó, B. (2022). Spanish Melon Landraces: Revealing Useful Diversity by Genomic, Morphological, and Metabolomic Analysis. *International Journal of Molecular Sciences*, 23(13), 7162. <https://doi.org/10.3390/ijms23137162>

Fredes, A., Roselló, S., Beltrán, J., Cebolla-Cornejo, J., Pérez-de-Castro, A., Gisbert, C., & Picó, M. B. (2017). Fruit quality assessment of watermelons grafted onto citron melon rootstock. *Journal of the Science of Food and Agriculture*, 97(5), 1646–1655. <https://doi.org/10.1002/jsfa.7915>

Gonda, I., Burger, Y., Schaffer, A. A., Ibdah, M., Tadmor, Y., Katzir, N., et al. (2016). Biosynthesis and perception of melon aroma. In *Biotechnology in Flavor Production* (pp. 281–305).

Huang, C. H., Zong, L., Buonanno, M., Xue, X., Wang, T., & Tedeschi, A. (2012). Impact of saline water irrigation on yield and quality of melon (*Cucumis melo* cv. Huanghemi) in northwest China. *European Journal of Agronomy*, 43, 68–76. <https://doi.org/10.1016/j.eja.2012.05.008>

Karaağaç, O., & Balkaya, A. (2013). Interspecific hybridization and hybrid seed yield of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) lines for rootstock breeding. *Scientia Horticulturae*, 149, 9–12. <https://doi.org/10.1016/J.SCIENTA.2012.10.021>

Kawaide, T. (1985). Utilization of rootstocks in cucurbits production in Japan. *Japanese Agricultural Research Quarterly*, 18(4), 285–288.

Koehler, P. E., & Kays, S. J. (1991). Sweet potato flavor: Quantitative and qualitative assessment of optimum sweetness. *Journal of Food Quality*, 14(3), 241–249.

Kolayli, S., Kara, M., Tezcan, F., Erim, F. B., Sahin, H., Ulusoy, E., & Aliyazicioglu, R. (2010). Comparative study of chemical and biochemical properties of different melon cultivars: Standard, hybrid, and grafted melons. *Journal of Agricultural and Food Chemistry*, 58(17), 9764–9769. [https://doi.org/10.1021/JF102408Y/ASSET/IMAGES/LARGE/JF-2010-02408Y\\_0002.JPG](https://doi.org/10.1021/JF102408Y/ASSET/IMAGES/LARGE/JF-2010-02408Y_0002.JPG)

Kyriacou, M. C., Rouphael, Y., Colla, G., Zrenner, R., & Schwarz, D. (2017). Vegetable Grafting: The Implications of a Growing Agronomic Imperative for Vegetable Fruit Quality and Nutritive Value. *Frontiers in Plant Science*, 8, 741. <https://doi.org/10.3389/fpls.2017.00741>

Lázaro, A., Fernández, I. C., Borrero, M. J., Cabello, F., López-Sesé, A. I., Gómez-Guillamón, M. L., & Picó, B. (2017). Agromorphological genetic diversity of Spanish traditional melons. *Genetic Resources and Crop Evolution*, 64(7), 1687–1706. <https://doi.org/10.1007/s10722-016-0466-0>

Lecholocholo, N., Shoko, T., Manhivi, V. E., Maboko, M. M., Akinola, S. A., & Sivakumar, D. (2022). Influence of different rootstocks on quality and volatile constituents of cantaloupe and honeydew melons (*Cucumis melo* L.) grown in high tunnels. *Food Chemistry*, 393, Article 133388. <https://doi.org/10.1016/j.foodchem.2022.133388>

Leida, C., Moser, C., Esteras, C., Sulpice, R., Lunn, J. E., de Langen, F., & Picó, B. (2015). Variability of candidate genes, genetic structure and association with sugar accumulation and climacteric behavior in a broad germplasm collection of melon (*Cucumis melo* L.). *BMC Genetics*, 16(1), 1–17. <https://doi.org/10.1186/s12863-015-0183-2>

López-Sesé, A. I., Staub, J. E., & Gómez-Guillamón, M. L. (2003). Genetic analysis of Spanish melon (*Cucumis melo* L.) germplasm using a standardized molecular-marker array and geographically diverse reference accessions. *Theoretical and Applied Genetics*, 108(1), 41–52. <https://doi.org/10.1007/s00122-003-1404-z>

