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1 **Genetic dissection of aroma biosynthesis in melon and its**
2 **relationship with climacteric ripening**

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22 **Highlights**

23 • Two melon commercial varieties produced 88 different VOCs.

24 • Melon rind and flesh contributed differently to fruit aroma.

25 • *ETHQV8.1*, a climacteric ripening QTL, has an impact in the biosynthesis of VOCs.

26 • Candidate genes and QTLs have been identified as controlling VOCs production in

27 melon.

28 • New tools for breeding fruit flavor in melon have been developed.

29

30 **Abstract**

31 Aroma is an essential trait in melon fruit quality, but its complexity and genetic basis is still

32 poorly understood. The aim of this study was the identification of quantitative trait loci (QTLs)

33 underlying volatile organic compounds (VOCs) biosynthesis in melon rind and flesh, using a

34 Recombinant Inbred Line (RIL) population from the cross 'Piel de Sapo' (PS) x 'Védrantais'

35 (VED), two commercial varieties segregating for ripening behavior. A total of 82 VOCs were

36 detected by gas chromatography-mass spectrometry (GC-MS), and 166 QTLs were identified.

37 The main QTL cluster was in chromosome 8, collocating with the previously described

38 ripening-related QTL *ETHQV8.1*, with an important role in VOCs biosynthesis. QTL clusters

39 involved in esters, lipid-derived volatiles and apocarotenoids were also identified, and

40 candidate genes have been proposed for ethyl 3-(methylthio)propanoate and benzaldehyde

41 biosynthesis. Our results provide genetic insights for deciphering fruit aroma in melon and

42 offer new tools for flavor breeding.

43

44 **Keywords:** aroma, climacteric ripening, *Cucumis melo* L., GC-MS, QTL mapping, VOCs.

45 **1. Introduction**

46 Melon (*Cucumis melo* L.) is an important crop worldwide, with a global production of more
47 than 27 million tonnes in 2018 (fao.org/faostat). Fruit quality is a complex trait determined by
48 several fruit characteristics and is essential for consumer's appreciation. Melon is a very
49 diverse species within the *Cucurbitaceae* family, comprising accessions that differ in color,
50 texture, aroma, flavor and nutritional value (Pitrat, 2017). Melon is also an interesting crop to
51 study fruit ripening, as the species includes climacteric and non-climacteric accessions.
52 Climacteric ripening consists in a concomitant increase of the respiration rate and ethylene
53 production at the onset of the ripening process, whereas in non-climacteric fruits both remain
54 at a basal levels during ripening (Lelievre, Latche, Jones, Bouzayen, & Pech, 1997). Ethylene
55 has a direct impact in aroma production (Manríquez et al., 2006; Shalit et al., 2001), which is
56 one of the most important determinants of flavor and consumer preferences.

57 Fruit aroma depends on the concentration and the combination of volatile organic compounds
58 (VOCs), which are genetically determined, but also highly influenced by environmental and
59 post-harvest factors (Wyllie, Leach, Wang, & Shewfelt, 1994). More than 500 different VOCs
60 have been identified in melon, and depending on the technique used more than 100
61 compounds can be found in a single accession (Esteras et al., 2018; Obando-Ulloa, Ruiz,
62 Monforte, & Fernández-Trujillo, 2010; Pang et al., 2012). VOCs can originate from amino acids,
63 fatty-acids or terpenes, and can be classified according to their chemical group. Esters,
64 alcohols and aldehydes are the most abundant VOCs in melon, but other types of compounds
65 such as benzenoids, ketones, furans, lactones, monoterpenes, sesquiterpenes or
66 apocarotenoids are also found (Gonda et al., 2016). Each volatile has a different impact in the
67 final aroma of the fruit depending on the individual odor threshold. Several melon accessions

68 have been studied combining gas chromatography-mass spectrometry (GC-MS) analyses with
69 odor evaluations to determine the compounds that contribute the most to the unique flavor
70 of melon varieties (Pang et al., 2012). Some key VOCs identified are nonanal, 6-nonenal (Z),
71 2,6-nonadienal-(E,Z), hexyl acetate, ethyl 2-methylpropanoate or phenylethyl alcohol. Each of
72 these VOCs provides specific aroma and flavor to melon fruits, some of them being
73 denominated as melon-like, cucumber-like, fruity, floral, sweet, green or even foul. For
74 instance, most esters give fruity and sweet notes, whereas aldehydes and alcohols give green,
75 fresh, cucumber-like notes (Gonda et al., 2016; Pang et al., 2012).

76 The volatile profile of climacteric and non-climacteric fruits differs both qualitative and
77 quantitatively (Esteras et al., 2018; Moing et al., 2020; Obando-Ulloa et al., 2008). For
78 instance, esters such as hexyl acetate and ethyl acetate are highly abundant in climacteric
79 melons, whereas aldehydes like pentanal are more common in non-climacteric melons
80 (Obando-Ulloa et al., 2008). Moreover, the diversity and amount of VOCs produced in
81 climacteric melons is usually higher than in non-climacteric ones.

82 Melon is a diploid species ($2n=2x=24$) with a relatively small genome (357Mbp). The
83 availability of a melon reference genome (Garcia-Mas et al., 2012), dense genetic maps
84 (Pereira et al., 2018), germplasm collections and mapping populations such as Recombinant
85 Inbred Lines (RILs) and Introgression Lines (ILs) have eased the identification of quantitative
86 trait loci (QTLs) associated to fruit traits such as morphology, carotenoid content or ripening
87 behavior (Obando-Ulloa et al., 2010; Pereira et al., 2018, 2020). RILs have shown to be
88 powerful populations to detect QTLs in melon and other species as they usually present a high
89 number of recombinations and represent diverse allelic combinations. Other powerful
90 available tools in melon are databases such as Melonet-DB (Yano, Nonaka, & Ezura, 2018),

91 fruitENCODE (<http://www.epigenome.cuhk.edu.hk/encode.html>) and Melonomics
92 (<http://www.melonomics.net>), which contain transcriptomic and epigenomic data, and the
93 melon variome map (Zhao et al., 2019).

94 Biosynthesis pathways to several volatiles have been partially elucidated in melon although
95 the complete biosynthetic pathways from amino acids, fatty acids and terpenes remain
96 unknown. Aldehydes are hydrogenated to form alcohols (Manríquez et al., 2006), and alcohols
97 are then acetylated to produce esters (Yahyaoui et al., 2002). The aminotransferases
98 *CmBCAT1* and *CmArAT1* have been described to catalyze amino acids deamination, the first
99 step of the production of several branched and sulfur VOCs (Gonda et al., 2010, 2013). In the
100 phenylpropanoid pathway, *CmCNL* and *CmBAMT* participate in the synthesis of cinnamoyl-
101 CoA and methyl benzoate, respectively (Gonda et al., 2018). In the fatty acid pathway,
102 lipoxygenases (LOX), hydroperoxide lyases, isomerases and dehydrogenases play a role in the
103 biosynthesis of C6 and C9 aliphatic aldehydes and alcohols from linoleic and linolenic acids
104 (Schwab, Davidovich-Rikanati, & Lewinsohn, 2008). Several LOX genes have been identified in
105 melon (Tang, Zhang, Cao, Wang, & Qi, 2015). For other aliphatic VOCs, α and β -oxidation have
106 been proposed as possible biosynthetic pathways (Schwab et al., 2008). In the terpenoid
107 pathway, the terpene synthases *CmTpsDul* and *CmTpsNY* modulate the accumulation of
108 sesquiterpenes (Portnoy et al., 2008), whereas the cleavage dioxygenase *CmCCD1* cleaves
109 carotenoids to produce several apocarotenoid VOCs such as geranylacetone and β -ionone
110 (Ibdah et al., 2006).

111 In this study, we analyzed the volatile profile of a RIL population developed from a cross
112 between two melon elite varieties, 'Piel de Sapo' (PS) and 'Védrantais' (VED) which segregates
113 for many fruit quality traits, including aroma and ripening behavior (Pereira et al., 2020). QTL

114 mapping was performed using metabolite data obtained from both rind and flesh tissues,
115 integrating phenotypic data from GC-MS and genotypic data previously obtained by
116 genotyping by sequencing (Pereira et al., 2018). We hypothesize that both PS and VED may
117 present functional alleles for the VOC synthesis, some of them related to ripening behavior.
118 The aim of this work is to identify genetic factors controlling aroma production in melon which
119 could be integrated into breeding programs for fruit flavor improvement.

120

121 **2. Materials and methods**

122 **2.1. Plant material**

123 The 89 RIL population, with 82 RILs in the F8 generation and seven in the F7, was developed
124 from a cross between the non-aromatic '*Piel de Sapo*' T111 (PS) (*C. melo* ssp. *melo* group
125 *inodorus*) and the aromatic '*Védrantais*' (VED) (*C. melo* ssp. *melo* group *cantalupensis*, French
126 Charentais-type) by Pereira et al. (2018). The plants were grown at Caldes de Montbui
127 (Barcelona) under greenhouse conditions, allowing only one melon per plant. The fruits were
128 harvested at the ripe stage as previously defined (Pereira et al., 2018). The fruits analyzed in
129 this study were harvested in summer 2015 (T3) and summer 2016 (T4 and T5), using a total of
130 three biological replicates for the 89 RILs (one per harvest time) and 9 biological replicates for
131 PS, VED and the hybrid PS x VED (three per harvest time) (Pereira et al., 2018).

132 **2.2. Sample preparation for volatile extraction**

133 Frozen samples of rind and flesh from the three biological replicates for each RIL were ground
134 up and stored at -80 °C. All biological replicates were independently analyzed by GC-MS. Two
135 grams of tissue were weighed and added to frozen chromatography 20 ml vials (Thermo Fisher
136 Scientific®, Waltham, MA, U.S.) containing 7 ml of a saturated NaCl solution and 15 ppm of 3-

137 hexanone used as internal standard. Samples were sealed with silicone septa and stored at 4
138 °C in the dark for no longer than a week, until GC-MS analysis was performed.

139 **2.3. Aroma analysis by GC-MS**

140 Solid-Phase Micro-Extraction (SPME) was carried out using a 7890A gas chromatograph
141 coupled to a 5975C mass spectrometer and a GC PAL 80 autosampler (Agilent Technologies®,
142 Santa Clara, CA, U.S.). The chromatograph was equipped with an SPME fiber (50/30 µm
143 DVB/CAR/PDMS, Merck®, Darmstadt, Germany) and a Sapiens-X5MS capillary column (30
144 m/0.25 mm/0.25 µm, Teknokroma®, Sant Cugat del Vallès, Spain). Samples were pre-
145 incubated 15 min to 50 °C at 250 rpm, and then the SPME fiber was exposed 30 min to the
146 vial headspace. Sample injection was performed in splitless mode, starting at a temperature
147 of 50 °C for 1 min, increasing 5 °C/min to 280 °C, and holding that temperature for 5 min (total
148 time 52 min). The carrier gas was helium at a head pressure of 13.37 psi. The source
149 temperature of the mass spectrometer was set at 230 °C and the quadrupole temperature at
150 150 °C, with no delay in the detection.

151 We performed an untargeted analysis for VOCs of rind and flesh samples. VOCs were
152 identified by comparison of their mass spectra with the NIST 11 library (NIST/EPA/NIH) and by
153 their Kovats retention index. The Kovats retention index was calculated using a mix of alkanes
154 (C₇-C₄₀ in hexane, Merck®, Darmstadt, Germany) under the same chromatographic conditions.
155 We created a list of compounds identified in several samples by applying two quality criteria:
156 a calculated Kovats index within a confidence interval of ±10 comparing to tabulated standard
157 values, and a minimum MS quality of 80. Compounds not matching both criteria were
158 excluded from the analysis. For individual samples, when the proximity of two compounds
159 lowered the MS quality, a cutoff of 50 was considered.

160 The content of each compound was estimated by comparison to the 3-hexanone internal
161 standard peak area. Compounds were coded following a similar criterion to Freilich *et al.*
162 (2015), grouping them based on their chemistry, tissue and name. Data was organized in four
163 different subsets: each biological replicate individually and the mean of each line. Means were
164 only considered when data of the three replicates was available. Medians of the biological
165 replicates were also calculated.

