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Evaluation of Genotype and Environment Effects on Taste and Aroma Flavor Components of Spanish Fresh Tomato Varieties

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ABSTRACT: Taste and aroma related compounds have been analyzed in a collection of four traditional varieties and two tomato hybrids, representing a wide variability in fruit shape and color, grown in different environments: screenhouse and open field. Protected cultivation tended to show lower sugar concentration (fructose and glucose) but similar acid contents (citric, malic, and glutamic acids). The decreased levels of sucrose equivalents and the similar ratios of sucrose equivalents to citric or glutamic acid contents indicated that protected cultivation, despite being useful to reduce the incidence of pests and viral diseases, reduces the organoleptic quality. Additionally, it doubles the interaccession variability and increased the level of intra-accession variability. In the case of aroma, the genotypic effect was considerably higher than the environmental component on the 12 main volatiles analyzed. Only hexanal and methyl salicylate were significantly affected by environment, while 10 out of 12 volatiles were affected by the genotype. Biplot analysis showed that, even in considerably different environments, it is possible to identify genotype-dependent main aroma profiles. In the case of 13 background volatiles, the environment showed no significant effects and the genotypic effect was lower, though it is possible to identify genotypic trends in background notes.

KEYWORDS: *Solanum* section *Lycopersicon*, sugar, acid, volatile, variability, quality, genetic resources

INTRODUCTION

During the last decades, consumer complaints regarding tomato quality have become commonplace.[1] Several factors related to the production process or the genetic material may explain this loss of quality. Usually, fruits are harvested too early in the green stage and they are later artificially ripened, while it has been reported that vine ripened tomatoes have higher levels of volatile compounds[2] and a better fruity and tomato-like flavor. In addition, during the development of breeding programs the main objectives have been higher productivity, resistance to diseases, external appearance, and long shelf life, but organoleptic quality has received little attention. As a result, the high productivity of commercial varieties affects flavor negatively, as high plant fruit loads have been related to lower sugar concentration and lower overall quality.[3] Furthermore, the use of delayed ripening genes to obtain long shelf life may also affect quality due to detrimental changes in aroma profiles.[4] Even consumers may be contributing to this "lack of flavor", as tomatoes are usually refrigerated before being fully ripe,[1] which results in lower aroma volatiles[5] and altered flavor.

In this context, flavor characterization of tomato fruits is essential either in the search of sources of variation for breeding programs focused on quality traits or for the direct selection of the most suitable varieties and environmental conditions for quality markets. Although sensory evaluation is the best method to characterize fruit quality, these tests are expensive, time-consuming, and require a panel with a considerable number of experts, and panellists often constitute the first source of variation.[6] Thus, when a high number of samples are to be analyzed, this type of evaluation is substituted by chemical determinations.

The extreme importance of variation in sugars and acids content to genotypic flavor differences has been repeatedly reported in the tomato.[7] These compounds not only contribute to the sweetness and sourness of tomatoes, but they are also major factors in overall flavor intensity.[7] Although simple determinations such as total soluble solids content, pH, or the titratable acidity have been related to organoleptic quality, advances in analytical methods have enabled more precise determinations of single compounds. The role in preference and acceptability of the contents of the reducing sugars fructose and glucose, the citric and malic acids, and the ratio between reducing sugars and acids has been well established.[7,8] Bucheli et al.[9] have also highlighted the role of glutamic acid and its ratio to total sugar content in fruit acceptability.

Aroma analysis has only recently gained more importance, due to its highly complex nature and the complexity of analytic techniques. More than 400 volatile compounds have been described in tomato,[10] though mainly 20 compounds, including hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenal, 1-hexanol, (*Z*)-3-hexen-1-ol, 2-isobutylthiazol, 6-methyl-5-hepten-2-one, geranyl-acetone, and β-ionone,[11] would be important in the determination of the characteristic tomato flavor. Nevertheless, minor volatiles

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Table 1. Description of the Accessions Assayed

accession	local name	fruit shape	fruit color	fruit ribbing
CDP8908	Muchamiel	flattenned	red-orange	strong
CDP0916	Morada	slightly flattened	pink	intermediate
CDP8075	Valenciano (blanca subtype[a])	heart shaped	red-orange	weak
CDP9944	Valenciano	heart shaped	red-orange	weak
Cambria	Commmercial hybrid	rounded	red	very weak
Bond	Commmercial hybrid	slightly flattened	red	weak-intermediate

[a] Bigger size than standard Valenciano.

with negative logodor units may still be important to determine specific tomato flavor as background notes.[12] Some of the main compounds are not only a determinant for aroma descriptors but also affect the perception of sweetness and sourness.[8]

Despite the efforts made in the study of tomato flavor, little work has been carried out using traditional varieties of tomato, which are reputed to have excellent organoleptic quality. In fact, traditional varieties of tomato have found a place in specialized markets where consumers are willing to pay up to 4 times the price of commercial varieties in order to recover the true flavor of tomatoes.[13] In Spain there is an important diversity of this crop as the result of centuries of cultivation from its introduction in Europe during the first half of the 16th century.

