


Article

Melon Genetic Resources Characterization for Rind Volatile Profile

Cristina Esteras ¹, Jose Luis Rambla ², Gerardo Sánchez ³, Antonio Granell ⁴ and María Belén Picó ^{1,*}

- ¹ Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain; criesgo@upvnet.upv.es
- ² Departamento de Ciencias Agrarias y del Medio Natural, Universitat Jaume I. Av. Vicent Sos Baynat s/n, 12071 Castellón de la Plana, Spain; jorambla@uji.es
- ³ Estación Experimental Agropecuaria San Pedro, Instituto Nacional de Tecnología Agropecuaria (INTA), Ruta Nac. N°9 Km170, 2930 San Pedro, Argentine; sanchez.gerardo@inta.gob.ar
- ⁴ Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas (CSIC), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain; agranell@ibmcp.upv.es
- * Correspondence: mpicosi@btc.upv.es

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Abstract: A melon core collection was analyzed for rind volatile compounds as, despite the fact that they are scarcely studied, these compounds play an important role in consumer preferences. Gas chromatography coupled to mass spectrometry allowed the detection of 171 volatiles. The high volatile diversity found was analyzed by Hierarchical Cluster Analysis (HCA), giving rise to two major clusters of accessions. The first cluster included climacteric and aromatic types such as *Cantalupensis*, *Ameri*, *Dudaim* and *Momordica*, rich in esters; the second one mainly included non-climacteric non-aromatic types such as *Inodorus*, *Flexuosus*, *Acidulus*, *Conomon* and wild *Agrestis*, with low volatiles content, specifically affecting esters. Many interesting accessions were identified, with different combinations of aroma profiles for rind and flesh, such as Spanish *Inodorus* landraces with low aroma flesh but rind levels of esters similar to those in climacteric *Cantalupensis*, exotic accessions sharing high contents of specific compounds responsible for the unique aroma of *Dudaim* melons or wild *Agrestis* with unexpected high content of some esters. Sesquiterpenes were present in rinds of some Asian *Ameri* and *Momordica* landraces, and discriminate groups of cultivars (sesquiterpene-rich/-poor) within each of the two most commercial melon horticultural groups (*Cantalupensis* and *Inodorus*), suggesting that the Asian germplasm is in the origin of specific current varieties or that this feature has been introgressed more recently from Asian sources. This rind characterization will encourage future efforts for breeding melon quality as many of the characterized landraces and wild accessions have been underexploited.

Keywords: *Cucumis melo*; volatiles; peel; diversity; quality genetic breeding

1. Introduction

Melon (*Cucumis melo* L., Cucurbitaceae) is a crop of high importance worldwide as its fruits are highly appreciated and mainly consumed as a dessert, but also as a vegetable like cucumber in several regions. This species displays a great genetic diversity which is exhibited in many aspects, especially in fruit traits such as size, shape, color, ripening behavior or netting [1]. Variation in other quality traits like nutrients content (sugars, acids and carotenoids among others) or aroma volatiles has also been reported [2–5]. Recently, Moing et al. [6] analyzed a dataset of over 80,000 metabolomic and elemental features, including 282 flesh volatile metabolites, in a melon collection of 51 accessions finding that

metabolic classification strongly supports phylogenomics, although not completely. *C. melo* is divided into two subspecies [7], subsp. *melo* and subsp. *agrestis*, and several classifications in varieties or horticultural groups have been described to date because of the existence of many intermediate types that complicate taxonomy [1,8].

Together with resistance to diseases, breeding for fruit quality is one of the main goals pursued in this crop. Quality aspects are genetically determined complex traits which are also affected by the environment [4,9]. Perception of aroma depends on both qualitative and quantitative differences in volatile organic compounds (VOCs) of different chemical nature, and also on the different odor threshold of each compound [10].

The melon flesh volatile profile has already been deeply studied, basically in commercial genotypes, but also including a few representatives of other horticultural groups [9,11–21], and the comprehension of the pathways and genes involved in their biosynthesis is currently being prompted using genomic, transcriptomic and biochemical approaches and also segregating populations [22–28]. Recently, the flesh aroma profile of a melon core collection of 71 accessions representing the whole species diversity has been analyzed [5]. Two-hundred compounds were detected in the flesh, which allowed a clustering coherent with the horticultural type and geographical origin of accessions, and also showed the huge variation still underexploited in many non-commercial melons and the existence of many intermediate types regarding ripening behavior, a feature linked to aroma profile. Consistently with previous assays [3,12,22,29–32] aromatic climacteric types were rich in esters while non-aromatic non-climacteric types presented a higher content in lipid-derived aldehydes and alcohols.

Most of the fruit quality studies conducted to date have focused on flesh aroma, but melon rind aroma is important for consumer attraction at the market. Although rind aroma does not affect the edible part of the fruit, except in cases of melons consumed unripe such as cucumbers or those with edible skins such as Makuwa melons, it does affect the consumer quality perception. However, rind aroma has not been yet extensively studied, with a few studies only analyzing few accessions [12,33] and, accordingly, the available variation for this quality trait has not been exploited. Aubert and Pitrat [12] studied with detail the rind aroma of only one genotype, the Dudaïm Queen Anne's Pocket Melon, as this variety is known to have a particularly strong aroma among melons. They found that VOC levels in the rind were higher than in the flesh, and that eugenol, thioether esters and lactones were likely the main contributors to its unique aroma, especially in the rind, as their abundance and low odor threshold suggests. Subsequently, Portnoy et al. [33] stated that sesquiterpenes were mainly present in climacteric mature melon rinds in contrast with their absence in non-climacteric ones after analyzing a set of 16 genotypes, mainly *Cantalupensis-Reticulatus* and *Inodorus* types.

In this context, the novelty of this work is precisely the uncovering of the rind aroma volatiles diversity in a melon core collection representing the existing variability in the *C. melo* species, including many horticultural groups and wild melons not previously studied. Flesh aroma of this germplasm has been recently analyzed by Esteras et al. [5] and herein rind results are presented to emphasize not only the variability detected in the species regarding rind VOCs, but also the importance of considering the rind aroma fingerprint in breeding programs, in order to enhance consumers' attraction and marketability and also to maintain/avoid specific rind profiles during the breeding process using diverse germplasm.

2. Materials and Methods

2.1. Plant Material

A total of 72 accessions belonging to the melon core collection conserved at the Institute for the Conservation and Breeding of Agricultural Biodiversity of the Universitat Politècnica de Valencia, Spain (UPV-COMAV) genebank and previously characterized for flesh volatile profile by Esteras et al. [5] were selected for the study of rind VOCs.

Although recently Pitrat [1] reported a classification of 19 melon groups including wild, feral and cultivated types, in this work we used a simplified version [8]: *Cantalupensis* and *Reticulatus* often

considered the same group (cantaloupes, muskmelons), Inodorus (winter melons, casaba melons), Ameri (Asian landraces), Flexuosus (snake melons), Chate (cucumber melons), Dudaim (pocket melons), Momordica (snap melons), Acidulus (acid melons), Conomon, Makuwa, and Chinensis often referred to as Conomon (pickling oriental melons), and Tibish (African domesticates). The collection presented herein includes accessions from different origins belonging to both subspecies and all the varieties based on Pitrat [8] (Table 1). The selection of this germplasm was mainly based on previous molecular and morphological characterization assays [34–36]. Additionally, wild and exotic accessions were prioritized as they are of special interest since they had not been analyzed previously and contain variability not present in the main commercial types.