166 Information about the odor of the compounds detected was extracted from the literature.

167 **2.4. QTL analysis**

168 QTL mapping was conducted with MapQTL 6® performing both interval mapping (IM) and the
169 Kruskal-Wallis test (K-W). The genetic map used was described in Pereira *et al.* (2018) and was
170 constructed using genotyping-by-sequencing data from the same RIL population. MapChart®
171 was used for the map representation. For the statistical support of the QTL mapping, a
172 permutation test with 1,000 iterations and 95% confidence was performed. QTLs with a LOD
173 score ≥ 2.75 in at least one of the four subsets were preselected. The final map included only
174 QTLs that reached the 2.75 threshold in the means subset, or a score ≥ 2.50 in two subsets. K-
175 W scores were used to confirm the QTLs detected by IM. Candidate genes were selected using
176 genome version v3.6.1 and according to functional annotation data from the Melomics
177 database v4.0 (<https://www.melomics.net/>), orthologs found in PLAZA 4.0 (Van Bel *et al.*,
178 2018) and transcriptomic data from the fruitENCODE database
179 (<http://www.epigenome.cuhk.edu.hk/encode.html>).

180 **2.5. Statistical analysis**

181 The software R (v4.0.0) (<https://www.r-project.org/>) with the RStudio interface (v1.1.463)
182 (<http://www.rstudio.com/>) was used to perform statistical analysis and to represent the data.

183 A Shapiro-Wilk test was performed to check the normality of the data and a correlation test
184 was performed using the Spearman's rank correlation coefficient. Z scores were calculated to
185 standardize the data since they deviated from normality. PCA was performed with "ggfortify",
186 correlation was calculated with "Hmisc" and "corrplot" packages, and plots were generated
187 with "gplots", "factoextra" and "ggplot2".

188

189 **3. Results**

190 **3.1. General VOC analysis**

191 Melon fruits from the RIL population produced 82 different VOCs, 79 were present in rind and
192 all were present in flesh. According to their chemical structure, the list comprised 47 esters,
193 13 aldehydes, seven alcohols, six terpenes, four benzenoid derivatives, three ketones and two
194 furans (Table 1). The distribution of all VOCs in the RIL population was not normal ($p<0.05$)
195 but left-skewed. Both rind and flesh produced a great quantity of esters and aldehydes. Rind
196 samples contained a higher quantity of these compounds compared to flesh (Supplementary
197 Table S1-S4).

198 **Table 1.** VOCs identified by Kovats index and mass spectrum in the PS x VED RIL population.

Compound	NIST nomenclature	Code ^a	RI ^b	CAS
<i>Alcohols</i>				
2-Methylbutanol	1-Butanol, 2-methyl-	ALX_1B2M	729	137-32-6
1-Hexanol		ALX_1HXO	864	111-27-3
1-Octen-3-ol		ALX_103O	977	3391-86-4
Benzyl Alcohol		ALX_BZOL	1034	100-51-6
3-Octen-1-ol, (Z)-		ALX_3O1OZ	1066	20125-85-3
1-Octanol		ALX_1OCOL	1069	111-87-5
Phenylethyl Alcohol		ALX_PHEOL	1114	60-12-8
<i>Aldehydes</i>				
3-Methylbutanal	Butanal, 3-methyl-	ADX_B3M	647	590-86-3
2-Methylbutanal	Butanal, 2-methyl-	ADX_B2M	658	96-17-3
Pentanal		ADX_PNL	695	110-62-3
Hexanal		ADX_HXL	800	66-25-1
Heptanal		ADX_HPL	901	111-71-7
Benzaldehyde		ADX_BZALD	959	100-52-7
Octanal		ADX_OCL	1002	124-13-0
Benzeneacetaldehyde		ADX_BZACALD	1042	122-78-1

2-Octenal, (E)-		ADX_2OLE	1063	2548-87-0
6-Nonenal, (Z)-		ADX_6NNLZ	1103	2277-19-2
Nonanal		ADX_NNL	1104	124-19-6
2,6-Nonadienal, (E,Z)-		ADX_26NDLEZ	1152	557-48-2
Decanal		ADX_DCL	1205	112-31-2
<i>Benzenoids</i>				
Toluene		BX_TOL	761	108-88-3
1,3-Dimethylbenzene	Benzene, 1,3-dimethyl-	BX_BZ13DM	862	108-38-3
Styrene		BX_STYR	890	100-42-5
1-Methoxy-4-methylbenzene	Benzene, 1-methoxy-4-methyl-	BX_BZ1M4M	1019	104-93-8
<i>Esters</i>				
Ethyl Acetate		EX_EAC	615	141-78-6
Ethyl propanoate	Propanoic acid, ethyl ester	EX_PEE	707	105-37-3
n-Propyl acetate		EX_NPA	709	109-60-4
Methyl butanoate	Butanoic acid, methyl ester	EX_BME	717	623-42-7
Ethyl 2-methylpropanoate	Propanoic acid, 2-methyl-, ethyl ester	EX_P2MEE	751	97-62-1
2-Methylpropyl acetate	Acetic acid, 2-methylpropyl ester	EX_A2MPE	767	110-19-0
Methyl 2-methylbutanoate	Butanoic acid, 2-methyl-, methyl ester	EX_B2MME	772	868-57-5
Ethyl butanoate	Butanoic acid, ethyl ester	EX_BEE	801	105-54-4
Butyl acetate	Acetic acid, butyl ester	EX_ABE	812	123-86-4
Ethyl 2-methylbutanoate	Butanoic acid, 2-methyl-, ethyl ester	EX_B2MEE	846	7452-79-1
3-Methylbutyl acetate	1-Butanol, 3-methyl-, acetate	EX_1B3MA	873	123-92-2
2-Methylbutyl acetate	1-Butanol, 2-methyl-, acetate	EX_1B2MA	877	624-41-9
Propyl butanoate	Butanoic acid, propyl ester	EX_BPE	898	105-66-8
Ethyl pentanoate	Pentanoic acid, ethyl ester	EX_PNEE	901	539-82-2
Pentyl acetate	Acetic acid, pentyl ester	EX_APNE	913	628-63-7
3-Methyl-2-buten-1-ol acetate	2-Buten-1-ol, 3-methyl-, acetate	EX_2B1O3MA	921	1191-16-8
Methyl hexanoate	Hexanoic acid, methyl ester	EX_HXME	923	106-70-7
Propyl 2-methylbutanoate	Butanoic acid, 2-methyl-, propyl ester	EX_B2M.PE	943	37064-20-3
2-Methylpropyl butanoate	Butanoic acid, 2-methylpropyl ester	EX_B2MPE	953	539-90-2
Ethyl (methylthio)acetate		EX_EMTHAC	983	4455-13-4
Butyl butanoate	Butanoic acid, butyl ester	EX_BBE	995	109-21-7
Ethyl hexanoate	Hexanoic acid, ethyl ester	EX_HXEE	1000	123-66-0
3-Hexen-1-ol, acetate, (Z)-		EX_3HX1OAZ	1007	3681-71-8
Hexyl acetate	Acetic acid, hexyl ester	EX_AHXE	1016	142-92-7
1,2-Propanediol, diacetate		EX_12PLDA	1027	623-84-7
Cyclohexyl acetate*	Acetic acid, cyclohexyl ester*	EX_ACHXE	1039	622-45-7
2-Methylbutyl butanoate	Butanoic acid, 2-methylbutyl ester	EX_B2MBE	1057	51115-64-1
2,3-Butanediol diacetate		EX_23BOLDA	1061 & 1075	1114-92-7
Ethyl 2,4-hexadienoate	2,4-Hexadienoic acid, ethyl ester	EX_24HXEE	1094	2396-84-1
Propyl hexanoate	Hexanoic acid, propyl ester	EX_HXPE	1094	626-77-7
Ethyl heptanoate	Heptanoic acid, ethyl ester	EX_HPEE	1098	106-30-9
Ethyl 3-(methylthio)propanoate	3-(Methylthio)propanoic acid ethyl ester	EX_3MTHPEE	1100	13327-56-5
Heptyl acetate	Acetic acid, heptyl ester	EX_AHPE	1112	112-06-1
Ethyl (+)-3-acetoxybutyrate*		EX_E3AXB	1114	27846-49-7
1,3-Butanediol, diacetate		EX_13BOLDA	1128	1117-31-3
2-Methylpropyl hexanoate	Hexanoic acid, 2-methylpropyl ester	EX_HX2MPE	1150	105-79-3
Phenylmethyl acetate	Acetic acid, phenylmethyl ester	EX_APHME	1165	140-11-4
Ethyl benzoate	Benzoic acid, ethyl ester	EX_BZEE	1171	93-89-0
Diethyl butanedioate	Butanedioic acid, diethyl ester	EX_BDEE	1181	123-25-1
Ethyl 4-octenoate	4-Octenoic acid, ethyl ester	EX_4OEE	1186	138234-61-4
Butyl hexanoate	Hexanoic acid, butyl ester	EX_HXBE	1191	626-82-4
Ethyl octanoate	Octanoic acid, ethyl ester	EX_OEE	1198	106-32-1
Octyl acetate	Acetic acid, octyl ester	EX_AOE	1212	112-14-1
Ethyl benzeneacetate	Benzeneacetic acid, ethyl ester	EX_BZAEE	1245	101-97-3
2-Methylbutyl hexanoate	Hexanoic acid, 2-methylbutyl ester	EX_HX2MBE	1253	2601-13-0
2-Phenylethyl acetate	Acetic acid, 2-phenylethyl ester	EX_A2PHEE	1257	103-45-7
Ethyl decanoate	Decanoic acid, ethyl ester	EX_DCEE	1397	110-38-3
<i>Furans</i>				
2-Ethylfuran	Furan, 2-ethyl-	FX_F2E	700	3208-16-0
2-Pentylfuran	Furan, 2-pentyl-	FX_F2PN	992	3777-69-3

<i>Ketones</i>					
2-Methyl-3-pentanone	3-Pentanone, 2-methyl-	KX_3PN2M	743	565-69-5	
4-Heptanone*		KX_4HPA	868	123-19-3	
6-Methyl-5-hepten-2-one	5-Hepten-2-one, 6-methyl-	KX_5HP2O	985	110-93-0	
<i>Terpenes</i>					
Eucalyptol		TX_EUL	1030	470-82-6	
β-Cylohomocitral		TX_βCYHCL	1254	472-66-2	
Megastigma-4,6(Z),8(Z)-triene		TX_MEGA	1324	71186-25-9	
Caryophyllene		TX_βCPHL	1421	87-44-5	
Cis-Geranylacetone		TX_CGERAC	1445	3879-26-3	
δ-Cadinene		TX_δCAD	1527	483-76-1	

199 ^aX = R for rind, F for flesh

200 ^bRI = retention index

201 *Newly described in melon (to our knowledge)

202 We first analyzed the volatile profile of the parental lines. PS displayed a lower number of
 203 VOCs than VED in both rind (30 and 66, respectively) and flesh (27 and 71) tissues
 204 (Supplementary Table S1). The hybrid (Hyb) accumulated more compounds in rind (70) than
 205 in flesh (58). In all three lines, benzaldehyde was one of the most abundant compounds in
 206 rind, although the mean levels varied from 12914 ng g⁻¹ of frozen tissue in PS to 56057 ng g⁻¹
 207 of frozen tissue in VED. VED and Hyb, both climacteric, also produced high amounts of the C6
 208 lipid derived hexyl acetate (56132 ng g⁻¹ and 38153 ng g⁻¹ of frozen tissue, respectively). In
 209 flesh, lipid derived aldehydes such as nonanal (PS, 1364 ng g⁻¹) and octanal (Ved, 4220 ng g⁻¹
 210 and Hyb, 1530 ng g⁻¹) were predominant. The climacteric lines presented in addition high
 211 levels of ethyl hexanoate and butanoate derivatives in flesh. Some compounds were
 212 overproduced in Hyb fruits compared to PS and VED, such as heptanal and methyl hexanoate
 213 in rind, whereas other VOCs were underproduced like decanal in flesh.

214 VED produced significantly more esters, alcohols, aldehydes and terpenes than PS (p<0.05).
 215 For instance, VED samples produced 15 times more octanal and 40 times more benzyl alcohol
 216 in flesh, or 186 times more 2,3-butanedioldiacetate in rind. Furans and some ketones such as
 217 2-ethylfuran and 2-methyl-3-pentanone were produced principally in PS fruits, whereas
 218 benzenoids were more abundant in Hyb rind. The total amount of VOCs in flesh was more

219 variable than in rind, possibly due to a stronger environmental effect in flesh samples caused
220 by slight changes in ripening stage. VED main groups were esters (68-85 % in rind, 52-84 % in
221 flesh), followed by aldehydes (12-27 % in rind, 4-38 % in flesh) and alcohols (2-5 % in rind, 2-
222 14 % in flesh) (Supplemental Fig. S1). PS main groups were aldehydes (88-97 % in rind, 67-90
223 % in flesh), alcohols (1-3 % in rind, 1-4 % in flesh) and ketones (1 % in rind, 2-20 % in flesh).
224 Hyb melons had an intermediate phenotype between PS and VED but were more similar to
225 the VED profile.