The main objective of the present work was to develop the characterization of taste and aroma compounds of Spanish traditional varieties of tomatoes with reputed quality and high consumer acceptance and of control hybrids in two growing environments commonly used: open field and protected cultivation in a screenhouse, providing a high environmental contrast. This characterization enables the analysis of the relative importance of the environment, genotype, and its interaction on tomato flavor components.

This knowledge allows the optimization of the use of the growing environments in productions targeted to high-quality markets or of the selection in breeding programs, determining the real genetic potential of these genetic resources. It also contributes to ascertain the viability of developing high-quality cultivars with global adaptation characteristics or the necessity of developing environment-specific cultivars. The genotypic contribution to aroma profiles which has previously received little attention is also analyzed.

MATERIALS AND METHODS

Plant Materials and Experimental Design. Four accessions (CDP8908, CDP0916, CDP8075, and CDP9944) of Spanish traditional varieties of tomato from the genebank of the Institute for the Conservation and Improvement of Agrodiversity (COMAV) and two commercial hybrid varieties "Bond" and "Cambria" (Petoseed Co.Inc./Seminis, Almería, Spain) were used. The traditional varieties selected represented a wide diversity of fruit shapes and colors (Table 1). Accessions CDP8075 and CDP9944 belonged to two different subtypes of the same variety and were included to further analyze the effect of intravarietal variability.

The trials were carried out in two growing environments: in traditional open field conditions and under protected cultivation in a screenhouse (system usually used to prevent virus transmission by thrips and whiteflies). Traditional varieties are population varieties and thus represent a mixture of genotypes; therefore, for a precise evaluation of environment, genotype, and its interaction effects, clones of all the plants studied were used in each environment. Six mother plants per accession were grown by mid-February in glasshouse and several clones per plant were obtained in March. Two similar clones were selected from each plant and grown in a different growing system. For each growing system, six plants per accession were randomly distributed. The assay was carried out in Turís (39° 23′ N, 0° 42′ O; Valencia, Spain.). A spacing of 1.2 m × 0.4 m (2.1 plants m^{-2}) was applied. Plants were drip-irrigated, with the same doses and fertilization that are typically applied in tomato commercial plantations in the area of Valencia.

Fruit Sampling. Four representative fruits were collected from each plant at the mature-red stage (only from the first three trusses to minimize intraplant variability). Longitudinal wedges of the same weight were obtained from the fruits and ground and homogenized at low temperature in a laboratory blender (Micra D-8, Art-Labortechnik, Müllheim, Germany), and a bulked sample was obtained from each plant. The samples were kept frozen at −80 °C until analysis.

Analysis of Taste Components. The sugars fructose, glucose, and sucrose and the organic acids malic and citric and the glutamic acids were quantified following the method described by Roselló et al.[14] with modifications. Reagents and standards were purchased from Sigma-Aldrich Química S.A. (Madrid, Spain). Capillary electrophoresis was performed with a P/ACE MDQ (Beckman Instruments Inc., Fullerton, CA), controlled by the software 32 Karat V.5. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ) were used, with a 50 μm internal diameter, 363 μm external diameter, 67 cm total length, and 60 cm effective length. Capillaries were initially conditioned with consecutive rinses at 20 psi (137 895 Pa) and 50 °C: NaOH 1 N (5 min), NaOH 1 N (5 min), and deionized water (Elix 3, Millipore, Billerica, MA) (10 min). Following initial conditioning, the capillary was rinsed for 20 min at 20 °C with the separation buffer. Samples were thawed in darkness and then centrifuged at 2500 rpm (510*g*) for 5 min. The upper phase was diluted (1:10) in deionized water. The solution was filtered using 0.2 μm membranes and analyzed. The analysis conditions were as follows: hydrodynamic injection for 20 s at 0.5 psi; separation at −25 kV fixed voltage and 20 °C (separation buffer, 20 mM 2,6-piridin dicarboxylic acid (PDC) and 0.1% w/v hexadimethrine bromide, pH = 12.1). The capillary was rinsed with SDS (60 mM) for 3 min at 20 psi between samples, followed by the separation buffer at 20 psi for 3 min.

Analysis of Aroma Components. A total of 25 tomato volatiles have been determined in the present study: (*Z*)-3-hexenal, hexanal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*Z*)-3-hexen-1-ol, phenylacetaldehyde, 2-phenylethanol, methyl salicylate, 6-methyl-5-hepten-2-one, geranyl acetone, β-ionone, 2-isobutylthiazole, nonanal, (*E*)-2-octenal, guaiacol, eugenol, camphor, naphtalene, R-limonene, γ-terpinene, α-pinene, 6-methyl-5-hepten-2-ol, (*Z*)-citral, (*E*)-citral, and 1-hexanol. Reference aroma compounds were obtained from Sigma-Aldrich Química S.A. (Madrid, Spain, including Supelco and Fluka products) as pure compounds. Stock solutions of the aroma standards at 500 μg L^{-1} were prepared in acetone and stored at −18 °C. Working solutions were prepared by volume dilution in diethyl ether−hexane (1:1). The internal standard methyl salicylate-d_4 was of 99.5% purity and was purchased from SigmaAldrich, Sigma-Aldrich Química S.A. (Madrid, Spain).