Table 1. Accessions assayed for rind volatile profile.

Code	Local Name/Germplasm Bank Name	subsp.	Group	Origin
Ac-G22843Se	G22843 (PI 436534) ¹	<i>agrestis</i>	Acidulus	Senegal
Ac-TGR1551Zimb	TGR1551 (PI482420) ¹	<i>agrestis</i>	Acidulus	Zimbabwe
Ac-SRKSLan	SRK ²	<i>agrestis</i>	Acidulus	Sri Lanka
Ac-SVIInd	SVI ²	<i>agrestis</i>	Acidulus	India
Chi-VellInd	Velleri (PI 164320) ¹	<i>agrestis</i>	Chito	India
Con-GMJJa	Ginsen Makuwa (PI 420176) ¹	<i>agrestis</i>	Conomon-Makuwa-Chinensis	Japan
Con-BaishChi	Baishami ²	<i>agrestis</i>	Conomon-Makuwa-Chinensis	China
Con-FreeCJa	Freeman's Cucumber ³	<i>agrestis</i>	Conomon-Makuwa-Chinensis	Japan
Con-MielChi	Miel Blanc ³	<i>agrestis</i>	Conomon-Makuwa-Chinensis	China
Con-NanChi	Nanbukin ²	<i>agrestis</i>	Conomon-Makuwa-Chinensis	China
Con-PauPol	Paul ²	<i>agrestis</i>	Conomon-Makuwa-Chinensis	Poland
Con-SCKo	Songwhan Charmi (PI 161375) ¹	<i>agrestis</i>	Conomon-Makuwa-Chinensis	Korea
Con-ShiroJa	Shiro Uri Okayama ³	<i>agrestis</i>	Conomon-Makuwa-Chinensis	Japan
Mom-KhaInd	Kharbuja (CUM 438) ⁴	<i>agrestis</i>	Momordica	India
Mom-PI124Ind	PI 124112 ¹	<i>agrestis</i>	Momordica	India
Mom-MR1Ind	MR1 ³	<i>agrestis</i>	Momordica	India
Tibish-KSud	Tibish Khurtagat ²	<i>agrestis</i>	Tibish	Sudan
Ag-15591Gha	PI 185111 ¹	<i>agrestis</i>	wild melon	Ghana
Ag-CallInd	Callosus ³	<i>agrestis</i>	wild melon	India
Ag-TendSud	Tendelti ²	<i>agrestis</i>	wild melon	Sudan
Ag-WChInd	Wild Chibbar ³	<i>agrestis</i>	wild melon	India
Am-KafEgy	Kafr Hakim (PI 288233) ¹	<i>melo</i>	Ameri	Egypt
Am-korcaRus	Korça (CUM 168) ⁴	<i>melo</i>	Ameri	Russia
Am-TokTaj	Tokash ³	<i>melo</i>	Ameri	Tajikistan
Am-HassanTur	Hassanbey (PI 169368) ¹	<i>melo</i>	Ameri	Turkey
Am-KizilUzbe	Kizil-uruk ³	<i>melo</i>	Ameri	Uzbekistan
Am-AnaFran	Ananas 'D'Amerique' ²	<i>melo</i>	Ameri	France
Am-NesviGeor	Mucha Nesvi ²	<i>melo</i>	Ameri	Georgia
Am-YokIs	Yokneam ²	<i>melo</i>	Ameri	Israel
Can-EanaHun	Ezüst Ananasz (CUM 305) ⁴	<i>melo</i>	Cantalupensis-Reticulatus	Hungary
Can-PearlJa	Pearl (PI 266947) ¹	<i>melo</i>	Cantalupensis-Reticulatus	Japan
Can-GCHUSA	Golden Champlain (CUM 474) ⁴	<i>melo</i>	Cantalupensis-Reticulatus	USA
Can-TopUSA	Topmark ³	<i>melo</i>	Cantalupensis-Reticulatus	USA
Can-HBJUSA	Ar Hale's Best Jumbo ²	<i>melo</i>	Cantalupensis-Reticulatus	USA
Can-DulUSA	Dulce ³	<i>melo</i>	Cantalupensis-Reticulatus	USA
Can-Ogen2Is	Dvash ha ogen ³	<i>melo</i>	Cantalupensis-Reticulatus	Israel

Table 1. Cont.

Code	Local Name/Germplasm Bank Name	subsp.	Group	Origin
Can-Effa	Earl's Favourite ²	<i>melo</i>	Cantalupensis-Reticulatus	Japan
Can-GyFran	Gynadou ²	<i>melo</i>	Cantalupensis-Reticulatus	France
Can-NOFran	Nantais Oblong ²	<i>melo</i>	Cantalupensis-Reticulatus	France
Can-NCFran	Noir des Carmes ³	<i>melo</i>	Cantalupensis-Reticulatus	France
Can-PGRFran	Petit Gris de Rennes ³	<i>melo</i>	Cantalupensis-Reticulatus	France
Can-SemUSA	Seminole ³	<i>melo</i>	Cantalupensis-Reticulatus	USA
Can-SucrFran	Sucrin de Tours ²	<i>melo</i>	Cantalupensis-Reticulatus	France
Can-VedFran	Vedrantais ³	<i>melo</i>	Cantalupensis-Reticulatus	France
Can-WiUSA	Wi-998 ³	<i>melo</i>	Cantalupensis-Reticulatus	USA
Chate-CarIta	Carosello (CUM 363) ⁴	<i>melo</i>	Chate	Italy
Dud-QAPMGeorg	Queen Ann's Pocket Melon (PI 273438) ¹	<i>melo</i>	Dudaim	Georgia
Dud-QPMAfg	Queen's pocket melon ²	<i>melo</i>	Dudaim	Afganistan
Flex-Co20Ind	Snakemelon (CUM 225) ⁴	<i>melo</i>	Flexuosus	India
Flex-AryaInd	Arya ³	<i>melo</i>	Flexuosus	India
Flex-SnakeSA	Snake melón (CUM 353) ⁴	<i>melo</i>	Flexuosus	Saudi Arab.
La-KroFran	Kroumir ²	<i>melo</i>	indeterminate landrace	France
La-OgenBul	Ogen ³	<i>melo</i>	indeterminate landrace	Bulgaria
La-ZatIta	Zatta ²	<i>melo</i>	indeterminate landrace	Italy
La-CascaPor	Casca de Carvalho ³	<i>melo</i>	indeterminate landrace, probably Inodorus	Portugal
La-ErizoSp	Eriçó mallorquin ³	<i>melo</i>	indeterminate landrace, probably Inodorus	Spain
In-AmCañSp	Caña Dulce ³	<i>melo</i>	Inodorus	Spain
In-LaEscrSp	Escrito Oloroso ³	<i>melo</i>	Inodorus	Spain
In-BBescrSp	Blanco Escrito ³	<i>melo</i>	Inodorus	Spain
In-TeNinvSp2	Tendral Negro ³	<i>melo</i>	Inodorus	Spain
In-TeSp	Tendral ³	<i>melo</i>	Inodorus	Spain
In-PsPiñSp	Piel de sapo Piñonet ³	<i>melo</i>	Inodorus	Spain
In-TeNinvSp	Negro de Invierno ³	<i>melo</i>	Inodorus	Spain
In-BBredSp	Blanco Redondo ³	<i>melo</i>	Inodorus	Spain
In-RoMoch1Sp	Mochuelo ³	<i>melo</i>	Inodorus	Spain
In-ComunSp	Comun ³	<i>melo</i>	Inodorus	Spain
In-LaBolasSp	Bolas ³	<i>melo</i>	Inodorus	Spain
In-AmAoroSp	Amarillo oro ³	<i>melo</i>	Inodorus	Spain
In-CGBUSA	Casaba Golden Beauty ³	<i>melo</i>	Inodorus	USA
In-CrabPor	Crabranco ²	<i>melo</i>	Inodorus	Portugal
In-HoneyDewUSA	Honeydew green flesh ³	<i>melo</i>	Inodorus	USA
In-TDewUSA	Tam Dew ³	<i>melo</i>	Inodorus	USA

¹ Accessions provided by U.S. National Plant Germplasm System by USDA; ² Accessions kindly supplied by M. Pitrat (INRA); ³ Accessions from MELRIP project, core collection by Esteras et al. [34] and Leida et al. [35] currently conserved at the Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV)'s genebank; ⁴ Accessions provided by Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) genebank—Gatersleben.