226 The RIL population showed a left-skewed distribution and transgressive segregation for most
227 compounds. Interestingly, flesh was the only tissue to produce β -cyclocitral, cis-
228 geranylacetone and megastigma-4,6(Z),8(Z)-triene, the last one not being detected in any
229 parental line. The only VOCs found in all samples were benzaldehyde in rind and eucalyptol in
230 flesh, although nonanal and ethyl acetate were also produced by most lines in both tissues.
231 Ethyl decanoate in rind and pentanal in flesh were rare in the population, detected only in one
232 and 5 lines, respectively.

233 Correlations between VOCs in the RIL population were represented in a heatmap for both
234 tissues, rind and flesh (Fig. 1). Esters were positively correlated among them, forming two
235 clusters in flesh and 3 in rind. The cluster showing the strongest correlation comprised ethyl
236 esters. Another ester cluster included also some alcohols such as hexanol, 2-methylbutanol
237 and octanol, as well as eucalyptol in flesh and nonanal and decanal in rind. In addition, we
238 found clusters of positive correlation for aldehydes, phenylalanine derived and lipid derived
239 VOCs in rind, for acetates, nonanal derived VOCs and terpenes in flesh, plus some others with
240 diverse composition. Furans, ketones and benzenoids showed negative correlation with the
241 rest of the compounds.

242 **3.2. QTL mapping**

243 We found 166 QTLs for 63 different compounds, 77 of these QTLs were found in flesh and 89
 244 in rind. Among them, 13 QTLs were coincident between tissues. All QTLs were confirmed by
 245 both IM and K-W, except QTL *EF_3MTHPEE_3.1* that did not fit the IM thresholds but had a
 246 significant K-W value. QTLs were identified in all 12 melon chromosomes (Fig. 2 and Table 2).
 247 Of the total number of QTLs, 18 were found in all four analyzed subsets (individual replicates
 248 and mean), 28 were detected in three subsets, and 4 were detected in two subsets but not in
 249 the mean subset. In addition, 72 QTLs appeared in only one subset and in the mean, and 44
 250 were only found in the mean subset. The physical interval of the QTLs ranged from 5.9 Kb to
 251 25.3 Mb, with a median of 9.2 cM and 1.96 Mb in genetic and physical distance, respectively.
 252 The median number of genes within these intervals was 173. We defined 12 clusters of QTLs
 253 which included multiple overlapping QTLs for compounds chemically similar and/or belonging
 254 to the same biochemical pathway (Table 2 and Supplementary Table S5). One of them on
 255 Chr08 contained up to 92 different QTLs.

256 **Table 2.** QTLs detected in the PS x VED RIL population.

Chr	C ^a	QTL	Genetic position (cM)	Nearest marker ^b	Size (Mb) ^b	LOD mean ^c	LOD T3 ^c	LOD T4 ^c	LOD T5 ^c	K-W value	% Exp	Positive additive effect
1		<i>EF_E3AXB_1.1</i>	3.3	chr01_452338	1.2	2.54	0.79	2.53	1.35	<0.005	16.9	PS
		<i>EF_NPA_1.1</i>	122.2	chr01_36593213	0.7	2.75	1.66	1.30	1.31	<0.001	18.2	Ved
2	♂	<i>ER_BME_2.1</i>	23.7	chr02_1937609	1.1	2.55	0.82	2.20	3.33	<0.005	16.7	PS
		<i>EF_B2MME_2.1</i>	50.8	chr02_6015657	3.7	2.64	0.77	2.82	1.31	<0.005	14.6	PS
		<i>ER_1B2MA_2.1</i>	66.4	chr02_13326331	9.8	3.04	0.62	1.91	2.08	<0.005	19.7	PS
		<i>ER_B2MME_2.1</i>	61.5	chr02_9079639	5.5	3.08	1.95	2.07	2.06	<0.005	19.9	PS
		<i>EF_B2MME_2.2</i>	61.5	chr02_9079629	5.0	2.79	0.48	3.02	2.05	<0.0005	15.6	PS
		<i>EF_B2M.PE_2.1</i>	64.8	chr02_11199109	5.0	2.54	0.95	0.99	2.92	<0.005	17.0	PS
		<i>ER_B2MBE_2.1</i>	61.5	chr02_9079639	7.4	2.85	0.28	0.48	2.97	<0.0005	18.5	PS
		<i>ADR_BZACALD_2.1</i>	67.5	chr02_13326331	7.4	2.76	0.42	1.37	2.14	<0.001	18.0	PS
3	♂	<i>ALR_1HXO_3.1</i>	38.9	chr03_6150294	4.5	2.78	3.90	0.94	1.26	<0.0001	18.1	PS
		<i>FR_F2E_3.1</i>	119.0	chr03_30900408	1.8	2.87	0.79	0.75	0.04	<0.0001	18.7	PS
		<i>EF_EMTHAC_3.1</i>	119.6	chr03_30962715	0.4	3.99	1.51	1.82	3.02	<0.0001	24.0	Ved
		<i>EF_3MTHPEE_3.1</i>	119.6	chr03_30962715	0.7	2.64	1.77	1.91	1.20	<0.0005	16.6	Ved

4	<i>EF_A2MPE_4.1</i>	4.9	chr04_1127842	0.8	3.05	1.56	0.55	1.65	<0.0005	18.9	Ved	
	<i>ER_NPA_4.1</i>	68.4	chr04_15256396	3.1	2.70	3.03	1.00	0.90	<0.005	17.7	PS	
	<i>ADR_BZALD_4.1</i>	72.4	chr04_15922403	0.9	3.74	3.95	1.71	1.54	<0.0001	23.6	PS	
	<i>KF_3PN2M_4.1</i>	75.0	chr04_16687925	1.5	2.90	2.12	0.96	1.70	<0.001	19.1	Ved	
C4	<i>EF_EMTHAC_4.1</i>	75.0	chr04_16687925	3.0	2.80	0.94	1.29	3.07	<0.005	17.5	PS	
	<i>ER_B2MME_4.1</i>	75.0	chr04_16687925	1.5	3.20	2.32	2.09	1.82	<0.005	20.5	PS	
	<i>ER_3MTHPEE_4.1</i>	75.0	chr04_16687925	3.0	2.96	2.15	2.11	1.32	<0.0005	19.2	PS	
	<i>ER_NPA_4.2</i>	86.7	chr04_20479692	1.8	2.75	1.37	1.17	1.88	<0.005	18.0	PS	
	<i>ER_A2MPE_4.1</i>	92.7	chr04_25893667	6.0	2.99	0.36	1.36	2.12	<0.005	19.3	PS	
5	C5.1	<i>ADF_BZALD_5.1</i>	0.0	chr05_182820	0.5	2.86	1.77	1.29	3.91	<0.0001	17.9	PS
		<i>ALF_BZOL_5.1</i>	1.0	chr05_182820	0.5	4.28	3.27	3.21	3.23	<0.0001	20.6	PS
		<i>EF_NPA_5.1</i>	6.5	chr05_729528	1.2	2.51	2.99	1.84	0.37	<0.005	16.9	PS
C5.2	<i>ALF_1B2M_5.1</i>	10.9	chr05_1085286	0.6	2.55	2.80	1.43	0.85	<0.005	17.0	PS	
	<i>ALR_1B2M_5.1</i>	12.5	chr05_1085286	0.6	3.39	4.16	0.50	1.46	<0.0005	21.6	PS	
	<i>ER_NPA_5.1</i>	8.9	chr05_911334	0.5	2.71	3.70	0.80	1.51	<0.0005	17.7	PS	
	C5.3	<i>ADF_NNL_5.1</i>	41.2	chr05_2516188	0.5	3.80	2.08	1.34	0.92	<0.0005	24.3	PS
		<i>ADF_NNL_5.3</i>	48.2	chr05_2846615	1.7	3.51	1.92	0.56	1.79	<0.001	24.3	PS
		<i>ADF_NNL_5.2</i>	44.6	chr05_2849664	0.0	4.11	1.95	1.32	1.29	<0.0005	25.9	PS
		<i>EF_BEE_5.1</i>	93.6	chr05_25326402	0.9	2.74	2.91	1.09	1.88	<0.001	17.2	Ved
6	C6.1	<i>ALR_301OZ_6.1</i>	21.7	chr06_2396961	1.8	2.83	2.42	2.03	1.16	<0.0001	18.7	Ved
		<i>ER_3HX1OAZ_6.1</i>	21.7	chr06_2396961	0.2	3.21	7.05	1.34	3.39	<0.0001	20.9	Ved
		<i>EF_3HX1OAZ_6.1</i>	28.0	chr06_3059995	1.3	5.10	3.31	3.03	2.74	<0.0001	29.6	Ved
C6.2	<i>ER_3HX1OAZ_6.2</i>	28.0	chr06_3059995	0.9	3.41	7.21	1.47	3.98	<0.0001	22.1	Ved	
	<i>ALR_1B2M_6.1</i>	143.3	chr06_34012274	0.8	3.29	1.34	0.82	1.52	<0.0005	21.1	Ved	
	<i>ALF_BZOL_6.1</i>	144.3	chr06_34012274	1.1	2.77	0.54	0.72	2.90	<0.005	18.4	Ved	
7	<i>EF_NPA_7.1</i>	3.0	chr07_551647	1.1	2.76	0.63	3.21	1.10	<0.005	16.5	Ved	
	<i>ER_BBE_7.1</i>	14.3	chr07_1055303	0.6	2.77	1.19	1.49	0.26	<0.005	18.1	Ved	
	<i>TF_6CYHCL_7.1</i>	56.1	chr07_18142671	18.7	2.89	0.03	2.37	2.14	<0.0005	18.0	Ved	
8	<i>ER_24HXEE_8.2</i>	102.7	chr08_29813774	25.3	2.47	3.02	2.17	2.77	<0.001	16.3	Ved	
	<i>ADR_B3M_8.1</i>	84.2	chr08_9446475	3.1	2.59	3.44	2.12	0.81	<0.0001	17.0	Ved	
	<i>EF_B2M.PE_8.2</i>	90.3	chr08_24466782	18.5	2.84	2.55	1.07	0.47	<0.0005	18.7	Ved	
	<i>ER_AOE_8.1</i>	84.2	chr08_9446475	0.8	3.24	3.18	2.52	1.59	<0.0001	20.8	Ved	
M_8.1	<i>ER_AHPE_8.1</i>	85.2	chr08_9634968	1.1	3.13	3.63	2.19	2.81	<0.0001	20.2	Ved	
	<i>ER_ABE_8.1</i>	86.2	chr08_9634968	1.1	3.81	0.42	2.70	2.73	<0.0001	24.0	Ved	
	<i>EF_B2M.PE_8.1</i>	86.5	chr08_9745430	8.2	3.91	2.80	1.54	1.09	<0.0005	24.8	Ved	
	<i>ER_B2M.PE_8.1</i>	86.5	chr08_9634968	1.1	3.36	2.74	1.39	0.48	<0.0001	21.5	Ved	
	<i>EF_3MTHPEE_8.2</i>	90.3	chr08_24466782	16.6	3.83	1.84	1.80	2.10	<0.0001	32.7	Ved	
M_8.2	<i>EF_24HXEE_8.1</i>	87.5	chr08_9941727	18.3	2.53	3.50	0.43	0.91	<0.0001	15.9	Ved	
	<i>ER_ABE_8.2</i>	90.3	chr08_24466782	18.3	3.26	1.00	1.86	2.65	<0.0001	20.9	Ved	
	<i>ALF_PHEOL_8.2</i>	91.3	chr08_24466782	18.3	4.74	3.61	1.22	3.18	<0.0001	27.8	Ved	
	<i>ER_23BOLDA_8.1</i>	87.6	chr08_9941727	20.3	2.82	2.03	3.65	2.12	<0.0001	18.3	Ved	
M_8.3	<i>TR_EUL_8.1</i>	86.2	chr08_9634968	0.5	6.70	7.53	3.52	0.12	<0.0001	38.3	Ved	
	<i>ALF_PHEOL_8.1</i>	86.5	chr08_9634968	0.5	6.71	2.86	1.65	4.32	<0.0001	37.0	Ved	
	<i>EF_HXME_8.1</i>	86.5	chr08_9634968	0.5	5.32	3.95	2.60	1.09	<0.0001	30.6	Ved	
	<i>ADR_OCL_8.1</i>	86.5	chr08_9634968	0.5	4.26	3.40	2.12	1.77	<0.0001	26.4	Ved	