Calcium chloride 97% (Riedel de Haen) was purchased from Supelco (Sigma-Aldrich Química S.A., Madrid, Spain).Organic solvents (hexane, ethyl acetate, diethyl ether) of trace residue analysis quality were purchased from Scharlab (Barcelona, Spain).

SPE cartridges (Supelco, Sigma-Aldrich Química S.A., Madrid, Spain) were prepared by the manufacturer packing 500 mg of Tenax TA (80−100 mesh,) in 6 mL polyetylene cartridges retained using two polyethylene frits.

The extraction system developed in a previous work[15] and consisted of a 50 mL Erlenmeyer flask attached to a glass cap with two connexion tubes: the inlet connected to a dry N_2 gas supply, and the outlet fitted to the Tenax trap. Dry nitrogen (99.7%) was used to carry out the purge process and was led to flow into the flask at a flow of 1 L min^{-1}. In total, 30 g of tomato sample together with a 5% (w/w) of $CaCl_2$ solution and with addition of 50 μL of 15 μg mL^{-1} methyl salicylate-d_4 (surrogate/internal standard) were magnetically stirred (350 rpm) and heated at 35 °C for 120 min in order to allow the volatile analytes to be retained in the Tenax trap (maintained at ambient temperature). The trap was removed and eluted with 3.5 mL of hexane−ether (1:1) mixture. The final volume extract was adjusted to 1 mL by means of a gentle stream of nitrogen.

Chromatographic determination was carried out using a Varian CP-3800 gas chromatograph (Varian Inc. Palo Alto, CA) coupled with a mass spectrometry detector (Saturn 4000, Varian Inc. Palo Alto, CA). Separation of the analytes was carried out on a 30 m × 0.25 mm DB-5MS (0.25 μm film thickness) Varian capillary column, using helium at 1 mL min^{-1} as the carrier gas. The temperature program was as follows: 45 °C for 5 min, then raised to 96 °C at a rate of 3 °C/min, then raised to 150 °C at a rate of 6 °C min^{-1}, and finally raised up to 240 °C at a rate of 30 °C min^{-1}, with a final isothermal stage of 1.5 min (total chromatographic analysis time of 36 min). Injection in the splitless mode of a volume of 1 μL (injection port temperature 200 °C) was carried out using an autosampler Varian 8400 (Varian Inc. Palo Alto, CA) equipped with a 10 μL syringe. The gas chromatograph was directly interfaced with the Varian 4000 mass spectrometer, ion trap, (Varian Inc. Palo Alto, CA) in the external ionization mode with an electron ionization energy of 70 eV in the positive ion mode. The transfer line temperature was established at 250 °C, and ion source and trap temperatures were adjusted to 200 °C.

Quantitation of analytes in the sample extracts was performed using a calibration curve obtained plotting relative areas to internal standard methyl salycilate-d_4 against concentration (nanogram $milliliter^{-1}$) as described by Beltran et al.[15] The quantification ion used for the internal standard methyl salicylate-d_4 was 155. This ion corresponded to the molecular mass of the compound after having exchanged the deuterium in the alcohol group for hydrogen, which occurs due to contact with the aqueous sample.

Derived Variables and Statistical Analysis. In order to provide a better understanding of the factors studied over the taste component of organoleptic quality, three composite variables were derived. Total sugars were expressed as sucrose equivalents, a term calculated from fructose and glucose contents multiplied by 0.74 and 1.73, respectively, and which is related to sweetness.[16] Other variables derived from solid soluble contents were the ratio of sucrose equivalents to citric acid content and the ratio of sucrose equivalents to glutamic acid content. In the case of aromas, the compounds quantified were grouped in two sets of variables. The first one included main aroma notes: 12 volatiles that have been previously described as important in the determination of tomato aroma and have positive or close to 0 logodor units.[12] In this case, the concentrations were expressed as logodor units, a variable more closely related to aroma perception. This set of compounds included the first 12 listed in the analysis of aroma section. The second set included the remaining 13 compounds representative of the aroma background. In this case, logodor units were not used as the odor threshold is much higher than the concentration and therefore the variable loses its purpose.