2.2. Cultivation and Sampling

Cultivation was conducted from May to July (2014) under common greenhouse conditions in Valencia (Spain), at COMAV's facilities. Three plants per accession were grown in a randomized design, and one fruit per plant was collected when mature, taking into account the different ripening behavior (climacteric and non-climacteric types). For rind aroma analysis, the rind was separated from the pulp

using a potato peeler. At least 5 g were weighed, frozen with liquid nitrogen and powdered with mortar and pestle, avoiding unfreezing, before adding 2 mL of saturated calcium chloride per 5 g of rind. After a careful homogenization, 5 mL-vials with the mixture were stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

2.3. Analysis Conditions

Immediately before analysis, samples were incubated at $30\text{ }^{\circ}\text{C}$ for 5 min. Approximately 1 g was weighed and transferred to a 7 mL vial, then 2 volumes of a saturated CaCl_2 solution were added and homogenized gently. One mL of the resulting mixture was transferred to a 10 mL screw cap headspace vial. Volatile acquisition, separation and detection were performed exactly as described for volatile analysis in melon flesh [5]. Volatile compounds were captured by means of solid phase microextraction on the headspace (HS-SPME) and analyzed by gas chromatography coupled to mass spectrometry (GC/MS). Volatile extraction was performed by means of a $65\text{ }\mu\text{m}$ polydimethylsiloxane/divinylbenzene (PDMS/DVB) SPME fiber (Supelco, Bellefonte, PA, USA). Samples were tempered at $50\text{ }^{\circ}\text{C}$ for 10 min, and then the fiber exposed to the headspace for 20 min at the same temperature. Volatiles were desorbed in the GC/MS injection port for 1 min at $250\text{ }^{\circ}\text{C}$ in splitless mode. Sampling and injection were performed automatically with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). Chromatographic separation and detection were performed in a 6890N gas chromatograph coupled to a 5975B mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5ms fused silica capillary column (60 m, 0.25 mm , $1\text{ }\mu\text{m}$) (J&W Scientific, Agilent). Oven programming conditions were $40\text{ }^{\circ}\text{C}$ for 2 min, $5\text{ }^{\circ}\text{C}/\text{min}$ ramp until $260\text{ }^{\circ}\text{C}$, and then $260\text{ }^{\circ}\text{C}$ for 5 min. Helium was used as carrier gas at a constant flow of $1.2\text{ mL}/\text{min}$. Electronic impact ionization was used with a 70 eV ionization energy and $230\text{ }^{\circ}\text{C}$ ionization temperature. Acquisition was performed in the scan mode in the m/z mass range 35–220 (seven scans/s).

Untargeted analysis of all the peaks in the chromatograms was performed by means of the MetAlign software (WUR, <http://www.metalign.nl>). For quantitation, one specific ion was selected for each compound. An admixture reference sample was prepared by mixing thoroughly equal amounts of each sample. A 1 mL aliquot of this admixture was analyzed every five samples as part of the injection series and used as a reference to normalize for temporal variation and fiber aging. Finally, the normalized results for a sample were expressed as the ratio of the abundance of each compound in that particular sample to those present in the reference admixture.

A tentative identification was performed for each compound by comparison of its mass spectrum with those in the NIST05 Mass Spectral Database. When available, mass spectral identity and coelution with pure standards (Sigma-Aldrich, Darmstadt, Germany) were used to unequivocally identify the compounds.

2.4. Data Processing and Statistical Analysis

Hierarchical Cluster Analysis (HCA) was carried out with the mean dataset in order to study the relationships among the accessions based on their volatile profile. The mean was calculated from the individual values generated for all the fruits per accession (3), except in cases not considered for not being in the optimum maturity stage for a quality analysis or when only two fruits were obtained. A total number of 201 samples (fruits) were included in the analysis. The ratio of the levels of each volatile in a sample to the average of all the genotypes analyzed was \log_2 transformed. HCA and the heatmap were performed using Acuity 4.0 software (Axon Instruments), with the distance metrics based on the Pearson correlation.

In addition, Analysis of Variance (ANOVA) for every VOC was carried out using Statgraphics Centurion XVII software (Statpoint Technologies, Inc, USA). Comparisons of pairs of means analyzed were performed using Least Significant Difference (LSD) test with a probability level of $p < 0.05$.

The ExpressionCorrelation plug-in implemented in the Cytoscape software v2.7.0 was used for the construction of a correlation VOC network using also the Pearson correlation coefficient as described previously [37].

3. Results and Discussion

3.1. Melon Rind Volatile Profile

The volatile compounds in the rind of a core collection consisting of 72 melon accessions representing diverse origins, varieties and ripening behavior, and including not only cultivated but also wild types, were profiled to better characterize fruit aroma variation across the species. A total of 171 volatiles were detected based on the untargeted analysis of the chromatograms in the set of 201 samples (Supplementary Table S1). After comparison of mass spectra and retention time with those of pure standards commercially available, 55 compounds were identified unequivocally, and another 53 were tentatively identified based on their mass spectra. The other 63 VOCs remained unknown, although a plausible chemical structure based on their mass spectrum was provided in some cases.

Annotation of the VOCs detected is specified in Supplementary Table S1A. To simplify the results, VOCs were classified into fourteen groups according to chemical nature and abundance as follows: acetate esters (AE), methyl esters (ME), ethyl esters (EE), propyl esters (PE), butyl esters (BE), other esters (E), apocarotenoids (A), branched chain amino acid related compounds (BCAA), lipid derivatives (LD), monoterpenoids (M), sesquiterpenes (S), sulfur compounds (SC), phenolics (P), and others (O) for minority chemical groups.

To assess genotype classification based on the volatile profile and correlation between the volatile compounds detected, HCA was conducted (Figure 1). Compounds with similar chemical structures and sharing biosynthetic pathways displayed high correlations and clustered according to their similar abundance profiles as occurred with melon flesh VOCs [5] and in other species [38–40]. In this set of samples, volatile compounds were arranged in eight clusters (Figure 1, Supplementary Figure S1). Clusters 1 and 2 were rich in esters, the first one in ethyl esters and the second one mainly in acetate esters, methyl, propyl and butyl esters, while cluster 3 grouped several sulfur compounds. Cluster 4 presented two subclusters, one rich in other esters, like some butanoates and hexanoates, and the other rich in sesquiterpenes like α -copaene or β -caryophyllene. Cluster 5 grouped a few compounds including phenolics, whereas cluster 6 included many monoterpenoids. Most lipid-derived volatiles were grouped in clusters 7 and 8, with cluster 7 also including some apocarotenoids, and cluster 8 some phenolics.

