Chemical Composition and Inheritance Patterns of Aroma in *Fragaria ×ananassa* and *Fragaria virginiana* Progenies

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The strawberry aroma is one of the most popular fruit flavors (Bood and Zabetakis, 2002); its chemical composition has been extensively studied (Mc Fadden et al., 1965; Pérez et al., 2002; Schreier, 1980). To date, over 300 different aromatic compounds have been identified (Latrasse, 1991; Zabetakis and Holden, 1997). Among them, esters and furanones are considered the main aroma determinants in fresh strawberries. Although aroma is a highly appreciated organoleptic attribute, studies to determine the genetic basis of strawberry aroma are scarce (Aharoni et al., 2000). They have been focused on identifying some specific genes, but little is known about the inheritance patterns of the aroma trait and the main compounds associated with it. The objective of this study was to determine the nature of aroma inheritance in segregating populations of strawberries and identify the most important aroma components.

Materials and Methods

A diallele cross (14 × 14) was made among cultivars and elite wild selections of *Fragaria ×ananassa* and *F. virginiana*. The progenies (174 genotypes) were planted at Michigan State University experimental farm in East Lansing. Aromas were identified in three replications of dark-red fruit on five to ten progenies of each family, with a HP 6890 gas chromatography (GC) system equipped with a fused silica Supelcowax-10 capillary column (30 m × 0.25 mm × 0.25 µm). Helium (1 mL·min⁻¹) was the carrier gas. The GC was coupled to a Leco Pegasus II mass spectrometer (MS). The identification of the main compounds was performed by comparing the mass spectra and retention time data with those of standards and using the Wiley GC-MS library. For each compound, quantification was performed by replotting the data, selecting the most abundant fragment and measuring the area under peaks. Heritability in narrow sense (h²) was calculated for 15 compounds, using mid-parent-offspring regression analysis (Falconer and Mackay, 1996). Aroma compounds were screened in fruit of each of the 174 genotypes including aldehydes (2-hexenal, hexanal); alcohols (3-hexen-1-ol, (Z)); furanones (furanol, mesifuran); esters (butyl acetate, ethyl acetate, hexyl acetate, methyl acetate, octyl acetate, 2-

methyl ethyl butanoate, ethyl butanoate, methyl butanoate, ethyl hexanoate, and methyl hexanoate). These compounds are often mentioned in the literature as relevant for strawberry aroma (Pérez et al., 2002; Zabetakis and Holden, 1997) and in preliminary studies, our team found 3-hexen-1-ol, (Z), furaneol, hexyl acetate, ethyl butanoate, and ethyl hexanoate in various foods (jelly and milk) and cosmetics (lipstick) with artificial strawberry aroma. To determine the relative contribution of the compounds to strawberry aroma, the aroma value [AV = concentration/odor threshold (OT)] was calculated (Devos et al., 1990; Larsen and Poll, 1992; www.levellingwell.com). Additionally, a panel of 5 to 7 persons sampled aromas of fresh fruit of the 174 genotypes, maintained in glass containers for 30 min at room temperature. They ranked each sample into one of six categories: 6 = superior aroma, 5 = strong aroma, 4 = medium aroma, 3 = moderately low aroma, 2 = low aroma, and 1 = no aroma. Correlation analysis determined the association between panel rank and the relative contribution of the selected aroma compounds.

To evaluate the relationships of aroma compounds diversity and panel's perception, Shannon's index (Shannon, 1948) was calculated for 72 esters and 2 furanones in a subsample of 25 genotypes previously ranked by the panel.

$$H = -\sum_{i=1}^s p_i \ln p_i$$

In our case p_i corresponded to the proportion of compound i relative to the total number of compounds by genotype.

Results

The aroma value (AV) indicates the relative impact of individual compounds to the overall aroma (Zabetakis and Holden, 1997). According to the AV level, three groups of compounds were identified in strawberry. The compounds: mesifuran, ethyl acetate, methyl butanoate, ethyl butanoate and ethyl hexanoate belong to the first group; these had the highest AV, ranging between 1007 and 4420 (Table 1). The second group included: methyl acetate, hexyl acetate, furaneol, and 2-methyl-ethyl-butanoate whose AV ranged between 26.1 and 56.5. Finally, the third group of compounds was formed by: hexanal, 3-hexen-1-ol (Z), methyl hexanoate,

butyl acetate, octyl acetate and 2-hexenal, with AV between 0.007 and 4.7.

Significant correlations ($p > 0.005$, Table 1) were found between panel rankings versus concentration and panel ratings versus AV for butyl acetate, hexyl acetate, 2-methyl ethyl butanoate, ethyl butanoate, methyl butanoate, ethyl hexanoate and methyl hexanoate. No significant correlation was found between furanones and panel ratings.

