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# Analysis of the Volatile Aroma Constituents of Parental and Hybrid Clones of Pepino (*Solanum muricatum*)

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The volatile constituents of 10 clones (4 parents with different flavors and 6 hybrids from selected crossings among these parents) of pepino fruit (*Solanum muricatum*) were isolated by simultaneous distillation−extraction and analyzed by gas chromatography−mass spectrometry (GC-MS). Odor-contributing volatiles (OCVs) were detected by GC−olfactometry−MS analyses and included 24 esters (acetates, 3-methylbutanoates, and 3-methylbut-2-enoates), 7 aldehydes (especially hexenals and nonenals), 6 ketones, 9 alcohols, 3 lactones, 2 terpenes, $\beta$-damascenone, and mesifurane. Among these compounds, 17, of which 5 had not been reported previously in pepino, were found to contribute significantly to pepino aroma. OCVs can be assigned to three groups according to their odor quality: fruity fresh (acetates and prenol), green vegetable (C6 and C9 aldehydes), and exotic (lactones, mesifuran, and $\beta$-damascenone). Quantitative and qualitative differences between clones for these compounds are clearly related to differences in their overall flavor impression. The positive value found for the hybrid−midparent regression coefficient for volatile composition indicates that an important fraction of the variation observed is inheritable, which has important implications in breeding for improving aroma. Significant and positive correlations were found between OCVs having common precursors or related pathways.

**KEYWORDS:** ***Solanum muricatum*; pepino; volatile compounds; flavor; parental clones; hybrid clones; GC-MS**

## INTRODUCTION

The success of the establishment of pepino (*Solanum muricatum*), an Andean domesticate (*1*), as a new crop in Europe depends on the development of new cultivars with improved fruit quality. Aroma is an important component of fruit quality in pepino and is a key factor in determining the type of use of the fruits. Cultivars yielding berries with fruity and exotic aromas are preferentially used as a fresh dessert fruit. On the other hand, cultivars with fruits releasing herbaceous/vegetable-like aromas are preferred for being used in vegetable salads in the same way as cucumber (*2*).

Because aroma is important for pepino fruit quality, it must be considered as a main trait in breeding programs aimed to improve quality. As in other fruits and vegetables (*3*), pepino aroma is the result of a complex, and specific, combination of individual volatile compounds. Thus, the isolation, identification, and quantification of the pepino fruit volatiles is important in studying the differences in aroma between cultivars. Furthermore, qualitative and quantitative differences between cultivars have been detected in many species (*3*, *4*). Therefore, the use of a representative degree of variation for aroma is required if one is interested in determining the compounds contributing to the aroma of pepino fruits and responsible for the differences in aroma between cultivars.

Despite the wide spectrum for aroma that can be found between cultivars, studies about this topic in *S. muricatum* are scarce. Shiota et al. (*5*) conducted a pioneer study of the volatile fraction of pepino, in which they identified up to 30 compounds from three cultivars of pepino. The main constituents of the volatile fraction in pepino were 3-methylbut-2-en-1-ol, 3-methylbut-3-en-1-ol, (*Z*)-non-6-en-1-ol, 3-methylbut-2-en-1-yl acetate, 3-methylbut-3-en-1-yl acetate, butyl acetate, and hexyl acetate (all of them related to fruity aromas) and a series of linear C9 aldehydes (related to green−herbaceous odors). Recently, it was confirmed that 3-methylbut-2-en-1-ol, 3-methylbut-3-en-1-ol, and their corresponding acetates were also main compounds in the volatile fraction of two cultivars of pepino (*6*).

Because of the limited genotypic diversity utilized in these experiments, compounds of the volatile fraction of other pepino

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**Table 1.** Agronomic and Morphological Traits of Interest of Parents and Hybrids of Pepino Fruits Evaluated

| genotype | yield (t ha$^{-1}$) | fruit wt (g) | shape (length/width) | soluble solids (°Brix) |
|---|---|---|---|---|
| parental clones | | | | |
| Sm-4 | 0[a] | 150–250 | 0.90–1.10 | 7.0–9.0 |
| Sm-23 | 40–65 | 250–400 | 3.00–3.50 | 5.0–6.5 |
| Sm-26 | 0–20 | 100–200 | 1.20–1.40 | 8.0–9.0 |
| Sm-29 | 20–30 | 100–250 | 2.30–3.00 | 8.0–9.0 |
| hybrid clones | | | | |
| Sm-4 × Sm-23 (c4)[b] | 30–50 | 150–250 | 1.50 | 8.0–8.5 |
| Sm-4 × Sm-23 (c5) | 30–60 | 100–200 | 1.70–1.80 | 8.0 |
| Sm-4 × Sm-23 (c16) | 50–75 | 200–350 | 1.30 | 7.0–8.0 |
| Sm-4 × Sm-29 (c30) | 30–40 | 200–350 | 1.80–1.90 | 8.0 |
| Sm-23 × Sm-26 (c25) | 30–50 | 100–200 | 2.20–2.70 | 8.0–8.5 |
| Sm-29 × Sm-26 (c27) | 25–45 | 150–250 | 2.10–2.20 | 9.0–10.0 |

[a] Unviable pollen, nil yield under natural self-pollination conditions. It needs hand pollination in order to set fruits. [b] c4 means clone 4 of this segregating hybrid generation.

cultivars might remain unidentified. Furthermore, the identification of odor-contributing volatiles (OCVs) of different *S. muricatum* cultivars from the total volatiles (*7*) is of great importance for investigating the differences in the aroma between cultivars.