The mixed linear model used for the analysis of i genotype in j environment and k block inside environment j was

$$Y_{ij} = \mu + G_i + E_j + \mathrm{GE}_{ij} + e_{ij}$$

where Y = phenotypic value with population mean μ and variance V_P; G = genotype effect with mean 0 and variance V_G; E = environment effect with mean 0 and variance V_E; GE = genotype × environment interaction effect with mean 0 and variance $V_{G\times E}$; e = residual effect with mean 0 and variance V_e. First, genotype, environment, and its interaction were considered as fixed factors in order to study their influence in the phenotypic expression of the characters. This analysis of variance was carried out using the generalized least square (GLS) estimation to avoid the problems generated by the lack of normal distribution of the data obtained and applying the Herderson method III. Later, all the factors were considered as random and predicted using the adjusted unbiased prediction (AUP) method. Standard errors of the statistics were obtained by the jackknife procedures,[17] and two-tail t-tests were performed for testing the significance of parameters obtained. The use of a resampling method was very restrictive in the identification of statistically significant effects but allowed the relative generalization of the results obtained. All the data analyses were performed with QTModel (v. 0.7) software (kindly provided by Prof. Jun Zhu, director of the Bioinformatics Institute, Zhejiang University, China).

Additionally, an estimate of variability was obtained for each environment and genotype; these estimates included the intra-accession and interaccession components. Intra-accession variability was calculated as the coefficient of variation per environment and genotype and expressed as a percentage. The mean values per environment were calculated as the mean of the coefficients of variation of all the accessions. Interaccession variability was calculated as the coefficient of variation of the means of the accessions, thus avoiding the consideration of intra-accessions data dispersion.

Finally, a graphical multivariate statistical analysis, using the biplot method, was done to more easily study the complex relationships between accessions assayed and the sets of aroma constituents.[18] In GGE biplot analysis, singular value decomposition (SVD) of the two-way data table of accessions (rows) and traits (columns) was used. In this SVD, the singular values are entirely partitioned into row eigenvectors to preserve the row metric in order to graphically compare genotypes. However, prior to SVD, the original two-way data table must be adequately preprocessed (centered and scaled). The data centering was done in order to use the best model to show differences between accessions:

$$Y_{ij} - \mu - \beta_j = \alpha_i + \phi_{ij}$$

where y_{ij} is the phenotypic value of each cell of the two-way trait table, μ the grand mean, G_i the accession (row) main effect, β_j the trait (column) main effect, and ϕ_{ij} the specific interaction between the last two factors. The Phenotype x trait view was used to better highlight the growing system comparisons.

Additionally, for an effective visualization of the "which is best for what" pattern of the aromatic constituents of the accessions tested, a polygon view of the GGE biplot was used. The polygon is drawn joining the accessions located fastest to the biplot origin (vertex accessions, which have the highest content in the aromas in his respective direction) and used to compare adjacent vertex accessions.[18] All GGE biplot analyses and graphics were carried out with the GGEbiplot software (licensed by Dr. Weikai Yan, Ottawa, Canada).

RESULTS AND DISCUSION

The organoleptic characteristics of the samples were analyzed considering two groups of variables: soluble components and

Table 2. Soluble Component Determinations Including Main Sugars and Acids Affecting Tomato Organoleptic Quality and Derived Ratios Involved in Fruit Acceptability[a]

accession	growing system	citric acid	malic acid	glutamic acid	fructose	glucose	sucrose equiv	ratio sucrose equiv/citric acid	ratio sucrose equiv/glutamic acid
Bond	OF	4169 ± 354	1437 ± 101	1623 ± 19.5	15 207 ± 572	10 955 ± 507	34 415 ± 1257	9.5 ± 0.8	28.8 ± 3.9
Bond	SH	3843 ± 411	1354 ± 148	895 ± 8.7	13 422 ± 582	10 980 ± 594	31 346 ± 1432	8.8 ± 0.4	39.5 ± 2.9
Cambria	OF	4159 ± 315	1337 ± 95	1627 ± 15.0	15 671 ± 697	11 052 ± 804	35 289 ± 1692	8.8 ± 0.4	26.8 ± 4.5
Cambria	SH	4745 ± 305	2222 ± 239	2464 ± 23.3	16 767 ± 916	11 600 ± 515	37 592 ± 1818	8.3 ± 0.6	16.8 ± 1.4
CDP0916	OF	2607 ± 279	1215 ± 104	1784 ± 13.2	14 137 ± 684	10 054 ± 633	31 897 ± 1631	15.5 ± 2.2	19.3 ± 1.6
CDP0916	SH	2595 ± 299	1824 ± 166	1976 ± 18.1	12 946 ± 567	7 394 ± 702	27 869 ± 1353	12.0 ± 0.8	15.9 ± 1.4
CDP8908	OF	3096 ± 230	1413 ± 51	1662 ± 24.1	15 933 ± 493	12 871 ± 588	37 089 ± 1258	12.9 ± 1.0	30.9 ± 5.1
CDP8908	SH	2421 ± 205	1827 ± 293	1273 ± 13.8	14 180 ± 654	10 007 ± 898	31 937 ± 1587	15.3 ± 2.0	31.8 ± 6.2
CDP8075	OF	4472 ± 330	1196 ± 175	1699 ± 20.9	14 976 ± 513	11 680 ± 876	34 551 ± 1313	8.3 ± 0.6	25.5 ± 3.2
CDP8075	SH	5430 ± 326	1816 ± 127	2258 ± 21.2	15 425 ± 839	10 905 ± 947	34 754 ± 1983	6.5 ± 0.2	16.3 ± 1.1
CDP9944	OF	2949 ± 216	1497 ± 80	2478 ± 21.0	15 154 ± 536	11 847 ± 880	34 983 ± 1411	13.0 ± 1.1	15.4 ± 1.1
CDP9944	SH	2625 ± 147	1670 ± 132	2195 ± 21.6	11 508 ± 534	8 855 ± 397	26 462 ± 1153	10.5 ± 0.7	14.2 ± 1.7