The network correlation analysis carried out with rind VOCs is shown in Figure 2. Only strong correlations, $r > 0.85$, are shown in a network defined by 98 nodes and 321 edges (all positive correlations). Similar to those observed in the flesh [5,23,28], VOCs mainly clustered according to chemical nature, this is: a group of acetate esters, a group of ethyl esters, a group including methyl, propyl and butyl esters, and a group of sesquiterpenes. Esteras et al. [5] and Freilich et al. [23] reported acetate and ethyl esters as the most interconnected families of flesh VOCs. However, in rind the most intercorrelated one was the sesquiterpene family (absent in flesh profile), although the three ester clusters (acetate esters, ethyl esters, and the cluster of methyl, propyl and butyl esters) also showed high correlations creating a dense network. No interconnections between esters and the sesquiterpene clusters were observed at the correlation considered ($r > 0.85$). This is coherent with the different chemical pathways producing esters from fatty acids or some amino acids, and to sesquiterpenes from the terpenoids pathway [24].

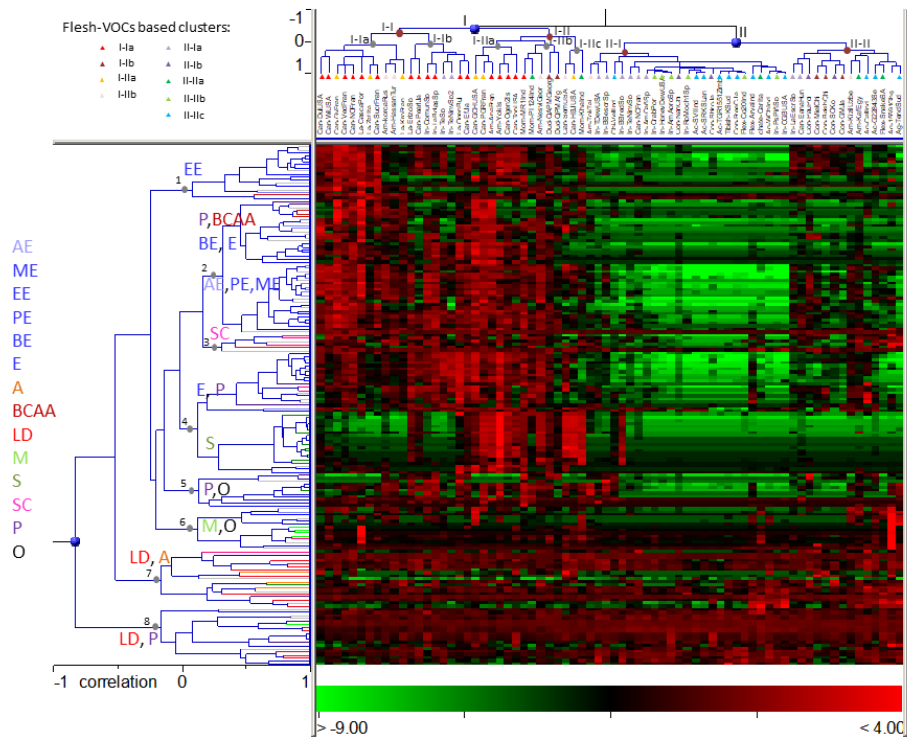


Figure 1. Hierarchical Cluster Analysis (HCA) and heatmap using rind volatile organic compound (VOC) data. Volatile clusters (1–8) and accessions clusters are indicated. HCA was constructed using the distance metrics based on the Pearson correlation. The red to green range of color in the heatmap indicates the level of each volatile in each sample according to the scale below (log 2 transformation of the ratio levels of each volatile in a sample/average of all the genotypes analyzed): light red for the highest values; light green, lowest; black, intermediate. Codes for VOCs are used (acetate esters AE, methyl esters ME, ethyl esters EE, propyl esters PE, butyl esters BE, other esters E, apocarotenoids A, branched chain amino acid related compounds BCAA, lipid derivatives LD, monoterpenoids M, sesquiterpenes S, sulfur compounds SC, phenolics P, and others O). Triangle colors are coded based on flesh-VOCs clusters by Esteras et al. [5].

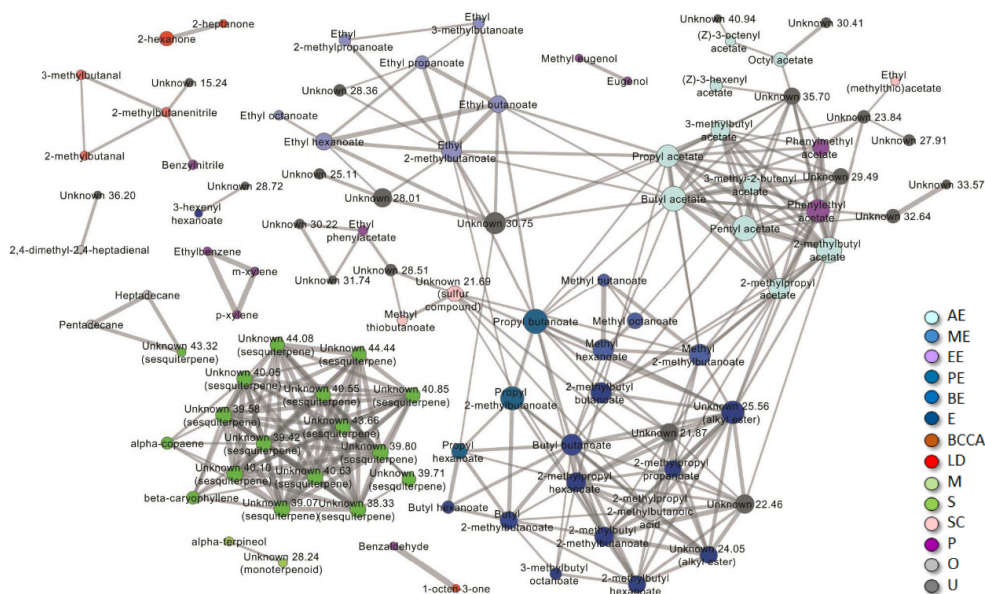


Figure 2. Correlation network analysis of the rind VOC data set. The nodes representing volatiles are colored according to the volatile chemical group as indicated in the image. Correlations over 0.85 are indicated. Line thickness indicates correlation strength: the wider the line, the stronger the correlation.

Sulfur and lipid derivatives, as well as monoterpenes and BCAA related compounds presented low correlation within and between family clusters.

3.2. Rind vs. Flesh VOCs Profile

The total number of VOCs detected in rind was slightly lower than that previously detected in flesh in this same melon collection [5] (171 vs. 200 VOCs, in rind and flesh, respectively; Supplementary Table S2A). However, although the rind presented a lower recount of esters, the main compounds responsible for fruity and sweet odor (50 vs. 66), of apocarotenoids (3 vs. 10) and of lipid-derived compounds (14 vs. 21), in the case of monoterpenoids, sesquiterpenes and phenolics their diversity was higher in rind compared to flesh (5 vs. 4, 19 vs. 1, and 17 vs. 9, for monoterpenoids, sesquiterpenes and phenolics in rind and flesh, respectively). The notable presence of sesquiterpenes in rind in contrast to flesh is in accordance with previous studies [24,33]. For BCAA and sulfur compounds, the number of volatiles detected stayed the same (five and seven, respectively). The highest reduction in rind with respect to flesh was detected in acetate and ethyl esters (14 vs. 24 and 10 vs. 18, for acetate and ethyl esters, in rind and flesh, respectively), while in propyl and butyl esters it remained the same and in volatiles classified as other esters even rind was slightly richer (14 vs. 11, in rind and flesh, respectively).