Also, information on the inheritance of the aroma and its constituents will provide valuable information for plant breeders. No data exist for pepino regarding this subject, but differences exist in the mode of inheritance for several individual volatiles in tomato (*8*), a species phylogenetically close to pepino (*9*).

In this work, we analyzed the volatiles of fruits from a collection of pepino clones, including parents differing in aroma, and hybrids between them by means of GC−olfactometry−MS. This will provide information not only on the compounds relevant for the different patterns of aroma in pepino but also on their inheritance.

## MATERIALS AND METHODS

**Plant Material.** Fruits from 10 clones of pepino (4 parents and 6 hybrids from selected crossings among these parents) were analyzed to detect the volatiles present in *S. muricatum* (**Table 1**). The population of parents represents a comprehensive spectrum of the different patterns of aromas in this species: from the intense green−herbaceous aroma of Sm-23 (used in salads as a vegetable) to the fruity and exotic aroma of Sm-29 (dessert fruit), including clones with intermediate odor quality (Sm-26 and Sm-4, intense green-fruity and light green-fruity, respectively). The six clonal hybrids are the result of an advanced program for the improvement of yield and soluble solids concentration (SSC), and they were selected from segregating hybrid offsprings obtained after the four parental clones had been crossed (*10*). Three of the six clonal hybrids studied were selected from the same segregating hybrid population [Sm-4 × Sm-23 (c4), Sm-4 × Sm-23 (c5), and Sm-4 × Sm-23 (c16)]. Plants were grown in a greenhouse in Valencia (Spain) under autumn−winter growing season conditions (*11*, *12*). Fruits were harvested when commercially ripe and analyzed 2 days later.

**Experimental Design.** Two samples per clone were analyzed, except for Sm-4 and Sm-4 × Sm-29 (c30), for which only one sample each could be analyzed. Each sample was prepared from three fruits, each of which came from a different plant of the same clone. Thus, a total of six plants were sampled per clone [three in the case of Sm-4 and Sm-4 × Sm-29 (c30)].

**Preparation of Samples and Extraction of Volatiles.** Extraction of volatiles was conducted as described by Shiota (*5*) according to the Lickens−Nickerson simultaneous extraction−distillation (SDE) for 2 h, utilizing diethyl ether as solvent.

Preliminary experiments with fruits from Sm-23 and Sm-29 (representatives of the two extreme aromas in pepino) were conducted to determine an adequate protocol for the preparation of the samples. Different sample weights (100, 250, and 500 g of fruit flesh), aqueous media (distilled water or a 10% NaCl solution), and preparations of the sample (pieces dipped into the liquid or as a puree obtained after homogenization with the aqueous medium in a commercial blender) were tested. Preparation of samples as a puree of 250 g of fruit flesh in distilled water (1:1) was chosen because it was found to represent the optimum combination. In addition, the analysis of the peel revealed that this part of the fruit was very poor in volatiles compared to the flesh, which was in agreement with the findings of Ruiz-Beviá et al. (*6*). Consequently, our work was focused on the flesh. SDE volatile extracts were concentrated to 1 mL on a Vigreux column (40 °C). Methyl decanoate (2.23 mg kg$^{-1}$ of fruit) was used as internal standard.

The use of enzyme-inactivating agents in the preparation of samples was found to be unnecessary. A preliminary gas chromatography−mass spectrometry (GC-MS) analysis, comparing SDE extractions, following the protocol described above, with solvent extraction (diethyl ether) of homogenates inhibited with methanol [preparation as puree of 250 g of fruit flesh in methanol (1:1)] revealed that the first method gives the best and reproducible yields of all volatiles.

**Analysis of Volatiles.** Volatiles were identified by GC-MS. A Siemens SiChromat II gas chromatograph directly coupled through a Live-T effluent splitter (1:1) to a Finnigan-MAT 8222 mass spectrometer and a sniffing-port and a SPB-5 fused silica capillary column (30 m length, 0.53 mm i.d., 1.5 $\mu$m film thickness; Supelco, Bellefonte, PA) were used. Injector and sniffing-port temperature was held at 250 °C. The column was programmed from 100 °C at a rate of 5 °C min$^{-1}$ to 250 °C. Helium was used as carrier gas at a flow of 3 mL min$^{-1}$ and split ratio of 1:5. The volume of the samples injected was 2 $\mu$L. Identification of volatiles was done by comparing mass spectra (ionization energy = 70 eV) and linear retention indices (*13*) with commercially available (Merck, Fluka, Sigma-Aldrich, Oril) or synthesized (see ref *14* and below) reference substances.

Quantitative analysis was performed with a Siemens SiChromat 1-4 gas chromatograph, equipped with the same type of column, carrier gas, and temperature conditions. The flame ionization detector temperature was 260 °C. Quantitative data were obtained by comparison to the peak area of the internal standard without considering response factors.