	<i>ALR_OCOL_8.1</i>	86.5	chr08_9634968	0.5	4.17	4.28	3.86	1.34	<0.0001	25.9	Ved
	<i>ALR_1HXO_8.1</i>	86.5	chr08_9634968	0.5	3.87	1.37	3.33	2.57	<0.0005	24.3	Ved
	<i>EF_3MTHPEE_8.1</i>	86.5	chr08_9634968	0.8	5.76	2.57	3.20	3.61	<0.0001	32.7	Ved
	<i>ER_EMTHAC_8.1</i>	86.5	chr08_9634968	0.8	5.05	4.71	4.63	3.40	<0.0001	30.5	Ved
	<i>ER_BZAAE_8.1</i>	86.5	chr08_9634968	0.8	4.95	4.44	3.58	5.19	<0.0001	30.0	Ved
	<i>ER_B2MME_8.1</i>	86.5	chr08_9634968	0.8	4.93	3.44	3.50	4.05	<0.0001	29.9	Ved
	<i>ADR_DCL_8.1</i>	86.5	chr08_9634968	0.8	4.79	3.29	3.11	4.45	<0.0001	29.2	Ved
	<i>ER_3MTHPEE_8.1</i>	86.5	chr08_9634968	0.8	4.64	2.41	3.68	3.54	<0.0001	28.4	Ved
M_8.4	<i>BR_STYR_8.1</i>	86.5	chr08_9634968	0.8	3.73	0.15	3.50	1.90	<0.0005	23.9	Ved
	<i>ADR_BZACALD_8.1</i>	86.5	chr08_9634968	0.8	3.70	4.66	2.65	2.95	<0.0001	23.4	Ved
	<i>ADR_NNL_8.1</i>	86.5	chr08_9634968	0.8	3.68	2.78	2.58	2.75	<0.0001	23.2	Ved
	<i>ER_AHXE_8.1</i>	86.5	chr08_9634968	0.8	3.38	2.08	2.05	3.02	<0.0001	21.6	Ved
	<i>ER_A2PHEE_8.1</i>	86.5	chr08_9634968	0.8	2.60	0.74	2.44	2.75	<0.0001	17.0	Ved
	<i>ER_E3AXB_8.1</i>	87.5	chr08_9941727	0.8	4.29	2.70	2.61	3.37	<0.0001	26.6	Ved
	<i>KR_4HPA_8.1</i>	87.5	chr08_24466782	0.8	0.21	0.18	3.22	5.50	<0.0001	1.5	PS
	<i>EF_BZAAE_8.1</i>	86.5	chr08_9634968	7.8	4.31	2.70	1.60	0.88	<0.0001	25.6	Ved
	<i>ALF_1B2M_8.1</i>	87.5	chr08_9941727	12.3	4.09	3.68	2.77	1.41	<0.0001	25.8	Ved
	<i>KF_3PN2M_8.1</i>	88.6	chr08_10225136	15.0	3.03	1.33	2.45	0.85	<0.001	19.8	PS
	<i>EF_1B2MA_8.1</i>	86.5	chr08_9634968	16.3	3.68	1.91	2.46	1.98	<0.0001	22.3	Ved
	<i>ER_24HXEE_8.1</i>	86.5	chr08_9634968	16.3	3.30	3.51	2.98	4.06	<0.0005	21.1	Ved
	<i>ER_HXEE_8.1</i>	87.5	chr08_9941727	16.3	4.26	5.58	3.98	3.69	<0.0001	26.4	Ved
	<i>EF_HXBE_8.1</i>	87.5	chr08_9745430	16.3	3.07	2.50	1.37	0.77	<0.0001	20.1	Ved
M_8.5	<i>EF_BBE_8.1</i>	88.6	chr08_10225136	16.3	3.78	1.65	1.40	2.06	<0.0001	24.1	Ved
	<i>ER_B2MEE_8.1</i>	88.9	chr08_10225136	16.3	4.58	2.68	1.93	5.67	<0.0001	28.1	Ved
	<i>ALF_1HXO_8.1</i>	88.9	chr08_10225136	16.3	2.50	2.19	3.79	0.08	<0.0001	15.8	Ved
	<i>ER_BEE_8.1</i>	90.3	chr08_24466782	16.3	4.96	1.93	3.94	5.68	<0.0001	30.0	Ved
	<i>ER_PNEE_8.1</i>	90.3	chr08_24466782	16.3	4.92	4.23	3.06	2.99	<0.0001	29.8	Ved
	<i>ER_E3AXB_8.2</i>	90.3	chr08_24466782	16.3	3.28	2.37	1.36	3.10	<0.0001	21.0	Ved
	<i>ER_HXME_8.1</i>	86.5	chr08_9634968	18.0	4.03	6.51	4.57	0.04	<0.0001	25.2	Ved
	<i>EF_HXPE_8.1</i>	86.5	chr08_9745430	18.0	2.79	3.15	1.27	0.95	<0.0001	18.4	Ved
	<i>EF_HX2MBE_8.1</i>	88.6	chr08_10225136	18.0	3.56	2.29	1.63	0.91	<0.0001	22.9	Ved
M_8.6	<i>ER_HPEE_8.1</i>	90.3	chr08_24466782	18.0	3.35	2.88	2.44	2.38	<0.0001	21.4	Ved
	<i>BR_STYR_8.2</i>	90.3	chr08_24466782	18.0	2.85	1.02	2.97	1.16	<0.001	18.8	Ved
	<i>ER_OEE_8.1</i>	90.3	chr08_24466782	18.0	2.64	1.68	1.62	2.91	<0.0001	17.3	Ved
	<i>ER_B2MME_8.2</i>	91.3	chr08_25723466	18.0	4.25	3.50	2.24	4.00	<0.0001	26.3	Ved
	<i>EF_HPEE_8.1</i>	88.9	chr08_10225136	20.0	4.02	3.40	1.58	1.68	<0.0001	25.4	Ved
M_8.7	<i>EF_HX2MPE_8.1</i>	90.3	chr08_24466782	20.0	2.88	1.82	1.11	1.15	<0.0001	19.0	Ved
	<i>ALR_OCOL_8.2</i>	90.3	chr08_24466782	20.0	4.53	3.19	0.08	<0.0001	9.8	Ved	
	<i>EF_DCEE_8.1</i>	93.5	chr08_27430936	20.0	3.09	1.22	2.19	1.20	<0.0001	19.1	Ved
	<i>EF_HPEE_8.2</i>	103.0	chr08_29813774	22.4	3.31	3.40	2.29	1.60	<0.0001	21.5	Ved
M_8.8	<i>ALR_OCOL_8.3</i>	103.0	chr08_29813774	22.4	1.26	4.19	2.82	0.03	<0.0001	8.7	Ved
	<i>ER_PEE_8.1</i>	90.3	chr08_24466782	16.1	4.02	1.68	2.23	3.18	<0.0001	25.1	Ved
M_8.9	<i>EF_B2MEE_8.1</i>	90.3	chr08_24466782	16.1	3.96	1.87	1.81	1.31	<0.0001	23.8	Ved

M_8.10	ER_EAC_8.1	90.3	chr08_24466782	17.8	3.89	2.04	1.87	4.22	<0.0005	24.4	Ved	
	EF_E3AXB_8.2	93.5	chr08_27430936	19.7	4.23	3.38	2.31	1.84	<0.0001	26.6	Ved	
	EF_E3AXB_8.1	90.3	chr08_24466782	15.8	4.93	2.40	1.47	1.99	<0.0001	30.3	Ved	
	EF_1B2MA_8.2	90.3	chr08_24466782	15.8	4.07	2.24	2.87	1.63	<0.0001	24.4	Ved	
	EF_HXEE_8.1	96.6	chr08_27868043	19.5	5.39	3.55	4.01	2.89	<0.0001	31.0	Ved	
M_8.11	EF_B2MPE_8.1	88.9	chr08_17287431	19.9	2.84	1.44	1.87	0.95	<0.005	18.7	Ved	
	BF_BZ13DM_8.1	96.6	chr08_27868043	19.9	2.97	2.02	1.60	2.33	<0.0005	18.5	PS	
	EF_PNEE_8.1	97.6	chr08_27868043	21.4	4.90	3.94	3.28	2.94	<0.0001	28.6	Ved	
	EF_BEE_8.1	91.3	chr08_24466782	22.5	3.06	1.21	2.38	1.79	<0.0005	19.0	Ved	
	ADR_NNL_8.2	91.7	chr08_25723466	12.1	2.53	3.24	1.99	1.73	<0.001	16.6	Ved	
M_8.12	ER_A2MPE_8.1	100.7	chr08_29419309	8.0	2.90	1.90	1.30	1.19	<0.0005	18.8	Ved	
	EF_HXME_8.2	95.6	chr08_27868043	5.0	5.12	4.97	2.38	1.40	<0.0001	29.7	Ved	
	ADR_BZACALD_8.2	91.7	chr08_25723466	5.3	2.65	3.98	2.07	3.23	<0.0001	17.3	Ved	
	EF_BZAAE_8.2	95.6	chr08_27868043	3.7	5.03	5.53	0.73	1.70	<0.0001	29.3	Ved	
	ER_HXME_8.2	96.6	chr08_27868043	3.7	2.87	7.77	1.02	0.01	<0.0001	18.7	Ved	
M_8.13	ADF_OCL_8.1	102.7	chr08_29419309	6.2	3.13	3.63	2.56	1.10	<0.0001	20.4	Ved	
	ER_ABE_8.3	100.7	chr08_29419309	2.4	3.77				<0.0001	23.8	Ved	
	ER_AHXE_8.2	99.6	chr08_29419309	1.9	2.77	3.56	1.77	1.23	<0.0001	18.1	Ved	
	ADR_BZACALD_8.3	100.7	chr08_29419309	1.9	4.77	6.68	2.41	4.27	<0.0001	29.1	Ved	
	EF_1B2MA_8.3	100.7	chr08_29419309	1.9	3.70	3.38	2.66	1.23	<0.0001	22.4	Ved	
M_8.14	TR_EUL_8.2	100.7	chr08_29419309	1.9	3.31	4.71	1.51	0.00	<0.0005	21.2	Ved	
	EF_AHXE_8.1	100.7	chr08_29419309	1.9	3.12	2.10	1.95	0.88	<0.0001	19.3	Ved	
	EF_B2M.PE_8.3	100.7	chr08_29419309	1.9	3.01	3.16	1.25	1.25	<0.005	19.7	Ved	
	EF_APNE_8.1	100.7	chr08_29419309	1.9	2.99	2.63	2.02	1.06	<0.0001	18.6	Ved	
	ER_PNEE_8.2	102.7	chr08_29813774	4.0	4.14	4.50	2.70	2.09	<0.0001	25.8	Ved	
M_8.15	ER_HXEE_8.2	102.7	chr08_29813774	4.0	3.66	6.04	2.76	2.36	<0.0001	23.2	Ved	
	ER_HPEE_8.2	102.7	chr08_29813774	4.0	3.30	2.34	2.76	1.90	<0.0001	21.1	Ved	
	ER_BZAAE_8.2	103.0	chr08_29813774	4.0	3.93	5.90	2.72	3.10	<0.0001	24.7	Ved	
	EF_E3AXB_8.3	103.0	chr08_29813774	2.5	3.46	2.54	0.85	4.13	<0.0005	22.3	Ved	
	EF_BEE_8.2	104.5	chr08_31341018	2.5	4.26	0.86	5.01	3.73	<0.0005	25.4	Ved	
9	EF_BZAAE_8.3	113.8	chr08_32190395	0.4	3.90	2.44	0.96	1.33	<0.0005	23.5	Ved	
	EF_BEE_8.3	114.3	chr08_32190395	0.4	4.50	1.66	4.24	3.90	<0.0001	26.6	Ved	
	ER_EAC_9.1	52.9	chr09_20327236	1.7	2.56	3.19	0.49	1.17	<0.001	16.8	PS	
	ER_BEE_9.1	64.3	chr09_21685526	2.3	2.75	1.74	1.02	1.31	<0.005	18.0	PS	
	TF_CGERAC_9.1	65.3	chr09_21754707	0.6	4.54	5.81	2.72	3.27	<0.0001	26.8	Ved	
C9	TF_6CYHCL_9.1	62.6	chr09_21387823	0.7	2.83	0.91	2.00	2.65	<0.0001	17.7	Ved	
	KF_5HP2O_9.1	64.3	chr09_21685526	0.5	8.36	11.65	6.69	7.68	<0.0001	45.7	Ved	
10	ER_PEE_10.1	9.1	chr10_1133000	1.3	2.52	3.17	0.20	1.19	<0.005	16.6	PS	
	ADF_HXL_10.1	31.0	chr10_3152004	2.4	3.07	0.61	0.17	2.51	<0.001	20.1	Ved	
11	BF_BZ13DM_11.1	3.9	chr11_7689143	11.1	4.82	0.44	3.17	2.86	<0.0001	28.2	PS	