[a] Means and standard errors. All compounds expressed in mg kg^{-1} except for ratios. OF, Open field; SH, screenhouse.

aromas. Both groups have considerably different natures and affect the perception of the flavor differently, so a separate presentation and discussion of the results obtained in each category seemed appropriate.

Taste Components. Main sugars (fructose, glucose, and sucrose) and organic acids (citric, malic, and glutamic acids) were determined (Table 2). Only traces of sucrose were detected in the analysis and were not further considered, an expected result due to the usual negligible concentrations of this sugar in red-ripe tomatoes.[7]

Regarding sugar accumulation, significant environmental, genotypic, and interaction effects were found (Table 3). The general mixed linear model used for the prediction of the growing environment, genotype, and interaction factor levels on the total phenotypic response showed that the fructose and glucose contents were significantly lower in the tomatoes grown under the screenhouse (Table 3). In our trials, the climatic conditions observed in the screenhouse represented a mean reduction of 26.7% of the average photosynthetically active radiation and an increase of 3.8% of the average day temperature, a common result for these kinds of protection structures.[19] Consequently, the effect of the reduction in the sugar content caused by plant shading[20,21] had a higher influence than the increased fruit sink power leading to higher dry matter contents associated with higher temperatures.[22] Regarding the genotype effect, cultivar Cambria showed a significantly high increment of fructose over the model mean content, with this sugar having a higher sweetening power.[7] On the contrary, traditional accessions CDP9944 and CDP0916 showed significantly lower fructose contents. Regarding glucose content, only CDP0916 showed significantly lower content than the mean. Additionally, a strong genotype × environment effect was detected in some cases. For example, the model estimates of the interactions for fructose content were not significant for Cambria, Bond, and CDP8075, indicating a good stability of these cultivars in the expression of the character in the environments studied. However, the traditional accession CDP9944 showed a high significant interaction with the open field due to its large adaptation to this traditional production system, whereas the interaction detected for accessions CDP0916 and CDP8908 increased their fructose content in protected cultivation. This behavior was similar in the case of glucose for CDP8908 and CDP9944 in screenhouse cultivation. Cultivar Cambria showed an expected high significant interaction value under the screenhouse (commercial hybrid adapted to protected production); on the contrary, accession CDP99443 had lower values in the open field (Table 3). This last accession belongs to a subtype of the variety "Valenciano" usually grown in greenhouses.

In the case of the main organic acids, the citric contents were not environment dependent (Table 3). Only significant genotype effects were observed, with considerably higher values in the accessions CDP8075 and the cv. Cambria (Table 3). No significant genotype × environment interactions were detected. On the contrary, malic acid contents were significantly affected by the growing system showing higher accumulation under protection. No important genotype effects were observed (all the predictions were 1000 or 2000 times lower than the environment effect). Significant interaction estimates were identified in accessions CDP0916, CDP8908, and CDP9944 and in cv. Bond. In all these cases, the interaction tended to counteract the environmental effect (Table 3), thus resulting in a relative stability. In general, as the main organic acids are concerned, these results are relatively consistent with those review by Dorais et al.,[21] as in the assays reviewed the effect of high intensity of photosynthetically active radiation (typical of open field conditions) increased soluble sugars but had little effect on organic acid accumulation.

Glutamic acid content was significantly genotype dependent (Table 3). Higher values were obtained in the accession CDP9944 and lower in the cv. Bond. The growing system had a significant but very limited effect (hundreds or thousands times lower than genotypic effects) on glutamic acid accumulation. Considerable genotype × environment interactions were found in all the genotypes but in cv. Cambria. They were of a similar magnitude as the genotype effect and highlighted the instability of the trait. Yamanaka et al.[23] reported much higher glutamic acid contents in open field cultivation than in the glasshouse. However, it should be noted that Davies and Hobson[20] clarified that the differences reported by Yamanaka et al. could be due either to season or to varietal effects and not to the differences in the environment. In our case, a strong genotypic and interaction effects (G + G × E) reveals an important general genetic-dependent response.