Large differences were found for compound abundance in the segregating population. The most aromatic samples (or genotypes) were those with highest diversity and abundance for esters. A significant correlation ($r = 0.89$, $p > 0.005$) was found between Shannon's Index and how strong an aroma was perceived by the panel. The most aromatic samples (13 genotypes) according to panel ratings, had H values ranging from 1.71 to 2.30 and 17 to 42 compounds, while the lowest rated fruit (12 genotypes) had H = 0 to 1.10 and 0 to 11 compounds.

The aroma classifications of the panel, showed a normal distribution for the six classes analyzed. Several of the individual compounds (Table 1), were significantly correlated ($P < 0.05$) with the ratings of aroma intensity determined by the panel: ethyl butanoate ($r = 0.53$), ethyl hexanoate ($r = 0.36$), butyl acetate ($r = 0.3$), hexyl acetate ($r = 0.28$), 2-methyl-ethyl butanoate ($r = 0.28$), 3-hexen-1-ol, (Z) ($r = 0.26$), methyl butanoate ($r = 0.21$), methyl hexanoate ($r = 0.2$), octyl acetate ($r = 0.19$), and furaneol ($r = 0.18$).

The heritability (Table 1) was high for 2-methyl ethyl butanoate (0.53) and 3-hexen-1-ol, (Z) (0.51), was medium for mesifuran (0.33), ethyl acetate (0.33), 2-hexenal (0.33), hexyl acetate (0.3) and low for furaneol (0.22), ethyl butanoate (0.22), butyl acetate (0.19), methyl acetate (0.17), methyl butanoate (0.12), ethyl hexanoate (0.11), and methyl hexanoate (0.05). The compounds hexanal and octyl acetate showed zero heritability. In these samples, the heritability was low ($h^2 = 0.04$) for aroma as a trait.

Discussion

There was consistency between panel classification and chemical diversity shown by Shannon's Index. According to AV levels, some of the most important compounds associated

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Table 1. Heritability (h^2), concentration, odor threshold (OT), and aroma value (AV) for 15 aroma volatiles from dark-red strawberry fruits.

Aroma volatiles	h^2 ^z	Concn (ppm)	OT (ppm) ^y	AV ^x
Aldehydes				
2-Hexenal	0.33	0.0007	0.1	0.007
Hexanal	0	0.4741	0.1	4.7
Alcohols				
3-Hexen-1-ol, (Z)	0.51	0.0106*	0.0039	2.7
Furanones				
Furaneol	0.22	0.4416*	0.01	44.2
Mesifuran	0.33	0.0442	0.00001	4420
Esters				
Butyl acetate	0.19	0.0775*	0.06	1.3
Ethyl acetate	0.33	12.8643	0.005	2573
Hexyl acetate	0.30	0.0949*	0.002	47.5
Methyl acetate	0.17	11.3096	0.2	56.5
Octyl acetate	0	0.0014*	0.0012	1.2
2-methyl-ethyl butanoate	0.53	0.0261*	0.001	26.1
Ethyl butanoate	0.22	1.0073*	0.001	1007
Methyl butanoate	0.12	1.6068*	0.001	1607
Ethyl hexanoate	0.11	0.3036*	0.0003	1012
Methyl hexanoate	0.005	0.2542*	0.1	2.5

^z h^2 = Heritability calculated for a sample size of 174 genotypes (*F. × ananassa* and *F. virginiana*). Significant correlations ($p < 0.05$) between panel ratings of aroma intensity and compound concentration are indicated in the concentration column.

^yDevos et al., 1990; www.leffingwell.com; Larsen and Poll, 1992.

^xAV = concentration/OT.

*Significant correlations at $p < 0.05$ between panel ratings of aroma intensity and compound concentration.

with strawberry aroma were mesifuran, ethyl acetate, methyl butanoate, ethyl hexanoate and ethyl butanoate, which agrees with previously published findings (Larsen and Poll, 1992; Pérez et al., 1992; Schreier, 1980). Moreover, we found all of these compounds in artificial strawberry aroma of some cosmetics and foods, indicating that they are important in the definition of aroma in strawberry.

Few studies have been done to determine the genetic control of aroma and its inheritance patterns in fresh fruit (Causse et al., 2001; Saliba-Colombani et al., 2001). Our results are coincident with those previously reported in other fruit, in that the aroma inheritance behaves as a complex trait.

Most aroma compounds studied showed variable heritabilities. The highest levels of heritability were shown by two compounds: 2- methyl-ethyl-butanoate ($h^2 = 0.53$) and 3-hexen-1-ol(Z) ($h^2 = 0.53$). Probably they are involved in the basal aroma in fresh strawberry

fruit. For example, it is known that 3-hexen-1-ol (Z) provides green notes to aroma for several species (Zabetakis and Holden, 1997)

Interestingly, the compounds with highest AV and abundance had medium to low heritabilities (0.11 to 0.33) in the fresh fruit analyzed. This result indicates that special breeding strategies, such as family selection and hybridization methods, should be applied to improve the aroma trait in strawberries. Heritability for aroma trait as a whole appears to be low; which would indicate that under the conditions of this experiment, the aroma character as well as the production of esters and furanones, are strongly influenced by the interaction between environment and genotype.

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