Clones having the highest concentration of the volatiles in the quantitative analysis [the four parents and hybrid Sm-29 × Sm-26 (c27)] were chosen to identify OCVs. Samples from these clones were analyzed by three trained panelists in a qualitative test using GC−olfactometry−MS. Compounds detected by the panelists in at least one of the clones evaluated were considered as OCVs in pepino.

**Synthesis of Reference Compounds.** (*Z*)-Non-6-en-1-yl acetate was prepared from (*Z*)-non-6-en-1-ol (Oril SA, Paris, France) and ethyl acetate by the addition of KOH according to the method of ref *14*. Methyl, butyl, 3-methylbutyl, 3-methylbut-2-enyl, and 3-methylbut-3-enyl 3-methylbut-2-enoates were prepared by the addition of ~10 mg of KOH and 20 mg of the corresponding alcohol (each obtained from Fluka Chemie GmbH) to 20 mg of senecioic acid (3-methylbut-2-enoic acid and 3,3-dimethylacrylic acid, Fluka). After 30 min, 50 $\mu$L of a HCl solution (10%) was added and the flask incubated at 100 °C for 15 min. The reaction products were identical by mass spectra and retention time compared to the corresponding compounds of pepino fruit [butyl 3-methylbut-2-enoate, *m*/*e* (%) 83 (100), 100 (74), 55 (38), 39 (24), 101 (24), 82 (21), 41 (20), 156 (18); 3-methylbutyl 3-methylbut-2-enoate, *m*/*e* (%) 83(100), 100 (43), 55(42), 43 (36), 70 (29), 101 (27), 71 (23), 170 (10)].

**Parental Effect and Correlations.** An estimate of the parental effect for the total volatiles and total OCVs was studied by mid-parent (MP)−hybrid regression analysis. Phenotypic correlations between OCVs were also calculated.

## RESULTS AND DISCUSSION

**Constituents of Pepino Volatiles.** A total of 53 volatiles, including esters, alcohols, aldehydes, ketones, and other com-

**Table 2.** Volatiles Identified in the Population of 10 Clones of Pepino Fruit

| compound | not reported previously[a] | sensory test[b] | retention idex[c] | identification[d] |
|---|---|---|---|---|
| esters | | | | |
| ethyl acetate | | ND | 608 | 1 |
| propyl acetate | | ND | 696 | 1 |
| butyl acetate | | (3) | 797 | 1 |
| pentyl acetate | | (3) | 904 | 1 |
| hexyl acetate | | (1) | 1006 | 1 |
| octyl acetate | * | ND | 1208 | 1 |
| (*Z*)-hex-3-en-1-yl acetate | * | ND | 999 | 1 |
| (*Z*)-non-6-en-1-yl acetate | | ND | 1308 | 1, 3 |
| 2-methylpropyl acetate | | ND | 753 | 1 |
| 3-methylbutyl acetate | | (5) | 862 | 1, 4 |
| 3-methylbut-3-en-1-yl acetate | | (5) | 872 | 1, 2, 3, 4 |
| 3-methylbut-2-en-1-yl acetate | | (5) | 914 | 1, 2, 3 |
| 2-methylene-butan-1,4-diyl diacetate | * | ND | 1255 | 1, 2 |
| (*Z*)-2-methylbut-2-en-1,4-diyl diacetate | * | ND | 1278 | 1, 2 |
| (*E*)-2-methylbut-2-en-1,4-diyl diacetate | * | ND | 1301 | 1, 2 |
| 3-methylbut-3-en-1-yl 3-methylbutanoate | | ND | 1115 | 1, 2, 3, 4 |
| 3-methylbut-2-en-1-yl 3-methylbutanoate | | ND | 1146 | 1, 2, 3, 4 |
| 3-methylbut-2-en-1-yl 2-methylbutanoate | * | ND | 1138 | 1, 2 |
| methyl 3-methylbut-2-enoate | | ND | 833 | 1, 3 |
| butyl 3-methylbut-2-enoate | | ND | 1121 | 1, 5 |
| 3-methybutyl 3-methylbut-2-enoate | * | ND | 1184 | 1, 4, 5 |
| 3-methylbut-3-en-1-yl 3-methylbut-2-enoate | | ND | 1195 | 1, 3, 4 |
| 3-methylbut-2-en-1-yl 3-methylbut-2-enoate | | ND | 1237 | 1, 3, 4 |
| 3-methylbut-2-en-1-yl 2-methylbut-2-enoate | * | ND | 1245 | 1, 2 |
| aldehydes and ketones | | | | |
| hexanal | | (5) | 789 | 1 |
| (*Z*)-hex-3-enal | * | ND | 782 | 1 |
| (*E*)-hex-2-enal | | (2) | 846 | 1 |
| (*Z*)-non-6-enal | | (5) | 1107 | 1 |
| (*E*,*Z*)-nona-2,6-dienal | | (5) | 1160 | 1 |
| (*E*)-non-2-enal | | (5) | 1165 | 1 |
| (*E*,*E*)-deca-2,4-dienal | * | ND | 1331 | 1 |
| acetoin (2-hydroxy-3-butanone) | * | ND | 700 | 1 |
| nonan-2-one | | ND | 1094 | 1 |
| undecan-2-one | * | ND | 1297 | 1 |
| tridecan-2-one | * | ND | 1499 | 1 |
| pentadecan-2-one | * | ND | 1703 | 1 |
| heptadecan-2-one | * | ND | 1902 | 1 |
| alcohols | | | | 1 |
| butan-1-ol | | ND | 651 | 1 |
| (*E*)-hex-2-en-1-ol | * | ND | 856 | 1 |
| (*Z*)-non-6-en-1-ol | | ND | 1172 | 1 |
| (*E*,*Z*)-nona-2,6-dienol | * | ND | 1170 | 1 |
| 2-methyl-3-buten-2-ol | | ND | 611 | 1 |
| 3-methyl-3-buten-1-ol | | ND | 716 | 1, 4 |
| 3-methyl-2-buten-1-ol | | (5) | 756 | 1 |
| pentadecan-2-ol | | ND | 1710 | 1 |
| heptadecan-2-ol | | ND | 1909 | 1 |
| miscellaneous | | | | 1 |
| $\beta$-damascenone | * | (5) | 1404 | 1 |
| 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (mesifuran) | * | (2) | 1064 | 1 |
| linalool | * | ND | 1104 | 1 |
| nerolidol | * | ND | 1574 | 1 |
| 4-nonanolide ($\gamma$- nonalactone) | * | (2) | 1387 | 1 |
| 4-decanolide ($\gamma$-decalactone) | * | (2) | 1494 | 1 |
| 5-dec-2-enolide (massoia lactone) | * | (2) | 1508 | 1 |