		<i>ER_B2MME_11.1</i>	6.5	chr11_10453734	13.1	2.83	2.29	0.91	1.65	<0.0005	18.4	Ved
		<i>ER_BEE_11.1</i>	5.9	chr11_10453734	11.5	<u>3.54</u>	1.52	1.66	2.11	<0.0001	22.5	Ved
		<i>EF_HXEE_11.1</i>	5.9	chr11_10453734	11.5	2.72	1.21	<u>3.96</u>	1.32	<0.0005	17.0	Ved
		<i>ER_PEE_11.1</i>	6.5	chr11_10453734	11.2	<u>3.52</u>	2.47	1.91	1.25	<0.0005	22.4	Ved
		<i>ER_EAC_11.1</i>	6.5	chr11_10453734	11.2	2.83	1.51	1.49	1.08	<0.0005	18.4	Ved
	C11.2	<i>EF_APNE_11.1</i>	88.3	chr11_32731899	1.2	4.21	1.07	2.06	<u>3.03</u>	<0.0001	25.1	PS
		<i>EF_AHXE_11.1</i>	88.3	chr11_32731899	1.2	2.75	0.27	1.83	<u>2.83</u>	<0.005	17.2	PS
		<i>EF_1B2MA_11.1</i>	88.3	chr11_32731899	1.3	2.71	0.98	1.93	2.54	<0.005	17.0	PS
		<i>ER_BPE_11.1</i>	88.5	chr11_32755551	1.3	4.31	1.76	1.38	<u>3.82</u>	<0.0001	26.7	PS
		<i>ER_AHXE_11.1</i>	88.5	chr11_32755551	1.3	4.30	1.76	1.60	<u>3.34</u>	<0.0005	26.6	PS
		<i>ER_ABE_11.1</i>	88.5	chr11_32755551	1.3	<u>3.97</u>	1.17	1.29	2.68	<0.0005	24.9	PS
		<i>ER_1B2MA_11.1</i>	88.5	chr11_32755551	1.6	3.47	2.02	1.56	1.81	<0.001	22.1	PS
		<i>ER_12PLDA_11.1</i>	88.5	chr11_32755551	1.9	<u>3.21</u>	2.74	0.35	1.14	<0.0001	20.6	PS
		<i>ER_2B1O3MA_11.1</i>	88.5	chr11_32755551	1.9	<u>3.05</u>	0.01	1.34	1.72	<0.005	19.7	PS
		<i>ER_3HX1OAZ_11.1</i>	90.5	chr11_32928874	2.0	2.55	1.51	0.98	<u>3.72</u>	<0.005	17.0	PS
		<i>ER_APNE_11.1</i>	88.5	chr11_32755551	0.6	4.30	1.59	1.92	<u>3.00</u>	<0.001	26.6	PS
		<i>ER_BBE_11.1</i>	88.5	chr11_32755551	1.1	2.73	0.31	0.69	<u>3.43</u>	<0.0005	17.9	PS
	12	<i>EF_12PLDA_11.1</i>	91.5	chr11_32928874	1.2	2.72	<u>3.30</u>	0.39	0.30	<0.0005	18.0	PS
		<i>EF_3HX1OAZ_11.1</i>	107.8	chr11_34107169	0.6	<u>3.51</u>	1.53	1.82	2.39	<0.0001	21.4	PS

257 ^aC = cluster, M = merged

258 ^b Physical positions and distances refer to the v3.6.1 melon reference genome

259 ^c LOD ≥2.50 are in bold, LOD ≥2.75 are underlined

260 The highest LOD values were found in clusters on Chr06, Chr08 and Chr09. On Chr06, we
 261 detected a stable QTL cluster (C6.1) with a LOD score of 7.21 related to lipid-derived VOCs,
 262 with three QTLs for 3-hexen-1-ol acetate (Z) at 19-32 cM in both flesh and rind, and one QTL
 263 for 3-octen-1-ol (Z) for rind. The narrowest QTL interval included 26 annotated genes and the
 264 positive additive effect of C6.1 was provided by the VED allele. The largest cluster was found
 265 on Chr08, formed by 92 QTLs overlapping between 71-121 cM. Many of these QTL were stable
 266 since they were detected in all subsets. We could identify 3 recurrent maximum LOD peaks,
 267 which were located around 86, 91 or 100 cM. These QTLs were merged (M) in black rectangles
 268 to help visualization (Fig. 2 and Table 2). The length of these QTLs ranged 0.41-25 Mb. The

269 positive effect was provided by the VED allele for all significant VOCs, except for 4-heptanone,
270 2-methyl-3-pentanone and 1,3-dimethylbenzene. QTLs in this cluster explained 15-30 % of the
271 variation. Another QTL cluster (C9) was found on Chr09 in flesh tissue for two terpenes and a
272 ketone derived from the carotenoid cleavage pathway. The cluster shares an interval of 0.51
273 Mb at 62-69 cM, a region that contains 73 annotated genes. The highest LOD value was 11.65
274 for 6-methyl-5-hepten-2-one and explained 45 % of the variance.

275 Other minor QTLs for compounds relevant for melon flavour were found on Chr02, Chr03,
276 Chr05 and Chr11. On Chr02, we found a cluster of 5 QTLs (C2) with a LOD peak of 3.08 between
277 61,5-67,5 cM. C2 included QTLs for isoleucine-derived esters, the PS allele contributing to
278 higher content of these VOCs. Another cluster (C3) was found at the end of Chr03, containing
279 two methionine-derived sulfur esters and overlapping in 0.39 Mb (containing 59 genes), with
280 a positive effect of the VED allele. The ethyl (methylthio)acetate QTL had a LOD above 3.00 in
281 one of the subsets, whereas the QTL for ethyl 3-(methylthio)propanoate was significant only
282 using K-W test. On Chr05, a cluster (C5.1) containing 109 genes was detected for benzaldehyde
283 and benzyl alcohol, two phenylalanine derived VOCs. The benzyl alcohol QTL was detected in
284 all subsets. Two more clusters were found on chromosome 5 (C5.2 and C5.3) for 1-butanol-2-
285 methyl and nonanal. In all these QTLs the positive additive effect was provided by the PS allele.
286 Two ester clusters were localized on Chr11, C11.1 and C11.2, containing predominantly ethyl
287 esters and acetates, respectively. The first cluster, with a positive effect of the VED allele, was
288 located between 0-11 cM and had a LOD peak of 3.54. The second cluster, with a positive
289 additive effect of the PS allele, was detected at 81-100 cM and had a LOD peak of 4.31.

290 **3.3. VOCs and ripening**

291 The RIL population segregates for climacteric ripening, as expected since the two parental
292 lines have opposite ripening behavior (Supplementary Table S2). The PS x VED RILs were
293 classified in a previous work as climacteric, non-climacteric and “unstable-climacteric”, the
294 last for those RILs that were climacteric only in some biological replicates (Pereira et al., 2020).
295 We first compared the three groups by doing a PCA using the mean subset (Fig. 3). Non-
296 climacteric RILs were grouped apart from the climacteric lines and showed less diversity, in
297 both rind and flesh. Climacteric RILs, which produced a more diverse profile and a higher
298 quantity of VOCs, presented higher dispersion. “Unstable-climacteric” RILs were distributed
299 within the two other groups. The same pattern was observed when each individual replicate
300 was analyzed (Supplementary Fig. S2). In rind tissue, the metabolites that represented the
301 largest positive and negative effects for each principal component were 2-ethylfuran and
302 hexyl acetate, respectively, in PC1 (X-axis), and ethyl octanoate and β -caryophyllene in PC2 (Y-
303 axis). In flesh, PC1 was determined principally by 2-methylbutyl acetate and 2-methyl-3-
304 pentanone, while PC2 was determined by 1-octen-3-ol and 2-methylpropyl butanoate.
305 The relative amount of each chemical group was calculated and compared between
306 climacteric and non-climacteric RILs for each subset (Fig. 4). Due to the dispersion of
307 “unstable-climacteric” lines in volatile content, they were excluded from the analysis. In
308 climacteric lines, esters were predominant (45-80% rind, 75-90% flesh), followed by aldehydes
309 (20-55% rind, 5-15% flesh). Non-climacteric lines had a more variable phenotype depending
310 on the tissue. In rind, aldehydes were the main group (50-95%), followed by esters (5-45%),
311 while in flesh both esters and aldehydes showed high and variable contents ranging from 20-
312 90% for esters and 5-60% for aldehydes. Therefore, an enrichment of esters was observed in
313 the flesh of non-climacteric RILs compared to rind tissue. Alcohols and the rest of the chemical
314 groups showed similar mass percentages in climacteric and non-climacteric RILs.

315

316 **4. Discussion**

317 Aroma is one of the main determinants of melon flavor. Insights into the genetic control of
318 VOC biosynthesis and regulation would facilitate its incorporation in breeding programs,
319 leading to varieties with superior flavor and better consumer's acceptance. The composition
320 of rind aroma has been neglected in some studies, despite its relevance for fruit quality and
321 acceptability and divergent VOC profiling. In this study, we used PS and VED which are both
322 elite commercial varieties with good flavor but differ in ripening behavior, fruit color and
323 volatile profile, among other fruit traits.

324 **4.1. Volatile profile**

325 From the 82 VOCs detected, three had not been previously identified in melon, to our
326 knowledge (Table 1). The number of VOCs found was in agreement with other GC-MS
327 experiments in melon that used SPME fibers (Shi et al., 2020). We found compounds that had
328 been previously described as key compounds for flavor in muskmelons and honeydews. For
329 instance, 6-nonenal (Z) and nonanal, which give melon-like and citrus notes, and ethyl acetate,
330 which evokes butterscotch (Kemp, Knavel, & Stoltz, 1972; Lignou, Parker, Oruna-Concha, &
331 Mottram, 2013; Wyllie, Leach, Wang, & Shewfelt, 1995). Other compounds like 2-methylbutyl
332 acetate, methyl 2-methylbutanoate, ethyl 2-methylpropanoate, hexyl acetate, ethyl
333 butanoate and ethyl hexanoate, which were more abundant in VED melons, have been
334 reported as important fruity, floral and sweet compounds (Lignou et al., 2013; Pang et al.,
335 2012; Wyllie et al., 1995). Another key compound found in the population was 2,6-nonadienal
336 (*E,Z*), which gives cucumber-like notes and is the main compound in honeydews (Perry, Wang,

337 & Lin, 2009). All the cited key VOCs were detected above its reported odor threshold,
338 suggesting that they play an important role in PS and VED final flavor.

339 Rind accumulated higher levels of VOCs, probably due to the lesser water content as
340 compared to flesh, and, remarkably, presented less variability than flesh tissues
341 (Supplementary Fig. S1 and Supplementary Table S1-2). The higher production of VOCs in VED
342 as compared to PS correlates with a more intense smell. Fruity aroma of VED melons comes
343 mainly from esters in both rind and flesh, in agreement with several studies that described
344 Cantaloupe aroma (Esteras, Rambla, Sánchez, Granell, & Picó, 2020; Esteras et al., 2018;
345 Obando-Ulloa et al., 2008). Conversely, the fresh aroma of PS comes from a high accumulation
346 of aldehydes, also shown in previous studies (Esteras et al., 2020, 2018; Obando-Ulloa et al.,
347 2008). Hyb melons also displayed many volatile compounds and had an intense fruity aroma,
348 likely conferred by high amounts of esters. The transgressive segregation showed for
349 heptanal, methyl hexanoate and decanal in Hyb melons exposed the effect of heterosis. The
350 volatile profile of the RILs was very diverse, although most lines were more similar to VED than
351 to PS, as the Hyb. Therefore, some VED alleles probably have a dominant effect on the final
352 volatile profile. A similar pattern was observed for climacteric ripening, which determines to
353 a high extent the volatile profile (Pereira et al., 2020).

354 Ethyl esters, acetates, and compounds coming from the same biosynthetic pathway were
355 positively correlated (Fig. 1). The ethyl ester correlation cluster could be explained by the
356 activity of alcohol acyltransferases able to transform several substrates (El-Sharkawy et al.,
357 2005; Yahyaoui et al., 2002). Another ester cluster including several alcohols could be related
358 to ethylene, as both alcohol dehydrogenases and acyltransferases are associated with
359 ethylene production (Manríquez et al., 2006; Shalit et al., 2001). The rest of correlation

360 clusters also grouped compounds from the same chemical group or the same biochemical
361 origin, being probably led by the same key enzymes. For instance, hexanoate or nonanal
362 derivatives are lipid derived VOCs (Schwab et al., 2008), β -caryophyllene and δ -cadinene are
363 terpenoids, and 2-methylbutanal or benzene derivatives belong to isoleucine and
364 phenylalanine pathways, respectively, through the *CmBCAT1* and *CmArAT1* enzymes (Gonda
365 et al., 2010). The inverse correlation of furans and certain benzenoids observed in PS, with
366 most esters abundant in VED, could be explained by the different ripening behavior of both
367 accessions.