Regarding levels of compounds, the total volatile content in rinds was 29.7% lower on average than in flesh (Supplementary Table S2A). The correlation coefficient among rind and flesh content in this germplasm collection was 0.85, which means that, in general terms, accessions with higher amounts of rind volatiles are also richer in flesh volatiles (Supplementary Table S2B). The correlation for each volatile present in both rind and flesh tissues (59 VOCs, excluding the compounds classified as unknown), was also calculated, being acetate esters, some phenolics like benzylnitrile and BCAA related compounds like 2-methylbutanenitrile were the ones with the highest correlation (Supplementary Table S2C). Many of the most abundant compounds in rind were present in the flesh, in some cases also in high quantities such as 2-methylpropyl acetate within acetate esters; methyl 2-methylbutanoate within methyl esters; ethyl hexanoate, ethyl butanoate and ethyl 2-methylbutanoate within ethyl esters; propyl butanoate within propyl esters; butyl butanoate within butyl esters, o-cymene within monoterpenoids; benzaldehyde, phenylethyl acetate and phenylmethyl acetate within phenolics; 2-methylbutanenitrile within BCAA related compounds or 1-octen-3-one, (E)-6-nonenal or hexanal within lipid derivatives. Within the latter group very low correlations rind-flesh were found (Supplementary Table S2C).

With regard to the volatiles reported by Gonda et al. [24] as the most important contributors for melon aroma (21 VOCs), 14 of them were identified in rind in the present work, including 11 esters with a sweet, fruity and floral odor, 2 lipid derivatives with a green, leaf odor; and 1 phenolic providing pine notes (Supplementary Figure S2). The comparison in the quantity of these volatiles present in rind and flesh is shown in Supplementary Figure S2. In general, flesh content was higher than rind content, although some exceptions were detected in the lipid derivatives (E)-2-nonenal and hexanal, and in some acetate esters for specific accessions such as the Portuguese landrace Casca de Carvalho or in some ethyl esters in the Italian landrace Zatta.

However, and interestingly, other VOCs were exclusively present in the rind such as pentadecane (also reported in melon rind by Moing et al. [6]), the lipid derivative 2-hexanone or the phenolic ethylbenzene, apart from sesquiterpenes, volatiles mainly associated to rind. Other VOCs such as the BCAA related 2-methylbutanal and 3-methylbutanal, the lipid derivatives undecanal and (E)-2-pentenal, the monoterpene α -terpineol, the methyl ester methyl butanoate or the phenolic phenylacetaldehyde were absent in the flesh of this collection, but had been previously reported in other melons [19,24,41]. These exclusive-rind or poorly present-in-flesh VOCs can be of interest in breeding, some of them being components of essential oils like undecanal in *Citrus* spp. and reported as flavoring agents like phenylacetaldehyde, or having a role as an antimycobacterial drugs or insecticides, like undecanal, α -terpineol and some sesquiterpenes.

3.3. Melon Core Collection Characterization Based on Rind VOCs

The huge differences in the rind volatile profile is in accordance with that reported in flesh [5] and is due to the fact that the melon germplasm assayed here represents the variability of the species. This interesting variability detected allowed the classification by HCA of the accessions based on similar rind volatile profiles that will be detailed later in this section.

The 72 accessions analyzed were classified by HCA in two major clusters (Figure 1). Cluster I included most of the aromatic accessions and was richer in VOCs, especially esters including acetates and methyl, ethyl, propyl and butyl esters, as well as some sulfur-containing compounds (clusters 1–5 of VOCs), and cluster II included low or non-aromatic accessions with small amounts of these compounds. However, both accession clusters I and II did not differ for most of the VOCs in compound clusters 7 and 8, basically lipid derivatives and phenolics, and some monoterpenoids of cluster 6 (Figure 1, Supplementary Figure S1). In the case of these monoterpenoids like α -terpineol, they were absent in all the accessions with a few exceptions in both aromatic (I) and non-aromatic clusters (II), while lipid derivatives such as 1-octen-3-one, heptanal and 2-heptanone, and phenolics such as benzaldehyde and acetophenone were detected in high amounts in all the germplasm collection. The uniformity in specific VOCs content between the two major accession clusters is even more notable for some compounds like 2-heptanone or m-xylene, in which no statistical differences among the genotypes have been found (*p*-values of 0.92 and 0.11, respectively; Supplementary Table S1C).

Interestingly, this first clustering is in agreement with the two major clusters described by Esteras et al. [5] based on flesh volatiles, basically dividing the germplasm collection into aromatic climacteric melons and low or non-aromatic non-climacteric types. Most cantaloupes and Ameri accessions were in the aromatic cluster I, together with Momordica, Dudaim types and some Spanish Inodorus, while the remaining Inodorus, Flexuosus, some Ameri, and the remaining subsp. *agrestis* types were in cluster II. Supplementary Figure S3 shows pictures of some of the most representative accessions in the clusters described below.

Two noticeable exceptions can be observed when comparing the grouping based on rind VOCs with that of flesh VOCs. Two Momordica accessions (PI 124112 and Kharbuja) classified in the flesh non-aromatic cluster, were in the aromatic cluster of rind VOCs. Conversely, three Conomon accessions, of the Chinensis and Makuwa horticultural groups, were in the flesh aromatic cluster but had low aroma rinds, with only moderate levels of ethyl esters (Figure 1, Supplementary Figure S1). These two groups, both Momordica and Conomon included other accessions with aromatic/non-aromatic in both flesh and rinds. This variability must be considered in melon breeding programs as these two horticultural groups represent the main sources for breeding resistance to diseases in melons [42,43].

3.3.1. Aromatic Accessions (cluster I)

The first cluster grouped basically the climacteric accessions, rich in esters: Cantalupensis-Reticulatus and Ameri types belonging to subsp. *melo*, Dudaim and Momordica types which have been considered intermediate forms between both subspecies, but also some Spanish Inodorus. Two subclusters were clearly differentiated due to the sesquiterpenes content. These compounds were almost absent in subcluster I-I and rather abundant in subcluster I-II (Figure 1, Supplementary Table S1)

- Non-sesquiterpene aromatic accessions (I-I)

Within the first subcluster (I-I), we found the esters richest accessions (especially ethyl esters) in the whole collection, American and French cantaloupes, climacteric European landraces, and Ameri melons from Eastern Europe (accessions of group I-Ia). In addition, in this subcluster (I-I), we found a set of Far East Cantalupensis and Spanish Inodorus presenting lower levels of these VOCs (I-Ib), but sharing with them the abundance of other esters, with even higher contents, and the absence of sesquiterpenes.