[a] According to ref *3*. 2-Methyl-3-buten-2-ol was identified by ref *5*, but is not listed in ref *3*. [b] ND = not detected at the sensory test. In parentheses is the number of clones (of a total of 5) in which the compound was detected at the sensory test. [c] Calculated according to ref *13*. [d] 1, by comparison of mass spectra and retention indices with authentic reference substances; 2, by mass spectra and retention index according to ref *14*; 3, by mass spectra according to ref *5*; 4, by mass spectra according to ref *15*; 5, by mass spectra (see Materials and Methods).

pounds, were identified (**Table 2**). The analyses of clones having a wide spectrum of volatiles allowed the identification of 24 compounds not previously reported in pepino: 8 esters, 5 ketones, 2 aldehydes, 2 alcohols, 3 lactones, 3 isoprenoids, and a furanone (**Table 2**).

The most abundant components in pepino flesh are esters. Among these esters 3-methylbutyl 3-methylbutanoate and 3-methylbut-2-en-1-yl 3-methylbut-2-enoate (**Table 2**) were identified in the venom of the European hornet (*Vespa crabro*) by Wheeler et al. (*15*). The 2-methylbutanoate and 2-methylbut-2-enoate (tiglinate) of 3-methylbut-2-en-1-ol (prenol), as well as the diacetates of the isomeric 2-methylbut-2-ene-1,4-diols, were described recently as new natural constituents of *Helenium aromaticum* by Kollmannsberger et al. (*14*), whereas to our knowledge the diacetate of 2-methylenebutane-1,4-diol was not found in nature up to now. Ketones and aldehydes (mainly C6 and C9 linear aldehydes) ranked second in number of compounds identified (**Table 2**). Nine alcohols were also identified,

**Table 3.** Odor Description of Each Odor-Contributing Volatile (OCV) Perceived at the Sniffing Port

| OCV | odor quality |
|---|---|
| group I | |
| butyl acetate | fruity, pear/banana-like |
| pentyl acetate | fruity, pineapple, banana oil-like |
| hexyl acetate | fruity, sweet, berry/pear-like |
| 3-methylbutyl acetate | fruity, fresh, pear/banana-like |
| 3-methylbut-2-en-1-yl acetate | fruity, green apple, banana |
| 3-methylbut-3-en-1-yl acetate | fruity, banana |
| 3-methylbut-2-en-1-ol | gassy fruity/green, lavender, fresh |
| group II | |
| hexanal | green |
| (*E*)-hex-2-enal | green, vegetable-like |
| (*E*)-non-2-enal | penetrating, green, vegetable-like |
| (*Z*)-non-6-enal | green, melon/cucumber-like |
| (*E*,*Z*)-nona-2,6-dienal | powerful, green, vegetable-like |
| group III | |
| $\beta$-damascenone | boiled apple/peach-like |
| mesifurane | caramel-like, burnt |
| $\gamma$-nonalactone | creamy, coconut-like |
| $\gamma$-decalactone | creamy, peach-like |
| massoia lactone | creamy, fatty, sweet, coconut-like |

**Table 4.** Total Volatiles (TVs) and Total Odor-Contributing Volatiles (TOCVs) (Micrograms per Kilogram) with Respective Midparent–Hybrid (MP–H) Correlations and Ratio of TOCVs to TVs (Percent) of the Clones Evaluated

| genotype | TVs[a] | TOCVs[a] | TOCVs/TVs |
|---|---|---|---|
| parents | | | |
| Sm-4 | 47339 bc[b] | 34218 b | 72 |
| Sm-23 | 15730 a | 11119 a | 71 |
| Sm-26 | 58612 c | 46498 c | 79 |
| Sm-29 | 50413 bc | 31679 b | 63 |
| hybrids | | | |
| Sm-4 × Sm-23 (c4) | 15749 a | 10678 a | 68 |
| Sm-4 × Sm-23 (c5) | 32356 ab | 18849 a | 58 |
| Sm-4 × Sm-23 (c16) | 20088 a | 10615 a | 53 |
| Sm-4 × Sm-29 (c30) | 30238 ab | 23059 ab | 76 |
| Sm-23 × Sm-26 (c25) | 35028 ab | 29649 b | 85 |
| Sm-29 × Sm-26 (c27) | 58155 c | 44484 c | 77 |
| MP–H correlation[c] | 0.789 NS | 0.905 * | |