368 **4.2. QTL mapping**

369 We found a similar number of QTLs (166) and segregating compounds (63) (Table 2 and Fig.
370 2) as Galpaz *et al.* (2018), which also used VOC profiling in a RIL population for QTL analysis
371 (FDR<10 %, 145 QTLs for 58 different compounds in 99 RILs). The median length of the QTLs
372 (9.2 cM and 1.96 Mb) is comparable with the 9.42 cM and 0.94 Mb obtained by Pereira *et al.*
373 (2018) using the same population for other traits. The maximum percentage of variance
374 explained by a QTL in our study was 45.7 %, near to a monogenic inheritance, but the majority
375 ranged from 15-25 %, which suggests a complex inheritance in VOCs synthesis. Similar values
376 have been reported in melon (Galpaz et al., 2018). Multiple QTLs on different chromosomes
377 controlled the amount of certain compounds, also indicating multigenic inheritance.

378 An important environmental effect was observed in the volatile composition of RILs, as 72
379 QTLs were found only in one year. Despite the environmental effect, 30.12 % of the QTLs were
380 detected in a minimum of two subsets. When using the mean subset, which should be more
381 robust, we found consistent results for the QTLs detected in more than one subset. We

382 decided to focus on the most reliable QTLs detected at least in two subsets and in those
383 forming clusters.

384 The detection of clusters of QTLs could be due to a common regulation, a common step in a
385 biochemical pathway or an enzyme capable of transforming several substrates, as had been
386 previously reported in melon (El-Sharkawy et al., 2005; Gonda et al., 2010). For instance,
387 several isoleucine derived VOCs were controlled by the QTL cluster C2 on Chr02. The length
388 of the confidence interval determined for each cluster was variable, ranging from 0.39 Mb to
389 22.44 Mb. Some of them contained 100 genes or less, such as the sulfur esters cluster on
390 Chr03 and the benzaldehyde and benzoic acid cluster on Chr05.

391 **4.2.1. Branched-chain amino acid aminotransferases as first-step enzymes in the volatile
392 biosynthesis**

393 The sulfur compounds ethyl(methylthio)acetate and ethyl 3-(methylthio)propanoate are
394 important odorants giving grassy and earthy aromas (Wyllie et al., 1994). A QTL cluster
395 modulating the production of these compounds was detected at the end of Chr03 partially
396 overlapping with a 2-ethylfuran QTL. This cluster includes the previously described *CmBCAT1*
397 gene (*MELO3C010776*) at 20-40 Kb from the maximum LOD peak. The aminotransferase
398 *CmBCAT1* is involved in the first step of the L-leucine, L-isoleucine and L-valine catabolic
399 pathways when expressed *in vitro* (Gonda et al., 2010). Previous studies in *Arabidopsis* have
400 shown that *BCAT4*, a homologue of *CmBCAT1*, participates in the methionine elongation
401 pathway (Schuster, Knill, Reichelt, Gershenzon, & Binder, 2006). Therefore, the melon enzyme
402 may participate in the deamination of L-methionine to form the precursor of ethyl 3-
403 (methylthio)propanoate. According to transcriptomic data from fruitENCODE
404 (<http://www.epigenome.cuhk.edu.hk/encode.html>), *MELO3C010776* is highly expressed in

405 VED flesh compared to PS. The two sulfur compounds were only detected in VED, and
406 interestingly, correlate with the ethylene production. Apart from *MELO3C010776*, an
407 ethylene-responsive transcription factor and an ACC synthase are present in this interval and
408 could also have an effect for this trait. In addition, this region overlaps with an aroma QTL
409 described by Pereira *et al.* (2020), which could be related to the *CmBCAT1* activity.

410 A *BCAT-like* gene (*MELO3C003454*) was found in the *EF_A2MPE_4.1* interval on Chr04. 2-
411 Methylpropyl acetate is an important odorant described by (Pang et al., 2012) and having a
412 floral aroma. In the presence of valine, higher quantities of 2-methylpropyl acetate were
413 produced (Gonda et al., 2010). This gene could have a similar activity to *CmBCAT1*. A BLAST
414 search revealed that *MELO3C003454* is similar to predicted chloroplastic D-aminoacid
415 aminotransferases in peach, strawberry and apple. Thus, a hypothetical activity of this enzyme
416 could be the deamination of D-valine into the precursor of 2-methylpropanal. The gene
417 underlying 2-methylbutanal derivatives' QTL cluster on Chr02 could also be an
418 aminotransferase, but no annotated BCAT-encoding gene has been found within this interval.

419 **4.2.2. Aldehyde oxidases and dehydrogenases**

420 Benzaldehyde and benzyl alcohol are also important odorants in melon, having almond and
421 floral-fruity aroma, respectively (Jordán, Shaw, & Goodner, 2001; Pang et al., 2012). These
422 compounds were controlled by a QTL on Chr05 in flesh, which includes three aldehyde
423 oxidases annotated less than 30 Kb downstream the maximum LOD peak. Although
424 benzaldehyde is produced at high quantities in rind, these enzymes could also be important
425 for flesh aroma. A BLAST analysis and a search for orthologues in PLAZA 4.0 (Van Bel et al.,
426 2018) revealed that *MELO3C014719* is an indole-3-acetaldehyde oxidase-like and is similar to
427 the *A. thaliana* AAO4 protein, which was reported to transform benzaldehyde into benzoic

428 acid in siliques (Ibdah, Chen, Wilkerson, & Pichersky, 2009). The transcriptomic data from
429 fruitENCODE supports this idea, as *MELO3C014719* is more expressed in VED flesh than in PS.
430 Both functional annotation and transcriptomic data point out *MELO3C014719* as a likely
431 candidate gene.

432 We identified another candidate gene within the 2-methylbutanol QTL interval, also on Chr05.
433 Although 2-methylbutanol is not a potent odorant, it is the precursor of 2-methylbutyl
434 acetate, described by Wyllie *et al.* (1995) and Lignou *et al.* (2013) as a key compound in melon
435 flavor giving banana and pear notes. *MELO3C014601*, an aldehyde dehydrogenase, could
436 catalyze the dehydrogenation of 2-methylbutanal to 2-methylbutanoate, and therefore
437 limiting the synthesis of the corresponding alcohol. However, the amount of alcohols and
438 acids also depends on the activity of alcohol acyltransferases (El-Sharkawy *et al.*, 2005).
439 Functional validation would be needed to confirm this candidate gene.

440 **4.2.3. Alcohol acyltransferases in ester formation**

441 Ester synthesis is known to be driven by alcohol acyltransferases. The characterization of
442 AAT1, AAT2, AAT3 and AAT4 showed their ability to transform a great variety of substrates (El-
443 Sharkawy *et al.*, 2005; Yahyaoui *et al.*, 2002). These enzymes were described as key steps in
444 the unique aroma of melon (Liu *et al.*, 2020). AAT1, AAT2 and AAT3 are located within the first
445 QTL cluster on Chr11 at 0-11.7 cM (C11.1). AAT1 is the most active enzyme and prefers C6
446 substrates, AAT3 prefers benzyl alcohol and acetate as substrates and AAT2 is inactive in VED
447 (El-Sharkawy *et al.*, 2005; Yahyaoui *et al.*, 2002). Interestingly, ethyl acetate and ethyl
448 propanoate were tested but not detected as products of these three enzymes (El-Sharkawy *et*
449 *al.*, 2005). Other AATs in the interval such as *MELO3C024764* and *MELO3C024769* may be
450 involved in the synthesis of these compounds.

451 Within the QTL cluster on Chr11 at 81-100 cM (C11.2), some metabolic enzymes and
452 transcription factors could be involved in the synthesis of esters, but no clear candidate gene
453 could be identified. Due to the interest of ester compounds, a fine mapping of this region will
454 be performed to help identifying possible candidates within the 234 genes contained in the
455 cluster (Table 2).

456 **4.2.4. Fatty-acid pathway**

457 Fatty-acid-derived VOCs such as 2,6-nonadienal and 6-nonenal (Z), which give honeydew and
458 cucumber notes, form part of the key VOCs identified in melon (Pang et al., 2012).
459 Lipoxygenases increase their expression at the final stages of fruit ripening. These enzymes
460 can also be enhanced by the addition of fatty acid precursors (Tang et al., 2015). On Chr05,
461 *EF_BEE_5.1*, a QTL for ethyl butanoate, another relevant odorant with ripe melon aroma
462 (Lignou et al., 2013), contains a cluster of 10 lipoxygenases and a γ -aminobutyrate
463 transaminase that could be involved in the formation of the butanoate backbone. In the
464 nonanal cluster C5.3, we identified 2 lipid-transfer proteins that could be involved in the first
465 steps of the synthesis. On Chr06, the QTL cluster C6.1 for *ER/F_3HX1OAZ_6.1/2* and
466 *ALR_3O1OZ_6.1* contains β -oxidation-related genes such as a 3-ketoacyl-CoA thiolase and a
467 synthase (Goepfert & Poirier, 2007). These candidate genes have also been found within
468 *ER_BBE_7.1* interval however its activity would need to be validated.

469 **4.2.5. Carotenoid derivatives**

470 Some terpenoids such as β -cyclocitral and 6-methyl-5-hepten-2-one are important odorants
471 in melon, giving tropical-like and peculiar aromas (Gonda et al., 2016; Pang et al., 2012). Their
472 biosynthesis starts from isopentenyl diphosphate (IPP) or dimethyl allyl diphosphate
473 (DMAPP), which can form chains to create the precursors of mono-, sesquiterpenes and

474 carotenoids. Two terpene synthases in 'Dulce' and 'Noy Yizre'el' accessions have been already
475 described in melon (Portnoy et al., 2008). Carotenoid cleavage dioxygenases such as CmCCD1
476 can cleave larger terpenes to generate VOCs such as geranylacetone, 6-methyl-5-hepten-2-
477 one and ionone (Ibdah et al., 2006; Vogel, Tan, McCarty, & Klee, 2008). In our study, we found
478 a QTL cluster with the highest LOD value for 6-methyl-5-hepten-2-one, and two other QTLs for
479 geranylacetone and β -cyclohomocitral. All of them are apocarotenoids, and the positive
480 additive effect is given by the VED allele. This QTL cluster is located on Chr09, and within its
481 interval we found the *CmOr* gene, *MELO3C005449*, responsible for the orange flesh color
482 (Tzuri et al., 2015). *CmOr* segregates in the RIL population, and we observed that only orange
483 fleshed lines (Pereira et al., 2018) accumulated apocarotenoids. Thus, *CmOr* is likely the
484 underlying gene for the apocarotenoid QTL cluster. This correlation between orange flesh
485 color and the presence of apocarotenoids was also seen in previous studies (Esteras et al.,
486 2018; Ibdah et al., 2006). A new QTL mapping including only the orange fleshed RILs did not
487 reveal any new QTLs for those VOCs.

488 **4.2.6. Aroma as an ethylene-dependent trait**

489 The association between aroma profile and climacteric ripening in melon has been largely
490 studied (Flores et al., 2002; Li et al., 2016; Obando-Ulloa et al., 2008; Pereira et al., 2020), since
491 both the aroma and other traits associated to ripening have a major influence on fruit quality.
492 The ethylene peak characteristic of climacteric ripening promotes ester and alcohol formation
493 (El-Sharkawy et al., 2005; Li et al., 2016; Manríquez et al., 2006). Indeed, the presence of
494 aroma has previously been used as a climacteric trait (Pereira et al., 2020).
495 As previously reported by Obando-Ulloa et al. (2008) and Esteras et al. (2018), we observed a
496 predominance of esters in climacteric lines and aldehydes in non-climacteric lines (Fig. 4).