Table 3. Statistical Significance of the Genotype, Environment, and Their Interaction Using a Mixed Model of Variance Components Analysis[a]

	citric acid	malic acid	glutamic acid	fructose	glucose	sucrose equivalents	ratio sucrose equiv/citric acid	ratio sucrose equiv/glutamic acid
G	Fpr < 10^{-15}	Fpr = 0.25	Fpr < 10^{-6}	Fpr < 10^{-3}	Fpr < 0.01	Fpr < 10^{-3}	Fpr < 10^{-10}	Fpr < 10^{-9}
E	Fpr = 0.88	Fpr < 10^{-5}	Fpr = 0.97	Fpr < 0.01	Fpr < 10^{-3}	Fpr < 10^{-3}	Fpr = 0.06	Fpr = 0.42
G × E	Fpr = 0.09	Fpr = 0.02	Fpr < 10^{-3}	Fpr < 0.01	Fpr = 0.03	Fpr = 0.01	Fpr = 0.14	Fpr = 0.01
				Model Estimates				
mean	3591***	1562***	1826***	14 597***	10 667***	33 148***	10.8***	23.5
				G				
Bond	395 ± 233*	−0.2 ± 0.1***	−371 ± 180**	−185 ± 266	187 ± 271	−191 ± 596	−1.5 ± 0.5***	9.3 ± 2.6***
Cambria	815 ± 198***	0.2 ± 0.1***	141 ± 100*	1005 ± 679*	416 ± 420	2085 ± 1454*	−2 ± 0.4***	−1.4 ± 2
CDP0916	−945 ± 191***	−0.1 ± 0.1	34 ± 73	−670 ± 468*	−1276 ± 868**	−2120 ± 1393*	2.8 ± 1***	−5.1 ± 1.4***
CDP8908	−791 ± 156***	0.1 ± 0.1	−234 ± 140*	280 ± 295	491 ± 475	855 ± 804	3.1 ± 0.9***	6.8 ± 3.4**
CDP8075	1292 ± 215***	−0.1 ± 0.1	99 ± 102	371 ± 333	397 ± 464	947 ± 853	−3.2 ± 0.4***	−2.2 ± 1.6
CDP9944	−766 ± 141***	0.2 ± 0.1	332 ± 182**	−801 ± 499**	−215 ± 342	−1576 ± 1075*	0.9 ± 0.6	−7.5 ± 1.6***
				E				
OF	−0.1 ± 0.3*	−205.7 ± 48.8***	−0.1 ± 0.01***	452 ± 265**	650 ± 239***	1306 ± 559**	0.4 ± 0.5	0.01 ± 0.001***
SH	0.1 ± 0.3*	205.7 ± 48.8***	0.1 ± 0.01***	−452 ± 265**	−650 ± 239***	−1306 ± 559**	−0.4 ± 0.5	−0.01 ± 0.001***
				G × E				
Bond*OF	224 ± 257	188.2 ± 118.3**	222 ± 166	353 ± 482	−551 ± 474	155 ± 1044	−0.5 ± 0.6	−2.5 ± 3.2
Bond*SH	15 ± 149	−203.6 ± 132.1*	−399 ± 171***	−535 ± 636	−681 ± 665	−1453 ± 1497	−0.7 ± 0.6**	3.5 ± 3.1
Cambria*OF	−225 ± 174*	−152.2 ± 101.8*	−79 ± 131	−296 ± 523	38 ± 486	−470 ± 1219	1.6 ± 1.8	−1.2 ± 1.3
Cambria*SH	42 ± 123	−03.9 ± 108.6	98 ± 191	619 ± 461	1099 ± 660**	1930 ± 1186*	−0.4 ± 0.8	1.4 ± 3.5
CDP0916*OF	4 ± 155	−162.6 ± 140.5	−259 ± 189	−574 ± 516	−17 ± 632	−985 ± 1230	−0.5 ± 0.5*	2.8 ± 2.4
CDP0916*SH	−71 ± 107	115.8 ± 86.1*	−402 ± 187**	1019 ± 545**	785 ± 720	2355 ± 1367**	−0.8 ± 0.9	−3.2 ± 1.3***
CDP8908*OF	−25 ± 214	−320.9 ± 164.1**	702 ± 152***	−586 ± 512	756 ± 531*	−385 ± 1134	−0.3 ± 0.4	9.7 ± 4.5***
CDP8908*SH	398 ± 303*	366.4 ± 215.6**	581 ± 214***	1802 ± 842**	1137 ± 648**	3963 ± 1828***	−0.4 ± 0.4	−4.6 ± 2.4**
CDP8075*OF	−253 ± 204*	115.4 ± 130.7	124 ± 150	−549 ± 500	−1436 ± 697***	−2083 ± 1233**	−0.2 ± 0.6	−2.8 ± 1.5**
CDP8075*SH	−442 ± 284	44.6 ± 200.6	−400 ± 158***	−26.6 ± 51.8	−561 ± 684	−900 ± 1249	2.0 ± 2.2	3.9 ± 4.1
CDP9944*OF	649 ± 461*	115.5 ± 123.4	387 ± 191**	104.1 ± 68.9*	453 ± 701	2126 ± 1608	−1.1 ± 1**	−4.5 ± 2.1***
CDP9944*SH	−316 ± 202*	−102.7 ± 111.6	27 ± 179	−202.8 ± 66.2***	−1021 ± 576**	−4252 ± 1523***	−0.3 ± 0.5	−2.7 ± 1.5**

[a] Genotype (G); environment (E; OF, open field; SH, screenhouse) and genotype × environment interaction (G × E). Values expressed in mg kg^{-1} except for ratios as means and standard errors. ∗, significant at 0.1 level; ∗∗, significant at 0.05 level; ∗∗∗, significant at 0.01 level.