[a] Average of samples of each clone. [b] Different letters indicate significant differences between clones for the Student–Newman–Keuls test at $P < 0.05$. [c] Correlation coefficient between hybrids and the respective midparent values, with NS and * indicating nonsignificant and significant at $P < 0.05$, respectively.

of which 3-methylbut-2-en-1-ol and 3-methylbut-3-en-1-ol have been reported, with their respective acetates, as main constituents of the volatile fraction of pepino (*5*, *6*). Additionally, 4-nonanolide ($\gamma$-nonalactone), 4-decanolide ($\gamma$-decalactone), 5-dec-2-enolide (massoia lactone), $\beta$-damascenone, nerolidol, linalool, and 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (mesifuran) (**Table 2**) have not been reported in pepino previously. All of them were detected and identified by GC-MS. $\beta$-Damascenone could be identified only in one sample by GC-MS. In the other samples investigated by GC−olfactometry−MS $\beta$-damascenone was identified tentatively by relating its characteristic odor (boiled apple/peach) at the sniffing port and the corresponding retention time with data of a reference sample. With its extremely low odor threshold (*16*) $\beta$-damascenone is often recognized sensorially far below its analytical detection limit during sniffing analyses.

**Volatile Compounds Contributing Significantly to Pepino Fruit Aroma (OCVs).** The sniffing qualitative analyses of the four parents and the hybrid Sm-29 × Sm-26 (c27) revealed that 17 volatiles, of which 5 had not been reported previously in pepino, are present in pepino fruits at levels high enough to be detected by the human nose and, therefore, contribute significantly to pepino aroma (OCVs).

Additionally, there is also some evidence that in some of the samples 4-vinylphenol and 4-vinylguaiacol may also contribute to the flavor. However, these phenols have to be regarded as artifacts of distillation (*17*) and are therefore not further considered. No reliable odor impressions were recorded for (*Z*)-hex-3-enal and linalool, which were masked by coeluting odoriferous compounds [hexanal and (*Z*)-non-6-enal, respectively].

OCVs were grouped according to their odor quality (**Table 3**). Group I was related to a fruity and/or fresh odor and included six acetates and 3-methylbut-2-en-1-ol. The volatiles of this group are usually related to pear/banana-like flavors and are naturally present in these two fruits (*3*). In this sense, 3-methylbutyl acetate is considered to be a key component of the banana flavor (*18*).

Group II is constituted by five C6 and C9 linear aldehydes, which contribute to pepino flavor with herbaceous/green notes (**Table 3**). These volatiles have been previously reported in pepino (*5*) and were detected by the panelists in the five clones tested, with the exception of (*E*)-hex-2-enal due to its lower concentration. (*E*)-Non-2-enal, (*Z*)-non-6-enal, and (*E*,*Z*)-nona-2,6-dienal showed strong and penetrating odors, which were immediately related to cucumber and/or melon at the sniffing port. In fact, they are considered as key components for the flavor of these vegetables (*3*). Hexanal contributes with a grassy note to the flavor of pepino fruits. These aldehydes are very common volatiles in higher plants having a high lipoxygenase activity and can be found in a wide range of fruits (*3*).

Group III is a miscellany of several volatiles that contribute to pepino flavor with exotic and distinctive notes (**Table 3**). The three lactones ($\gamma$-nonalactone, $\gamma$-decalactone, and massoia lactone) were described at the sniffing port as having a creamy odor resembling coconut oil/peach-like notes in agreement with their descriptions in the literature (*3*). In fact, $\gamma$-nonalactone is utilized in the formulation of synthetic coconut flavorings, and $\gamma$-decalactone is an important component for the flavor of peach and other *Prunus* fruits (*19*−*21*). Group III also includes two volatiles that contribute with delicate and exotic notes to the aroma of pepino: $\beta$-damascenone and mesifurane. The first one, which was detected by the panelists in the five clones tested (**Table 2**), is mainly responsible for the flavor of the essential oil of rose (*22*) and has also been reported as an important flavor compound in carotene-rich fruits such as tomato (*23*, *24*). The second is a relevant compound in the aroma of cultivated *Fragaria* (*25*, *26*) and pineapple (*27*).

The two most common names by which *S. muricatum* is known in English are pepino (Spanish word for cucumber) and melon-pear (*28*). Probably, the fact that the flavor of pepino fruit is mainly the result of volatiles reported to be relevant to the flavor of cucumber, melon, and pear, was the main reason for giving such names to this crop.

**Quantitative and Qualitative Variation of Volatiles between Clones.** There were important differences between clones for total concentration of volatiles and total concentration of OCVs (**Table 4**). The highest levels of total volatiles were found in parents Sm-26 (close to 60000 $\mu$g kg$^{-1}$) and Sm-29 (50000 $\mu$g kg$^{-1}$) and their hybrid Sm-26 × Sm-29 (c27). The lowest levels were found in parent Sm-23 and hybrid Sm-4 × Sm-23 (c4) (**Table 4**).