497 Moreover, esters were the principal components leading to the climacteric profile in the PCA
498 (Fig. 3). The high diversity in the VOC profile of climacteric lines had also been seen when
499 comparing climacteric and non-climacteric accessions (Esteras et al., 2018; Moing et al., 2020).
500 We have also noticed that the balance between esters and aldehydes remains constant,
501 summing up more than 90 % of the total amount of VOCs (Supplementary Table S2). This
502 balance could be explained by the ethylene-dependent nature of alcohol dehydrogenases and
503 alcohol acyltransferases (El-Sharkawy et al., 2005; Manríquez et al., 2006). Shi *et al.* (2020)
504 classified 39 melon cultivars according to their aldehyde/ester ratio and observed that several
505 cultivars had an intermediate phenotype. The evaluation of the aroma production in our RIL
506 population provides nuances to the previous classification between climacteric and non-
507 climacteric behavior, since some RILs classified as climacteric produce high levels of aldehydes
508 and others classified as non-climacteric produce abundant esters (Supplementary Table S2).
509 In addition, ester abundance was increased by PS alleles in Chr02 and Chr11. Esteras *et al.*
510 (2018) also detected a climacteric-like profile, linked to esters, in some *inodorus* accessions.
511 In fact, climacteric ripening is not an absolute trait, as it has been seen in the RIL population
512 showing different degrees of ethylene production (Pereira et al., 2020).
513 Many of our QTL intervals contain ethylene-related transcription factors that could play a role
514 in the production of VOCs. For instance, the cluster on Chr02 overlaps with ripening-related
515 QTLs that were previously described, such as QTLs for abscission layer formation, firmness,
516 ethylene production and earliness of aroma (Pereira et al., 2020). QTLs on chromosomes 3, 5,
517 6, 7, 8, 10 and 11 also overlap with harvest, fruit abscission, width of ethylene peak,
518 chlorophyll degradation and aroma QTLs (Galpaz et al., 2018; Obando-Ulloa et al., 2010;
519 Pereira et al., 2020). Specifically, 92 QTLs for very diverse compounds converged into the QTL
520 cluster on Chr08. These QTLs had high LOD scores and reproducibility in both 2015 and 2016

521 samples. In this region, *ETHQV8.1* has been recently described as a major regulator of
522 climacteric ripening in melon (Pereira et al., 2020). Since 66 % of the Chr08 QTLs described in
523 this work overlap with *ETHQV8.1*, including esters, aliphatic alcohols and phenylalanine
524 derivatives, these compounds are most likely ethylene dependent. The region that does not
525 contain *ETHQV8.1*, from 89-120 cM, included QTLs for ethyl esters and aldehydes. This region
526 overlaps with many benzenoid, terpenoid and sulfur compounds QTLs identified in Galpaz et
527 al. (2018), together with octanal and 3,6-nonadienal-(Z,Z) QTLs in Obando-Ulloa et al. (2010).
528 Our results confirm that Chr08 is playing an important role in aroma production in our
529 population.

530 **5. Conclusions**

531 Our work was focused on the genetics behind the biosynthesis of VOCs in melon to shed light
532 to the complex metabolic pathways that affect its flavor. We evaluated two melon tissues at
533 ripen stage, rind and flesh, since both affect consumer preferences in the market, but rind
534 VOCs profile is still poorly known. In this study, we described the aroma profile in a PS x VED
535 RIL population and its relationship with ripening behavior, mapping 166 QTLs to the melon
536 genome. A QTL cluster on Chr08 was the most important contributor to volatile biosynthesis,
537 mainly ethylene-related and overlapping with the major ripening QTL *ETHQV8.1* (Pereira et
538 al., 2020). Moreover, QTL clusters identified in Ch02, Chr05, Chr06 and Chr11 contributed to
539 decipher different VOCs synthesis pathways. These findings provide the ground for further
540 fine-mapping projects and the functional validation of the candidate genes proposed related
541 to aroma formation in melon.

542

543 **CRediT authorship contribution statement**

544 **Carlos Mayobre**: Methodology, Formal analysis, Writing - Original Draft. **Lara Pereira**:
545 Methodology, Writing – Review & Editing. **Abdelali Eltahiri**: Methodology, Formal analysis.
546 **Einat Bar**: Methodology. **Efraim Lewinsohn**: Writing – Review & Editing. **Jordi Garcia-Mas**:
547 Conceptualization, Writing – Review & Editing, Supervision, Funding Acquisition. **Marta Pujol**:
548 Conceptualization, Writing – Review & Editing, Supervision.

549 **Declaration of Competing Interest**

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

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563

564 **Appendix A. Supplementary Data**

565 **Supplementary Fig. S1.** Boxplot showing the amount of VOCs according to their chemical
566 group in rind (A) and flesh (B) samples of the parental lines (PS, VED) and the F1 (Hyb).

567 **Supplementary Fig. S2.** Principal component analysis (PCA) of the PS x VED RIL population
568 volatile profile. The upper part represents rind PCAs for T3 (A), T4 (B) and T5 (C) subsets; the
569 lower part represents flesh PCAs for T3 (D), T4 (E) and T5 (F) subsets. Different ellipses group
570 climacteric lines (C), non-climacteric lines (NC) and “unstable-climacteric” lines (UC).

571 **Supplementary Table S1.** Average content of volatile compounds detected in the parental
572 lines (PS, VED), the Hyb and in the RIL population for rind and flesh tissues.

573 **Supplementary Table S2.** Total VOC content according to the chemical classification and
574 ripening classification of RILs.

575 **Supplementary Table S3.** VOC content in ng g-1 frozen tissue of each VOC in rind.

576 **Supplementary Table S4.** VOC content in ng g-1 frozen tissue of each VOC in flesh.

577 **Supplementary Table S5.** Positions of the QTLs detected in the PS x VED RIL population.

578

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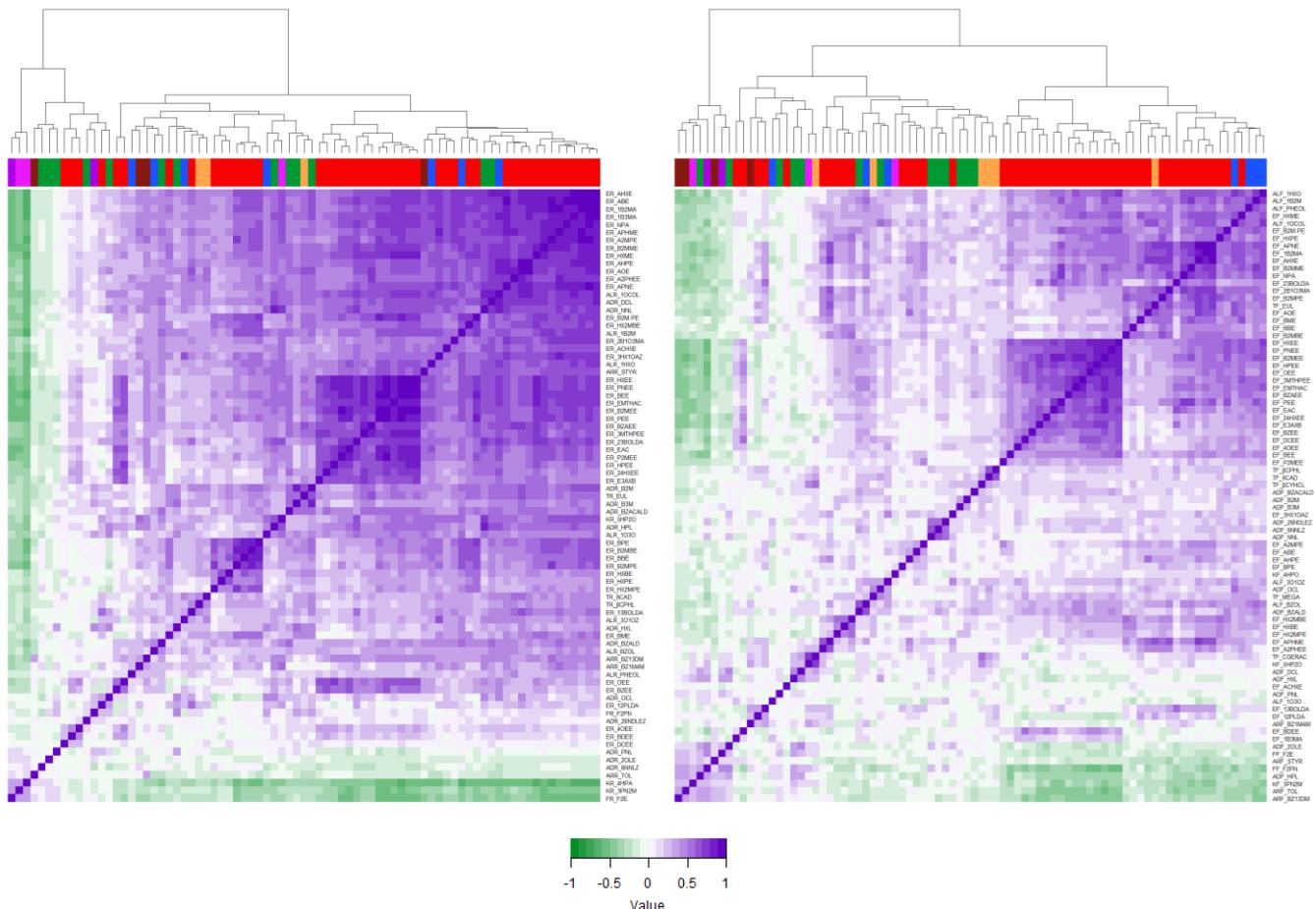


Fig. 1. Heatmap and dendrogram showing the correlation matrix by the Spearman's coefficient between VOCs in the RIL population for rind (A) and flesh (B).

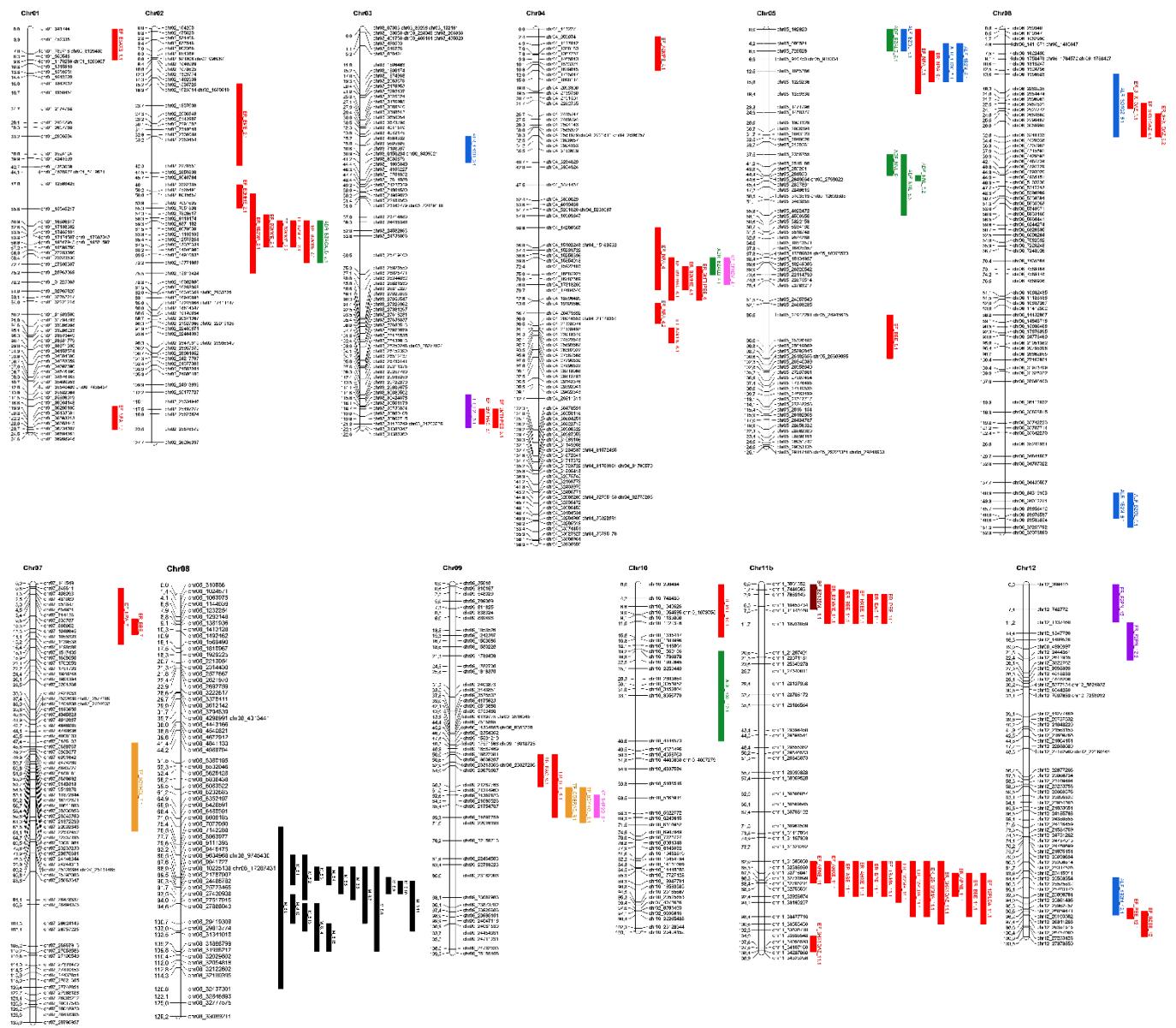


Fig. 2. Genetic map showing QTLs found in rind and flesh. QTLs for esters (red), alcohols (blue), aldehydes (green), terpenes (orange), benzenoids (brown), ketones (pink) and furans (purple) were represented selecting LOD-1 as upper and lower limit. Black QTLs represent merged (M) QTLs coincident for several compounds.