The first group (I-Ia), included French Charentais and American Reticulatus like Vedrantaïs, Nantais Oblong, Gynadou, Sucrin de Tours, and Dulce, and the Italian landrace Zatta with high levels of methyl, ethyl and butyl esters (methyl hexanoate, methyl butanoate, and methyl octanoate), nitriles (benzyl nitrile) and some BCAA related compounds, but also a few Ameri types from Eastern Europe with a lower level in ethyl esters, although with quite high contents of some phenolics (phenylacetaldehyde and benzyl nitrile), BCAA (3-methylbutanal, 2-methylbutanal), and sulfur compounds (methylthioacetate). Previously, the flesh VOCs pattern of Esteras et al. [5] grouped Gynadou and the Italian landrace Zatta with cantaloupes characterized by low ethyl esters content, but for rinds these two melons presented statistically the highest levels for many of them (LSD test, $p < 0.05$, Supplementary Table S3). Moreover, the previous flesh VOC profile included the Ameri landraces within the aromatic, but poor in ethyl esters, subcluster (Figure 1), whereas their rind profile is more similar to highly aromatic Cantalupensis. In both rind and flesh, Ameri types presented high contents of sulfur compounds.

The accessions of the cluster I-Ib, Japanese Earl's types, Spanish Inodorus and the Ogen landrace, were in general poorer in esters of clusters 1 and 2, and lacked phenolics, such as phenylacetaldehyde, and BCAA related compounds, such as 2-methylbutanal and 3-methylbutanal, in comparison to Cantalupensis melons of the I-Ia group, although were richer in other esters of cluster 4 (similarly to Cantalupensis, Momordica and Dudaim melons of subcluster I-II). For instance, the landrace Ogen (I-Ib), belonging to a group of melons used as parentals of the Galia melons, was, together with the cultivar Golden Champlain (I-IIa), the accession with the highest levels of butyl butanoate and butyl hexanoate (LSD test, $p < 0.05$, Supplementary Table S3).

As occurred in the flesh profile [5], the rind of some Spanish Inodorus landraces presented a higher level of esters compared to commercial Inodorus (mostly classified in the non-aromatic cluster II-I). In fact, the Spanish accessions of cluster I-Ib, Erizo, Común and Bolas, were also grouped with the most aromatic types based on flesh volatiles. Recently, Pitrat [1], splitted the Inodorus group into three new groups, Casaba, Inodorus and Ibericus, the latter including Spanish melons, both landraces of the Tendral, Rochet and Branco groups and the most commercial Piel de sapo or Amarillo, described as having low aroma and long shelf life. Our results show that not all Spanish landraces fit into this Ibericus group, as they have an intermediate climacterism and show both flesh and rind aroma more similar to Ameri and Cantalupensis types. In addition, these Spanish landraces presented higher amounts of some sulfur compounds, a common feature with Asiatic Ameri melons that can be in their origin.

The Earl's types from Japan (such as Pearl), now reclassified by Pitrat into the Earl's subgroup within the Inodorus, also share their rind profile with these Spanish landraces and the Ogen type, but not with the non-aromatic Honeydew or Tam Dew, also reclassified within the Inodorus group but in the Honeydew subgroup. These types, Earl's and Ogen, grouped separately from Charentais and Reticulatus, and typical Inodorus, being mixed with a few non-commercial Spanish, Eastern Europe and Asian Inodorus and Ameri (Ogen) or with a mixed group including Ameri, Flexuosus and other landraces (Earl's), based on the study with more than 200 Single Nucleotide Polymorphisms (SNPs) by Leida et al. [35]. Thus, the rind VOCs clustering is in agreement with this previous genetic classification.

Our results reveal a great variability within the Spanish types (Ibericus), displaying different profiles for rind and flesh. Leida et al. [35] in its population structure analysis also reported high variability in Spanish types, which mainly were located in a population apart from the remaining Inodorus from other origins. Furthermore, although the complete metabolic HCA by Moing et al. [6] consistently grouped together all the Inodorus accessions assayed, partial analysis with some types of compounds, especially rind semi-polar non-volatiles extracts, located the Tendral melon closer to some Cantalupensis-Reticulatus and Flexuosus genotypes than to other Inodorus, indicating that some Spanish Inodorus have chemical similarities to climacteric types.

The existence of *Inodorus* landraces with a volatile profile different from typical *Inodorus* and more similar to *Cantalupensis*, not only for flesh but also for rind is of interest for their exploitation in future breeding programs searching for more attractive types with long shelf-life.

- Sesquiterpene-rich aromatic accessions (I-II)

The second subcluster (I-II), characterized by the notable presence of sesquiterpenes, grouped some Ameri and *Cantalupensis*-*Reticulatus* such as Dvash ha ogen, Topmark, Golden Champlain, Petit Gris de Rennes or Seminole (I-IIa and I-IIc), together with all the Indian *Momordica* and *Dudaim* accessions (I-IIb). The Ameri Yokneam variety from Israel (I-IIa), classified in the subgroups Ananas of the Ameri group, was the richest accession with respect to the amount of sesquiterpenes, with significant differences with the remaining accessions of the collection for many of the 19 sesquiterpenes detected, followed by the *Reticulatus* Ar Hale's Best Jumbo and Seminole and the *Momordica* Kharbuja (I-IIc) (LSD test, $p < 0.05$, Supplementary Table S3).

Erizo and Ogen melons, clustered with the non-sesquiterpene aromatic accessions (I-Ib), also presented moderate levels of sesquiterpenes. In fact, Ogen presented the highest content of α -copaene together with Yokneam (LSD test, $p < 0.05$, Supplementary Table S3).

In our work, 19 sesquiterpenes were detected, although only α -copaene and β -caryophyllene could be unequivocally identified. In the work by Portnoy et al. [33] 15 different sesquiterpenes were detected (14 of them identified), including these two former volatiles, which were also reported in the rind of *Dudaim* and in some *Cantalupensis* analyzed by these authors. None of the accessions analyzed by Portnoy et al. [33] except the *Reticulatus* Dulce presented α -farnesene, the only sesquiterpene detected by Esteras et al. [5] in fruit flesh, particularly in the Conomon and Makuwa types. α -farnesene was the unique sesquiterpene detected in the rind of Dulce by Portnoy et al. [33], although in the present study it was not detected. However, β -caryophyllene and a few unidentified sesquiterpenes were detected in this accession, but in a very low level.

The presence of sesquiterpenes in mature rinds had been previously associated only to some climacteric types like Eshkolit Ha'Amakim, in contrast to others like Dulce [33]. Our results show that climacteric accessions displaying the highest levels of esters did not produce any sesquiterpenes, or they were present only at very low levels (I-I). Therefore, our results are coherent with the two classes of *Cantalupensis* detected according to the presence of these volatiles (I-I and I-II): a group of high ester and low sesquiterpene and a group of moderate ester and high sesquiterpene.

Sesquiterpenes constitute a very diverse chemical group, often with strong and characteristic odors. They have been detected in other fruit rinds like in apples and oranges [44,45], and in many cases not only contributing to aroma but also with a defensive role against herbivores [45,46]. In fact, plant oils rich in sesquiterpenes and monoterpenes are used to control insect pests [47]. However, this ecological function of sesquiterpenes related to communication and defense [48] is currently unknown in melon.

The sesquiterpene-richest accessions were mainly *Cantalupensis*, divided in two groups based on ester and sulfur compound content: I-IIa with higher amounts in methyl, ethyl, propyl and butyl esters and sulfur compounds, and I-IIc. The group I-IIb (*Momordica* and *Dudaim*) presents a moderate content of sesquiterpenes, accompanied by a lower level in BCAA related compounds and phenolics, with the exception of eugenol and methyl eugenol, for which the *Momordica* MR1 presented the highest values in the core collection (LSD test, $p < 0.05$, Supplementary Table S3).