OCVs represent >50% of total volatiles, ranging from 53% in Sm-4 × Sm-23 (c16) to 85% in Sm-23 × Sm-26 (c25) (**Table**

**Table 5.** Intervals of Concentration (Micrograms per Kilogram) of Odor-Contributing Volatiles (OCVs) in the Volatile Fraction of Parental and Hybrid Clones[a]

| | parents | | | | hybrids | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| OCV | Sm-4 | Sm-23 | Sm-26 | Sm-29 | Sm-4 × Sm-23 (c4) | Sm-4 × Sm-23 (c5) | Sm-4S × Sm-23 (c16) | Sm-4 × Sm-29 (c30) | Sm-23 × Sm-26 (c25) | Sm-29 × Sm-26 (c27) |
| group I | | | | | | | | | | |
| butyl acetate | 500 | 284–350 | 1908–1961 | 4658–5581 | 785–2462 | 2900 | 660–1000 | 2351 | 1091–1135 | 2384–3176 |
| pentyl acetate | NQ[a] | NQ | NQ | 23–50 | NQ | NQ | NQ | NQ | NQ | 85–95 |
| hexyl acetate | 130 | 217–413 | 210–301 | 613–680 | 136–318 | 115–126 | 93–94 | 186 | 233–268 | 293–350 |
| 3-methylbutyl acetate | 188 | 123–238 | 537–798 | 1010–1038 | 79–370 | 100–145 | 53 | 222 | 247–272 | 966–990 |
| 3-methylbut-2-en-1-yl acetate | 9415 | 503–565 | 7680–8653 | 8403–9722 | 836–3473 | 300–327 | 137–394 | 5499 | 9639 | 12577–12738 |
| 3-methylbut-3-en-1-yl acetate | 17267 | 3804–4692 | 18500–21900 | 11500–12300 | 1851–4377 | 2182–4104 | 5009–6287 | 7390 | 5213–9853 | 15102–17370 |
| 3-methylbut-2-en-1-ol | 1062 | 333–493 | 1442–2075 | 2698–3158 | 161–1210 | 722–996 | 323–394 | 1302 | 524–849 | 2118–2823 |
| group II | | | | | | | | | | |
| hexanal | 241 | 2070 | 168–225 | 0–42 | 760–1506 | 441–1153 | 832–1115 | 696 | 1003–4703 | 650–677 |
| (*E*)-hex-2-enal | 156 | 4085 | 569–1586 | 105–406 | 1469 | 3501–4965 | 1271–2413 | 2322 | 2700–9386 | 3272–3856 |
| (*E*,*Z*)-nona-2,6-dienal | 475 | 1513 | 2616–9506 | 50–419 | 569 | 1723–4518 | 278 | 1306 | 1915–3268 | 1647–3308 |
| (*E*)-non-2-enal | 50 | 1643 | 1086–4835 | 0–272 | 445 | 1012–1846 | NQ | 676 | 2181–2782 | 976–1794 |
| (*Z*)-non-6-enal | 4734 | 122–361 | 2447–4014 | 50–60 | 498 | 2182–4211 | 378–498 | 1059 | 372–777 | 525–926 |
| group III | | | | | | | | | | |
| $\beta$-damascenone | NQ[b] | NQ | NQ | NQ | –[c] | – | – | – | – | NQ |
| mesifurane | NQ | NQ | NQ | 58–80 | NQ | NQ | NQ | NQ | NQ | 40–50 |
| $\gamma$- nonalactone | NQ | NQ | NQ | NQ | NQ | NQ | NQ | NQ | NQ | NQ |
| $\gamma$-decalactone | NQ | NQ | NQ | 20 | NQ | NQ | NQ | NQ | NQ | 20 |
| massoia lactone | NQ | 10 | NQ | 121–134 | 50 | 30 | NQ | 50 | 20 | 100 |

[a] Two samples per clone were analyzed except for Sm-4 and Sm-4 × Sm-29 (c30) in which only one sample was utilized. Each sample was prepared from three fruits, each from a different plant. [b] NQ = not quantified because concentration was too low during GC analysis. [c] Not investigated by GC–olfactometry–MS.

**4**). Similar to the total volatile fraction, significant differences were found between clones for the total concentration of OCVs. Parent Sm-26 and hybrid Sm-29 × Sm-26 (c27) showed the highest values (~45000 $\mu$g kg$^{-1}$), followed by Sm-4, whereas the lowest values were found in Sm-23 and the three hybrids between this clone and Sm-4 (<20000 $\mu$g kg$^{-1}$) (**Table 4**).

Several facts suggest that the differences among clones for both total volatiles and total OCVs might have an important genetic component. Hybrids [with the exception of Sm-4 × Sm-29 (c30)] showed concentrations intermediate or not significantly different from that of one or both parents. In addition, a high positive correlation between hybrids and mid-parent value was found for odor-contributing compounds (**Table 4**), indicating that the greater the average of a couple of parents, the higher the value of the respective hybrid.