In all the soluble components determined, a considerable level of variation was found. This variability was analyzed considering its components: intra-accession variability (mean differences among plants within accession) and interaccession variability (differences among the means of the accessions), being considerably higher than the intra-accession component (Table 4). The relevance of intra-accession variability was expected in the traditional varieties as these should be considered population varieties and thus genetically heterogeneous but not in the case of the commercial hybrid cultivars which should be genetically homogeneous. In this last case, the effect must be a consequence of microenvironmental differences. Therefore, this result emphasizes the important amount of variability due to the environment in soluble compounds affecting organoleptic quality.

Differences in the components of variation were detected between the two growing conditions. Almost twice as much interaccession variability was found when plants were cultivated in the screenhouse as in the open field. In the case of intra-accession variability, the differences between both growing systems were less important and could not be generalized for all the soluble components nor all the accessions (Table 4). Nevertheless, it seems that screenhouse cultivation increased the level of intra-accession variability, especially in the case of sugars. One plausible explanation would be that light intensity variability is considerably higher in the protected cultivation due to the shadowing effects of the structure. In this case, temperature distribution is also more variable than in the open field due to the reduced air flows that homogenize temperature.[24] It should also be noted that the levels of intra- and intervariability were much higher for organic acids than for sugars (Table 4). In all the cases, differences cannot be due to analytical variation, as the repeatability of the analysis methodology is lower than 3.3% for all the compounds.

Differences in variability were also detected among the accessions tested, as well as an interaction between the accession and growing system. Accession CDP9944 stood for lower intra-accession coefficient of variation in almost all the soluble components, while as previously stated the commercial hybrids showed a unexpected high degree of variation.

Obviously, the degree of inter- and intra-accession variability joined to the important genotype × environment interaction detected strengthen the idea that experimental design should be

Table 4. Coefficients of Variation of Soluble Component Determinations Including Main Sugars and Acids Affecting Tomato Organoleptic Quality and Derived Ratios Involved in Fruit Acceptability[a]

genotype	environment	citric acid	malic acid	glutamic acid	fructose	glucose	sucrose equiv	ratio sucrose equiv/ citric acid	ratio sucrose equiv/ glutamic acid	mean CV
Bond	OF	31.5	38	53.6	16.8	20.7	16.3	38.9	61.3	34.6
Bond	SH	48.8	47.8	43.3	19.4	24.2	20.4	19.7	33.4	32.1
Cambria	OF	28.5	30.3	37	17.8	29.1	19.2	16.7	66.6	30.7
Cambria	SH	38.2	24.5	40.5	24.8	15.5	20.5	29.1	35.2	28.5
VL199B	OF	36.3	45.4	31.5	20.5	26.7	21.7	61	35.4	34.8
VL199B	SH	38.5	48.9	38.8	18.6	40.3	20.6	26.8	37.6	33.8
VL242	OF	13.6	27.8	54.3	11.6	17.1	12.7	28.6	62	28.5
VL242	SH	59.9	31.7	40.5	17.3	33.6	18.6	49.9	73.3	40.6
VL243	OF	58.6	29.5	49.3	13.7	30	15.2	29.6	49.5	34.4
VL243	SH	28	24	37.5	21.8	34.7	22.8	14	26.8	26.2
VL246	OF	21.4	29.3	33.9	14.1	29.7	16.1	33.2	29.4	25.9
VL246	SH	31.6	22.4	39.3	18.6	17.9	17.4	26	46.8	27.5
mean intra-accession CV	OF	31.7	33.4	43.3	15.8	25.6	16.9	34.7	50.7	
mean intra-accession CV	SH	40.8	33.2	40.0	20.1	27.7	20.1	27.6	42.2	
mean intra-accession CV	both	36.2	33.3	41.6	17.9	26.6	18.5	31.1	46.4	
interaccession CV	OF	9.1	21.9	18.3	4.1	8.4	4.8	25.4	24.2	
interaccession CV	SH	15.3	35.4	34.7	12.0	15.9	12.2	31.1	47.9	
interaccession CV	both	19.3	28.2	26.7	9.4	13.7	9.9	27.4	35.6	

[a] Values expressed as percentages. OF, open field; SH, screenhouse.

carefully used in assays dealing with taste quality in tomatoes. This would be the case of selection programs for elite local varieties targeted to quality markets or of the selections of segregating populations performed in conventional commercial breeding programs. In this sense it would be essential to deal with a high number of individuals or clones, random block designs, and multienvironmental assays. Otherwise, biased conclusions might be obtained.