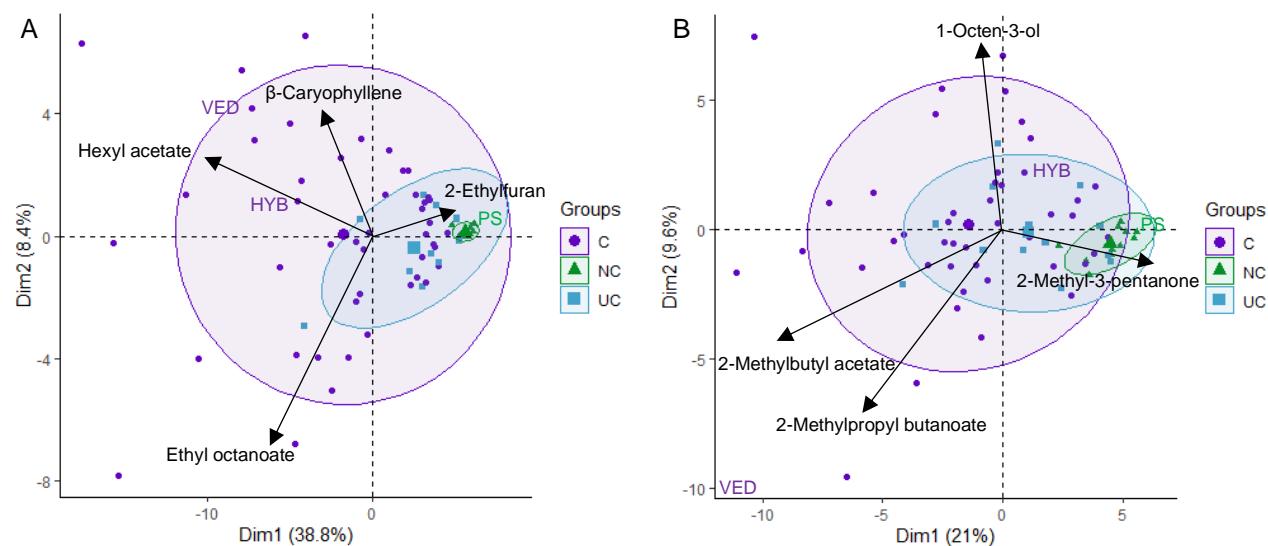


Fig. 3. Principal component analysis (PCA) of the PS x VED RIL population volatile profile in rind (A) and flesh (B). Different ellipses group climacteric lines (C), non-climacteric lines (NC) and “unstable-climacteric” lines (UC). The arrows represent the VOCs with the most positive and negative effect for the two principal components.

711

Climacteric RILs

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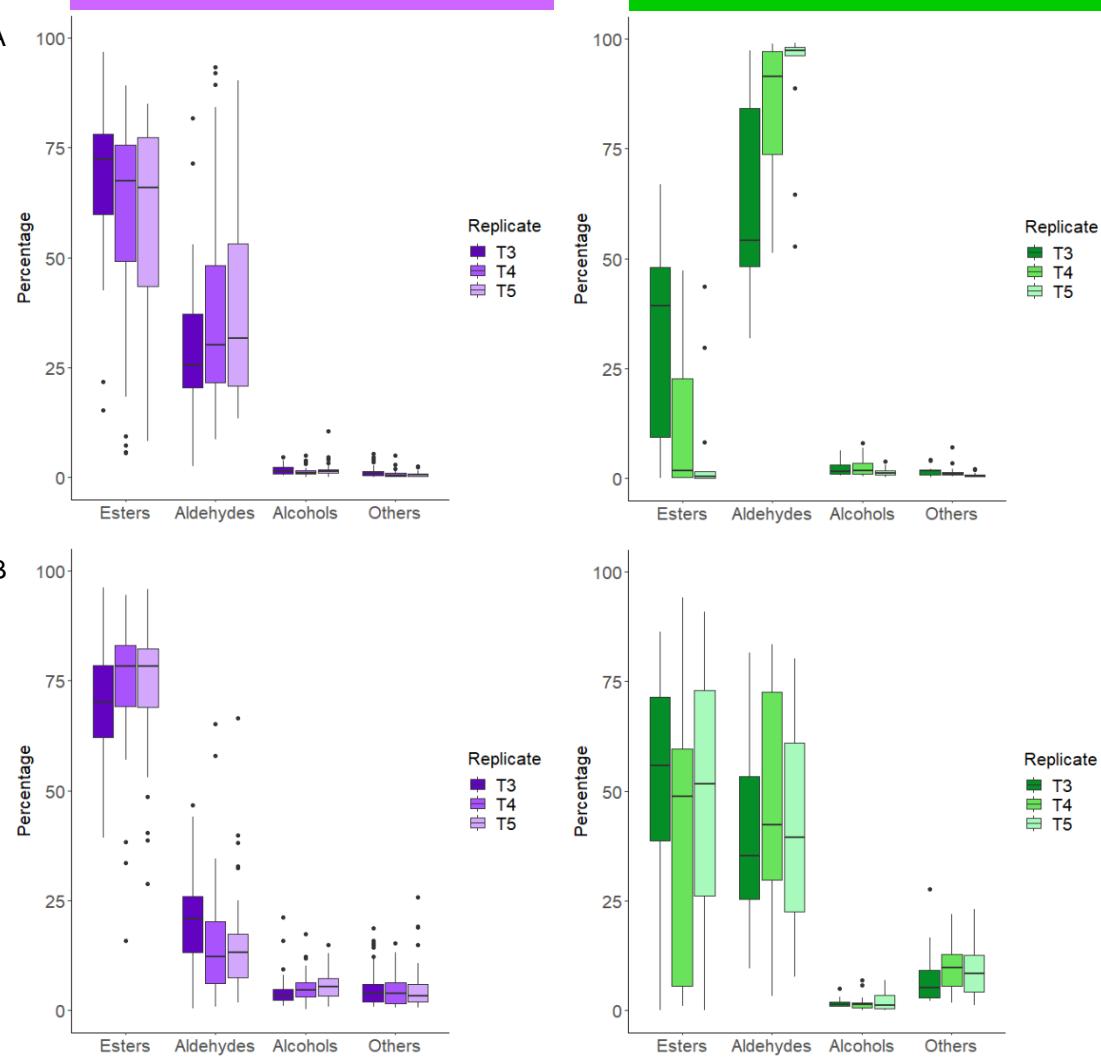
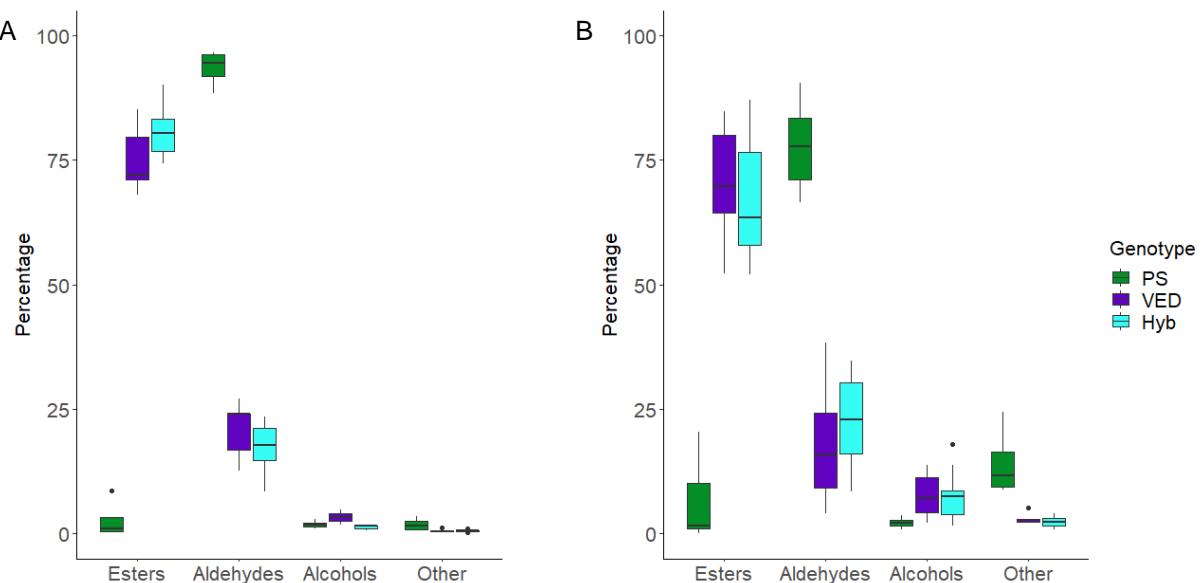
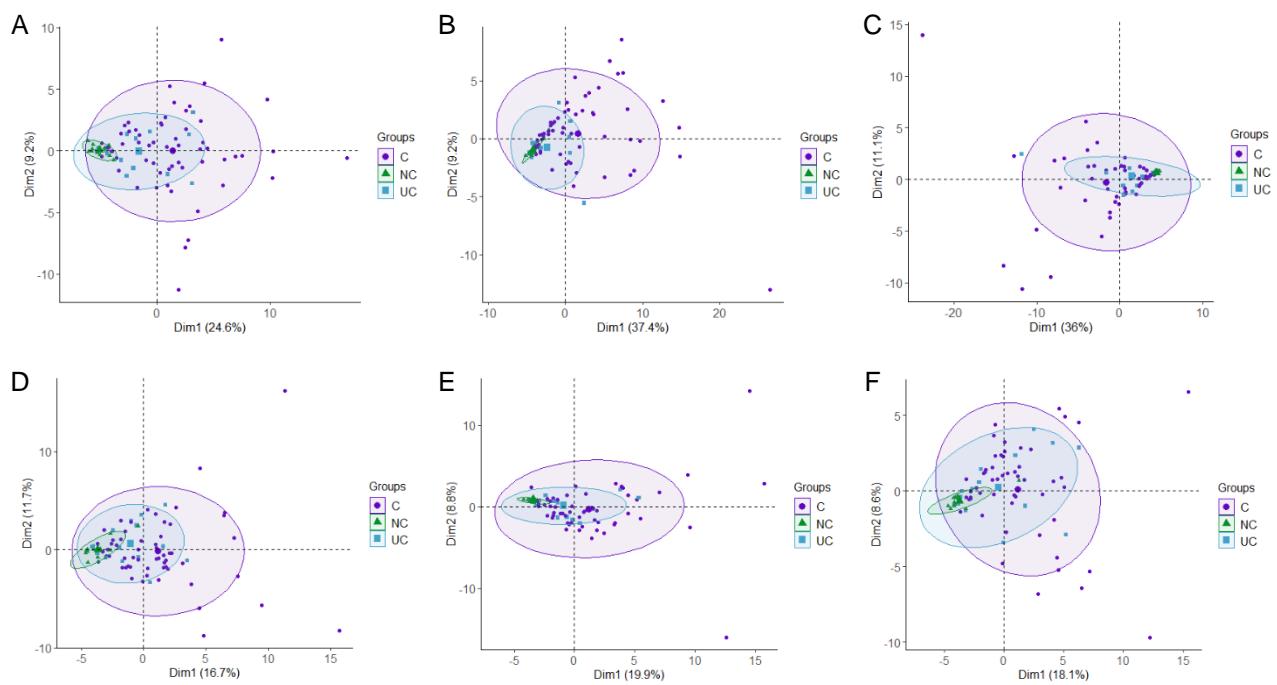


Fig. 4. Percentage of VOCs in RILs according to their chemistry, in rind (A) and flesh (B). Data from three subsets with plants performed in 2015 (T3) and 2016 (T4, T5) are represented.



Supplementary Fig. S1. Boxplot showing the amount of VOCs according to their chemical group in rind (A) and flesh (B) samples of the parental lines (PS, VED) and the F1 (Hyb).



Supplementary Fig. S2. Principal component analysis (PCA) of the PS x VED RIL population volatile profile. The upper part represents rind PCAs for T3 (A), T4 (B) and T5 (C) subsets; the lower part represents flesh PCAs for T3 (D), T4 (E) and T5 (F) subsets. Different ellipses group climacteric lines (C), non-climacteric lines (NC) and “unstable-climacteric” lines (UC).