Differences in the flavor of the parental clones can be explained by quantitative and qualitative differences in individual OCVs. Sm-29, with a fruity and exotic flavor, showed higher concentrations of compounds of group I than Sm-23, characterized by a green/vegetable-like flavor (**Table 5**). In fact, compounds of group I represented the most important fraction of the total concentration of volatiles (55%) and total concentration of OCVs (90%) in Sm-29 (**Tables 4** and **5**), and levels of 3-methylbut-2-en-1-yl acetate were 15 times higher in Sm-29 than in Sm-23. Moreover, all of the compounds of group III detected here were present in Sm-29, and this contributed to the final aroma of its fruits with exotic notes of coconut and peach. On the contrary, only $\beta$-damascenone was found to contribute significantly to the aroma of Sm-23 fruits (**Table 5**). Differences in the levels of linear aldehydes contribute further to explain differences between both clones, as Sm-23 showed higher levels than Sm-29 for the five aldehydes. In this way, hexanal levels in Sm-23 were 50 times higher than that in Sm-29 (**Table 5**).

Although Sm-26 showed higher values of total OCVs than Sm-29, and similar levels of compounds of group I (higher in 3-methylbut-3-en-1-yl acetate) (**Table 5**), its flavor is not as fruity and attractive as that of Sm-29. This may be related to the concentrations of volatiles from other groups. The range of compounds of group III in Sm-26 is not as wide as in Sm-29 and, furthermore, Sm-26 has a greater concentration of aldehydes than Sm-29 and even higher than Sm-23 in the case of (*E*,*Z*)-nona-2,6-dienal and (*Z*)-non-6-enal (**Table 5**). This greater concentration of group II compounds may mask the flavor of volatiles of group I.

In the case of Sm-4, only high values of 3-methylbut-2-en-1-yl acetate and 3-methylbut-3-en-1-yl acetate (similar to Sm-26 and Sm-29) and (*Z*)-non-6-enal were found (**Table 5**). In the case of the other OCVs, low levels were found in this clone (many of them among the lowest of the four parents) (**Table 5**). Thus, despite ranking third for the total OCVs, with values even higher than those of Sm-29 (**Table 4**), the flavor of this clone is the least intense of the four parents. This fact suggests that the presence of certain volatiles at adequate concentrations may be more important than the value of total volatiles (or even total OCVs) for the intensity of flavor in pepino fruit.

Hybrids, as parents, showed quantitative differences for OCVs. In addition, a strong relationship between parents and hybrids for the concentration of individual volatiles was found (**Table 5**). This suggests that the flavor has a complex genetic control, as it is inherited as a combination of different traits rather than as a single trait. Thus, hybrids involving parents Sm-26 and Sm-29 showed, in general, higher levels of group I volatiles than the three hybrids from crossing Sm-4 × Sm-23 (**Table 5**). In the case of aldehydes we found that in almost all cases the hybrid with the highest value of a particular aldehyde is always derived from the parent with the highest value (**Table 5**). Furthermore, individual compounds of group III were found only in hybrids derived from parents in which such compounds had also been identified (**Table 5**).

The hypothesis of a parental effect in the constitution of the flavor in the hybrids is also supported by the range of volatiles found in Sm-29 × Sm-26 (c27). This hybrid, selected from the crossing between parents with the highest concentrations of compounds of groups I and III, also showed the highest values of these compounds among the hybrids (**Table 5**).

**Table 6.** Correlation Coefficients between Odor-Contributing Volatiles (OCVs) of Groups I and II[a]

| compound[b] | PA | HA | 3-MBA | 3-M-2-BA | 3-M-3-BA | 3-M-2-Bol | hexanal | (*E*)-2-hexenal | (*E*)-2-nonenal | (*Z*)-6-nonenal | (*E*,*Z*)-2,6-nonadienal |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BA | 0.483$^{NS}$ | 0.743*** | 0.763*** | 0.489$^{NS}$ | 0.287$^{NS}$ | 0.856*** | –0.326$^{NS}$ | –0.365$^{NS}$ | –0.113$^{NS}$ | –0.316$^{NS}$ | –0.172$^{NS}$ |
| PA | | 0.485$^{NS}$ | 0.842*** | 0.814** | 0.888*** | 0.777** | –0.225$^{NS}$ | –0.193$^{NS}$ | 0.026$^{NS}$ | –0.385$^{NS}$ | –0.085$^{NS}$ |
| HA | | | 0.768*** | 0.475$^{NS}$ | 0.298$^{NS}$ | 0.753*** | –0.035$^{NS}$ | –0.339$^{NS}$ | 0.065$^{NS}$ | –0.548* | –0.187$^{NS}$ |
| 3-MBA | | | | 0.787*** | 0.689** | 0.950 *** | –0.269$^{NS}$ | –0.317$^{NS}$ | 0.187$^{NS}$ | –0.246$^{NS}$ | 0.128$^{NS}$ |
| 3-M-2-BA | | | | | 0.791*** | 0.769*** | –0.440$^{NS}$ | –0.476$^{NS}$ | 0.127$^{NS}$ | –0.182$^{NS}$ | –0.014$^{NS}$ |
| 3-M-3-BA | | | | | | 0.680** | –0.263$^{NS}$ | –0.339$^{NS}$ | 0.338$^{NS}$ | 0.244$^{NS}$ | 0.360$^{NS}$ |
| 3-M-2-Bol | | | | | | | –0.314$^{NS}$ | –0.379$^{NS}$ | 0.094$^{NS}$ | –0.192$^{NS}$ | 0.035$^{NS}$ |
| hexanal | | | | | | | | 0.868*** | 0.265$^{NS}$ | –0.250$^{NS}$ | 0.067$^{NS}$ |
| (*E*)-hex-2-enal | | | | | | | | | 0.387$^{NS}$ | –0.003$^{NS}$ | 0.285$^{NS}$ |
| (*E*)-non-2-enal | | | | | | | | | | 0.221$^{NS}$ | 0.909*** |
| (*Z*)-non-6-enal | | | | | | | | | | | 0.547* |