Momordica melons, a highly polymorphic group from India, were the only subsp. *agrestis* accessions in this aromatic cluster I. This is in agreement with its intermediate climacteric behavior and also the intermediate position between both subspecies reported in several genotyping studies [49,50]. In this sense, recent studies suggest that *Momordica* actually belongs to subsp. *melo* [6,51]. Additionally, Indian germplasm has been suggested as the origin of modern Mediterranean and Far-East melons [52]. The presence of sesquiterpenes in the rind of some *Cantalupensis* (both cantaloupes and *Reticulatus*) and Asian landraces but not in Charentais-type *Cantalupensis* and most *Inodorus* melons may

suggest either that the *Momordica* type has not participated in the development of these kind of melons or alternatively that low-sesquiterpene *Momordica* melons like PI 124112 have been involved. This high-sesquiterpene feature might also have been incorporated during the breeding process of modern varieties, as *Momordica* types are among the main sources of disease resistances introgressed in modern melons.

Another melon group which is considered intermediate between subspecies is *Dudaim*, also called mango melon, which is characterized by its highly aromatic fruits. In fact, the components of this peculiar strong aroma were analyzed by Aubert and Pitrat [12] because of its singularity. Unlike that which occurred in flesh volatiles [5], *Dudaim* genotypes were grouped with *Momordica* according to the volatile pattern in the rind, far from *Conomon* (*Makuwa*) types with which it shared the flesh profile (Figure 1).

Both *Dudaim* genotypes were especially rich in the phenolics methyl eugenol and eugenol, two compounds absent in most of the germplasm and previously reported at high concentrations in rind, even higher than in flesh, in Queen Anne's Pocket melon [12]. Some landraces like the Spanish Bolas and the Bulgarian Ogen (I-Ib), and the *Conomon* Paul (cluster II) also presented high levels of these phenylpropanes, even higher than the level in *Dudaim* in the latter. Recently, the flesh VOCs characterization of an introgression lines (ILs) collection derived from Ginsen *Makuwa*, as a donor parent, and the *Cantalupensis* *Vedrantais*, as a recurrent one, has demonstrated that specific *Makuwa* introgressions lead to an increase in eugenol content [28]. A moderate amount of this compound was detected in the flesh of *Makuwa* melons [5] and herein we report it also in rind (Supplementary Table S1). Eugenol is a notable flavor constituent in several spices like clove or fruits like wild strawberry, passionfruit or grapes, but also has been reported as an insect repellent and in plant pathogen defense strategies [53]. Conversely, methyl eugenol has been reported as an insect pollinator attractant [54]. The effect of these VOCs in the rind of these specific melon groups, basically *Dudaim* and *Momordica* (I-IIb) and *Inodorus* landraces (I-Ib), remains unclear, but suggests its importance in such different melon varieties, and the noticeable loss of these compounds in commercial types.

3.3.2. Low or Non-Aromatic Accessions (cluster II)

The second cluster (II) grouped basically non-climacteric types, a group of *Inodorus*, mixed with some subspecies *agrestis* types (*Conomon*, *Acidulus*, and *Tibish*) and some non-sweet melons, climacteric but consumed like cucumbers when immature (*Flexuosus-Chate*), in a first subcluster (II-I), and a second subcluster mainly with of *Conomon* and wild *Agrestis* melons (II-II).

- Sweet and non-sweet melons with non-aromatic rinds (II-I)

The first subcluster (II-I) presented a low level of VOCs, lacking most of the compounds detected except the ones found in nearly all the samples, like 1-butanol, butanal, monoterpenes, a few sulfur-derived compounds, most lipid-derived compounds, a few esters, and some phenolic compounds. Despite the fact that this cluster included nearly all the non-climacteric types, some climacteric accessions and intermediate types regarding ripening behavior were included here: the previously mentioned *Flexuosus* and *Chate* that, despite not being analyzed at complete maturity, were the ones with the highest level of VOCs in this subcluster, as was an *Ameri* accession from Central Asia. The *Ameri* has been reported as a group with high genetic variability [35,50]. Thus, not surprisingly, different *Ameri* varieties presented different rind volatile profiles, as also occurred with flesh aroma [5].

Within the non-climacteric types in this subcluster (II-I) we found many Spanish *Inodorus* (now *Ibericus*), such as Blanco or Piel de sapo Piñonet, but also international commercial types, such as Honeydew or Tam Dew, mixed with Indian and African *Acidulus*, and African *Tibish* melons, but with few differences in the VOCs content (LSD test, $p < 0.05$, Supplementary Table S3; Figure 1). It is worth highlighting that Blanco Redondo, a Spanish *Inodorus* melon, was the only melon in cluster II with a relatively high amount of sesquiterpenes. This kind of landraces with an *Inodorus* genetic background can be of interest in breeding commercial *Inodorus* types contributing new external aroma profiles.

The group including the Flexuosus Arya, the Chate Carosello, and the Inodorus Piel de sapo Piñonet, one of the most important types in the Spanish market, and the Casaba Golden Beauty presented the highest levels in the whole collection of some lipid derivatives like (E)-2-nonenal, together with some African Agrestis and the Flexuosus Snakemelon from the Conomon-Agrestis subcluster (II-II) (Supplementary Table S1, Supplementary Figure S1). This VOC, (E)-2-nonenal, is associated to cucumber-like odor in Inodorus melons [24], being present in the flesh of these types [5].

The only wild Agrestis in this subcluster was the Indian Wild Chibbar, which presented interestingly a very similar rind VOC profile to the Piel de sapo accession.

- Far Eastern Conomon and wild Agrestis had low aroma rinds (II-II)

The Conomon-wild Agrestis subcluster (II-II) presented an intermediate VOC profile between the aromatic accessions rich in esters in cluster I and the non-aromatic ones in subcluster II-I. These accessions were poor in esters, but presented significantly higher levels of some ethyl (like ethyl propanoate), propyl (like propyl butanoate) and acetate esters (like propyl acetate), sulfur volatiles, some lipid derivatives and several apocarotenoids in comparison to the Inodorus-Acidulus-Flexuosus subcluster II-I. Some climacteric types such as two Ameri accessions from Egypt and Uzbekistan and a Flexuosus from Saudi Arabia were also included in this subcluster. The Ameri and the Flexuosus accessions were clustered together with the wild Indian Callosus and two wild African types, in agreement with the high variability reported within Ameri group and the intermediate position of Flexuosus. The Spanish landrace Escrito oloroso, the only Inodorus genotype with this volatile profile, was clustered with the intermediate-climacteric medium-sweet Conomon-Makuwa accessions. In fact, the name Escrito oloroso means smelly in Spanish, in contrast with the remaining Inodorus of subcluster II-I lacking esters.

Although a group including Conomon, Chinensis and Makuwa types has been considered in the current work, the non-climacteric Conomon and the intermediate-climacteric Makuwa can be differentiated mainly based on their ripening and sweetness [1], and classification of other metabolomics also divides these two groups [6]. In this sense, Esteras et al. [5] also reported two groups based on flesh volatiles, the Conomon-Chinensis accessions (Songwhan Charmi, Paul, Shiro Uri Okayama, Nanbukin) within the non-aromatic cluster and the Makuwa accessions (Ginsen Makuwa and Baishami) mixed with Dudaim in the aromatic cluster (flesh_II-Ib and flesh_I-Ib, respectively; Figure 1). Our results imply that these Far East landraces are very similar regarding rind aroma, although the flesh profile can be very different and seems to be influenced by the different climacteric behavior (Makuwa vs. Conomon-Chinensis).