Németh, D., Balázs, G., Bodor, Z., Zaukuu, J. L. Z., Kovács, Z., & Kappel, N. (2020). Food quality attributes of melon (*Cucumis melo* L.) influenced by grafting. *Progress in Agricultural Engineering Sciences*, 16(S1), 53–66. <https://doi.org/10.1556/446.2020.10006>

Park, D. K., Son, S. H., Kim, S., Lee, W. M., Lee, H. J., Choi, H. S., & Huh, Y. C. (2013). Selection of Melon Genotypes with Resistance to Fusarium Wilt and Monosporascus Root Rot for Rootstocks. *Plant Breeding and Biotechnology*, 1(3), 277–282. <https://doi.org/10.9787/pbb.2013.1.3.277>

Perpiñá, G., Cebolla-Cornejo, J., Esteras, C., Monforte, A. J., & Picó, B. (2017). 'MAK-10': A Long Shelf-life Charentais Breeding Line Developed by Introgression of a Genomic Region from Makuwa Melon. *HortScience*, 52(11), 1633–1638. <https://doi.org/10.21273/HORTSCI2068-17>.

Pico, B., Thompson, A. J., Gisbert, C., Yetisir, H., & Bebeli, P. (2017). Genetic resources for rootstock breeding. *Vegetable Grafting: Principles and Practices*, Vol. 1, pp. 22–69. CABI Valencia, ES.

Pitrat, M. (2016). Melon Genetic Resources: Phenotypic Diversity and Horticultural Taxonomy. In R. Grumet, N. Katzir & J. García-Mas *Genetics and genomics of the Cucurbitaceae*. (pp. 25–60) Springer. [https://doi.org/10.1007/7397\\_2016\\_10](https://doi.org/10.1007/7397_2016_10).

Reganold, J. P., & Wachter, J. M. (2016). Organic agriculture in the twenty-first century. *Nature Plants*, 2, 15221. <https://doi.org/10.1038/nplants.2015.221>

Roig, C., Fita, A., Ríos, G., Hammond, J. P., Nuez, F., & Picó, B. (2012). Root transcriptional responses of two melon genotypes with contrasting resistance to Monosporascus cannonballus (Pollack et Uecker) infection. *BMC Genomics*, 13. <https://doi.org/10.1186/1471-2164-13-601>

Rouphael, Y., Kyriacou, M. C., & Colla, G. (2018). Vegetable Grafting: A Toolbox for Securing Yield Stability under Multiple Stress Conditions. *Frontiers in Plant Science*, 8, 2255. <https://doi.org/10.3389/fpls.2017.02255>

Soteriou, G. A., Papaiannnis, L. C., & Kyriacou, M. C. (2016). Indexing melon physiological decline to fruit quality and vine morphometric parameters. *Scientia Horticulturae*, 203, 207–215. <https://doi.org/10.1016/j.scientia.2016.03.032>

Tedeschi, A., Lavini, A., Riccardi, M., Pulvento, C., & d'Andria, R. (2011). Melon crops (*Cucumis melo* L. cv. Tendral) grown in a mediterranean environment under saline-sodic conditions: Part I. Yield and quality. *Agricultural Water Management*, 98(9), 1329–1338. <https://doi.org/10.1016/j.agwat.2011.04.007>

Verzera, A., Dima, G., Tripodi, G., Condurso, C., Crinò, P., Romano, D., & Paratore, A. (2014). Aroma and sensory quality of honeydew melon fruits (*Cucumis melo* L. subsp. *melo* var. *inodorus* H. Jacq.) in relation to different rootstocks. *Scientia Horticulturae*, 169, 118–124. <https://doi.org/10.1016/j.scientia.2014.02.008>

Zarid, M., Bueso, M. C., & Fernández-Trujillo, J. P. (2020). Seasonal effects on flesh volatile concentrations and texture at harvest in a near-isogenic line of melon with introgression in LG X. *Scientia Horticulturae*, 266, Article 109244. <https://doi.org/10.1016/J.SCIENTIA.2020.109244>