[a] NS, *, **, and *** indicate nonsignificant and significant at $P < 0.05$, 0.01, and 0.001, respectively. [b] BA, butyl acetate; PA, pentyl acetate; HA, hexyl acetate; 3-MBA, 3-methylbutyl acetate; 3-M-2-BA, 3-methylbut-2-en-1-yl acetate; 3-M-3-BA, 3-methylbut-3-en-1-yl acetate; 3-M-2-Bol, 3-methylbut-2-en-1-ol.

Examples of the typical segregation of the hybrid offsprings of pepino (*10*) were also found. As clones of this species are usually highly heterozygous, the hybrid population derived from a crossing between two different clones includes genetically different individuals (*29*). As a consequence, a high degree of variation (segregation) can be found among hybrids of the same parents and, even, some individuals may show higher levels than any parent (transgressive) for many traits (*10*, *30*). Thus, differences among the three hybrids derived from Sm-4 and Sm-23 for total volatiles and total OCVs (**Table 4**) and individual volatiles (**Table 5**) may be explained by the heterozygous nature of parents. On the other hand, hybrid Sm-29 × Sm-26 (c27) was transgressive to their respective parents for 3-methylbut-2-en-1-yl acetate, hexanal, and (*E*)-hex-2-enal, whereas Sm-4 × Sm-23 hybrids were transgressive for butyl acetate (**Table 5**). Obtention of transgressive individuals for flavor compounds is a very important point for the genetic improvement of the flavor, because it indicates that selection of individuals with improved flavor resulting from specific combinations of genes from parental clones can be obtained.

**Correlations between Odor-Contributing Volatiles.** Only OCVs of groups I and II were considered in the estimate of phenotypic correlations. Compounds of group III were not included because their concentrations were not quantified or were very low. Nineteen significant correlations between compounds of groups I and II were found. All significant correlations but one were positive (within a range of 0.547−0.950) and were detected between compounds from the same group (most of them between compounds of group I) (**Table 6**), whereas the only negative correlation was found between hexyl acetate (group I) and (*Z*)-non-6-enal (group II). This suggests that development of clones for either dessert or salad use will be facilitated because simultaneous selection for high concentration of fruity volatiles and low concentration of vegetable-like flavors is possible in the first case, whereas the contrary is possible in the second case.

Many correlations can be explained because most of these compounds are metabolites of related pathways, so that they have precursors in common. Thus, it was found that concentrations of 3-methylbutyl acetate, 3-methylbut-2-en-1-yl acetate, 3-methylbut-3-en-1-yl acetate, and 3-methylbut-2-en-1-ol (prenol) were positively correlated (**Table 6**). The conversion of hexanoate (precursor of hexyl acetate) into butyrate (precursor of butyl acetate) by $\beta$-oxidation (*31*) may explain the positive correlation between butyl acetate and hexyl acetate.

Higher plants (e.g., cucumber, melon, pear) can form C6 and C9 aldehydes from unsaturated C18 fatty acids such as linoleic or linolenic acid through sequential reactions by lipoxygenase and fatty acid hydroperoxide lyase (*32*, *33*). This is a branch of a highly divergent metabolic pathway, called the phytooxylipin pathway. In this pathway, lipoxygenase produces 9- and 13-hydroperoxides of both linoleic and linolenic acids. Then, hydroperoxide lyase releases C6 and C9 linear aldehydes from 13-hydroperoxides and 9-hydroperoxydes, respectively. In the case of C6 aldehydes, hexanal (from linoleic acid) and (*Z*)-hex-3-enal (linolenic acid) are found, and the latter is isomerized to (*E*)-hex-2-enal, which has been reported in strawberry and tomato (*34*−*36*) and may account for the positive correlation found between hexanal and (*E*)-hex-2-enal.

The study of a clonal collection representative of different flavors in *S. muricatum* has allowed the identification of a wide spectrum of volatile constituents in this species. These volatiles can be assigned to three groups according to their odor quality: fruity/fresh (acetates and prenol), green (C6 and C9 aldehydes) and exotic (lactones, mesifuran, and $\beta$-damascenone). Quantitative and qualitative differences between clones for these compounds are clearly related to differences in their final flavor. In this way, fruits with higher levels of acetates, prenol, and constituents with exotic notes and low levels of aldehydes are more suited for use as a dessert fruit, whereas the contrary is true for fruits destined to be used in salads. The levels of volatiles of hybrids are greatly influenced by the parents, and this offers opportunities for selection and development of clones with improved flavor, as it is possible to select clones with more intense fruity (or green) flavor from planned crossings.

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