In a recent flesh volatiles analysis with the Makuwa IL collection, Perpiñá et al. [28] found several VOCs patterns in the ILs, mostly reducing the amount of esters and apocarotenoids with respect to the aromatic Vedrantais recurrent parent despite bearing small introgressions from the Makuwa melon. In view of our results, the analysis of rind aroma should be considered when using Conomon-Makuwa accessions in breeding, since rind profile can change dramatically, and to focus only on flesh traits can lead to a loss of fruit quality during the process.

For some volatiles, accessions in this subcluster II-II presented similar or even higher amounts than aromatic types. For instance, the African wild type PI 185111 shared with the landrace Casca de Carvalho (I-Ia) the highest level of the collection for several monoterpenoids such as α -terpineol (LSD test, $p < 0.05$, Supplementary Table S3), a volatile providing a floral odor. As previously mentioned, many monoterpenes and sesquiterpenes are thought to have defense roles. The presence in this wild accession of high levels of monoterpenoids in contrast to the remaining accessions may be an interesting starting point for future studies about possible resistant/repellent responses against pests. This accession has been reported as resistant to soil pathogens [55,56], although to date no studies have focused on its behavior in relation to insects. Surprisingly, this wild PI 185111, with no good scent, also presented one of the highest levels for some acetates (6-nonenyl acetate) and ethyl esters (ethyl

propanoate) in the collection (LSD test, $p < 0.05$, Supplementary Table S3), showing that non-cultivated types present more variability than expected and can have also high levels of some esters.

With respect to wild *Agrestis*, it is important to note the distinction between African and some Indian melons based on just a few VOCs such as sulfur compounds like methylthioacetate, high in the African *Agrestis* PI 185111 and Tendelti, or phenolics like ethyl phenylacetate, abundant in Indian *Callosus*. Studies using molecular markers [34,52] had previously reported this genetic differentiation between both *Agrestis* groups. However, the Indian Wild Chibbar presented a richer volatile profile (II-I) than *Callosus* and the African *Agrestis*, suggesting a greater diversity in Indian types.

3.4. Perspectives in Elucidation of Genomic Regions Involved

The study of the rind aroma profile of such a diverse collection encourages the use of specific accessions as interesting genetic resources in breeding. For instance, the use of the exotic Dudaim Queen Ann's Pocket Melon as donor parental in the development of ILs with the recurrent Piel de sapo resulted in a Piel de sapo line with a stronger external aroma [57]. New VOC profiles can be obtained employing segregating populations that not only can lead to interesting breeding lines, but also to the detection of genomic regions associated to specific volatiles. Regarding flesh aroma, the ILs derived from Ginsen Makuwa x Vedrantaïs have shown several volatile profiles, including lines with high levels of aldehydes or eugenol, although with a common reduction in alkyl esters and apocarotenoids due to Makuwa introgressions [28]. These authors have also suggested several genomic regions and candidate genes controlling the VOC profiles detected. Previously, effects of donor alleles on the qualitative trait of external aroma in this IL collection were also reported [58] as well as in other populations using Vedrantaïs and Piel de sapo melons [59]. However, few quantitative studies about rind volatiles have been performed with the aim to elucidate the genomic regions involved. Thus, due to the ample variability reported herein, a Genome Wide Association study (GWAS) including this core collection would be of interest in the future in order to find Quantitative Trait Loci (QTLs) and candidate genes controlling the amount of rind VOCs in the different melon types.

4. Conclusions

In this work we present the most exhaustive analysis of rind volatile profile in melons up to date. Our results regarding rind aroma corroborate the huge qualitative and quantitative diversity present in the species *C. melo*, with some exclusive rind volatiles not present in the flesh. Statistical analyses highlight some varietal groups and non-commercial genotypes as types of interest because of their different profiles or attractive external odors. In this sense, Spanish *Inodorus* presented high variation, combining aromatic and non-aromatic rind and flesh aroma profiles, which may be of interest in breeding more aromatic types in long-shelf life genetic backgrounds. Climacteric types also presented several VOC profiles with the sesquiterpene content as the key differentiation factor that could also be of interest in breeding for new external aroma types or even for crop protection. For instance, *Cantalupensis* accessions, divided in previous studies into *Cantalupensis* and *Reticulatus* or according to flesh color, constituted in this work four main groups, rich or not in sesquiterpenes and with higher or intermediate content of esters. Exotic melons like some *Conomon*, with high content of some phenolics associated to the characteristic odor of Dudaim, or wild *Agrestis*, richest in some esters, showed an unexpected variability which has been underexploited to date.

Additionally, the VOCs detected have been useful as biomarkers to classify and characterize this germplasm collection, in a similar manner as previously reported with volatile compounds in the flesh. The main clusters described in this work are mainly in agreement with previous phylogenetic studies, suggesting that rind volatiles are good markers to calculate genetic distances between accessions. However, differences in some groups have been detected. In *Conomon*, for example, non-aromatic rind is reported although great variation exists based on flesh profile with aromatic and non-aromatic types.

Moreover, the species variability represented in aroma-profile clusters should also be a factor to bear in mind during breeding programs, as accessions with similar or distinct profiles can be selected

as parentals affecting fruit quality and perception. However, further studies need to be carried out to determine the threshold perception of many of the VOCs detected, especially in sesquiterpenes, and thus, the association of specific compounds with the attractive rind scent, or in other cases with possible pest repellent odor.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/10/1512/s1>, Table S1: Rind volatile compounds detected in the fruits analyzed. A. Annotation of VOCs detected. B. Fruits analyzed for rind volatile profile and VOCs detected. C. Statistics for VOCs and accessions. Table S2: Comparison analysis between rind and flesh VOCs in the germplasm collection assayed. A. VOCs detected in the melon germplasm collection rind in this study and previously reported in flesh. B. Total content of VOCs per accession, for rind and flesh, and the correlation coefficient between total amount of VOCs in both tissues. C. Mean total values per VOCs, for rind and flesh tissues, and the correlation coefficient for each VOCs and for the set of VOCs present in both tissues in the total germplasm collection. Table S3: Least Significant Difference (LSD) test for each VOC detected in the germplasm collection with a probability level of $p < 0.05$. Figure S1: Detail of the clusters observed with the Hierarchical Cluster Analysis and heatmap calculated. Color associated to chemical group: purple (phenolics), pink (sulfur compounds), maroon (BCAA), red (lipid derivatives), light blue (acetate esters), dark blue (other esters), green (monoterpenoids), dark green (sesquiterpenes), orange (apocarotenoids), grey (others), and black (unknown). Figure S2: Bar graphs showing the mean content per accession assayed, in both rind and flesh, for the melon volatiles reported as with high impact in melon aroma by Gonda et al. [24]. A. Impact acetate esters VOCs flesh vs. rind. B. Impact ethyl and methyl esters VOCs flesh vs. rind. C. Impact lipid derivatives VOCs flesh vs. rind. D. Impact phenolic VOC flesh vs. rind. VOCs codes specified in Table S1. Figure S3: Hierarchical Cluster Analysis showing images of the fruits for some representative accessions in the main subclusters. (a) Vedrantais (b) Yokneam (c) Tendral 10/3/2020Negro (d) PI124112 (e) Queen’s pocket melon (f) Tam Dew (g) Amarillo oro (h) TGR1551 (i) Tibish Khurtagat (j) Snakemelon (k) Piel de sapo Piñonet (l) Nanbukin (m) PI 185111.

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