In general, the protected cultivation in screenhouse, with lower light intensity and higher temperature, tended to offer lower sugar concentrations with similar acid contents and, thus, probably a lower organoleptic quality. This type of cultivation seemed not to considerably affect the intra-accession variability, though it would increase varietal differences. Nevertheless, in order to have a clearer view of the effect of accession and growing system over organoleptic quality, new variables with a previously demonstrated relation with fruit acceptability were derived from the individual determinations.

In this sense, Baldwin et al.[8] concluded that tomato acceptability was positively correlated with total sugar content expressed as sucrose equivalents, a variable that also showed a strong correlation with sweetness perception. Tomato acceptability has additionally been related with the ratio between this sucrose equivalent and total acidity.[8] In order to calculate a similar ratio in the present study, the citric acid content was used instead of total acidity by considering the correlation observed between these variables in other studies[20,25] and also between citric acid and sourness.[7] In previous experiments using part of the same varieties, the sugar content expressed as sucrose equivalents and the ratio of sugar content to citric acid showed high correlations with preference and flavor intensity, whereas the inclusion of malic acid in the ratio showed lower correlations.[13] Malic acid is about 14% more sour than citric acid,[26] though its lower concentration (approximately 10 times lower than citric acid) limits its influence on the tomato taste. In addition, the ratio between sucrose equivalents and glutamic acid was calculated as this ratio has also been reported to be correlated with fruit acceptability.[9]

Sucrose equivalents were significantly affected by the genotype, growing system, and their interaction (Table 3). The cv. Cambria showed the highest values, a consistent result with its higher contents in fructose as this sugar has a higher impact in sweetness perception. In general, screenhouse cultivation diminished the amount of sucrose equivalents, though considerable interactions of different magnitude were identified in the traditional varieties (Table 3).

Only the genotype significantly affected the ratio of sucrose equivalents to citric acid. Higher estimates were obtained for the accessions CDP0916, CDP9944, and CDP0916 (Table 3). In the case of the ratio sucrose equivalents to glutamic acid, the genotype and the interaction had a significant effect but not the environment. Accession CDP8908 and cv. Bond obtained significantly higher values for this ratio.

The pattern of variability found in these new variables was different than that of the original ones. In the case of sucrose equivalents, higher intra-accession than interaccession variability was observed, though in the case of the ratios sucrose equivalents to citric or glutamic acid, the coefficients of variation for each component of variance were similar. Higher interaccession variability in the three variables was found in the screenhouse, and this was also true for sucrose equivalents intra-accession variability but not in the case of the ratios that showed lower intra-accession variability when the plants were grown in the screenhouse. Again, screenhouse cultivation tended to emphasize differences among accessions.

In general, the lower values of sucrose equivalents and the similar values of the ratios sucrose equivalents to citric acid and

Table 5. Determinations of Main Aroma Compounds[a]

accession	growing system	(*Z*)-3-hexenal	hexanal	(*E*)-2-hexenal	(*E*)-2-heptenal	(*Z*)-3-hexen-1-ol	phenylacetaldehyde	2-phenylethanol	methylsalicylate	6-methyl-5-hepten-2-one	geranylacetone	*β*-ionone	2-isobutylthiazole
Bond	OF	n.d.	317.9 ± 0.1	46.7 ± 20.7	10.0 ± 3.8	236.7 ± 137.0	5.8 ± 2.0	n.d.	253.8 ± 86.0	331.6 ± 81.7	24.5 ± 5.4	24.6 ± 7.3	14.0 ± 4.4
Bond	SH	n.d.	n.d.	33.6 ± 9.9	2.8 ± 1.3	32.1 ± 9.1	10.7 ± 5.7	2.9 ± 2.9	263.9 ± 64.3	231.1 ± 63.9	17.4 ± 4.8	23.9 ± 5.9	7.4 ± 2.9
Cambria	OF	16.8 ± 16.8	548.2 ± 25.0	67.8 ± 19.9	4.7 ± 1.3	125.2 ± 95.3	5.9 ± 4.8	n.d.	49.3 ± 49.3	116.4 ± 61.2	27.2 ± 5.7	8.0 ± 6.7	25.3 ± 5.5
Cambria	SH	18.9 ± 18.9	703.7 ± 137.8	77.3 ± 24.1	10.9 ± 5.2	226.2 ± 138.8	1.9 ± 1.2	1.6 ± 1.6	170.9 ± 56.8	286.6 ± 75.2	24.7 ± 2.3	8.9 ± 4.7	17.6 ± 5.2
CDP0916	OF	n.d.	257.4 ± 41.0	50.4 ± 15.2	n.d.	103.4 ± 30.5	3.4 ± 0.4	11.6 ± 3.9	144.0 ± 32.8	223.4 ± 68.0	18.2 ± 2.8	8.1 ± 1.9	6.5 ± 2.8
CDP0916	SH	n.d.	189.8 ± 27.1	141.8 ± 26.9	n.d.	353.4 ± 194.8	7.1 ± 1.4	22.7 ± 4.4	238.5 ± 34.3	255.9 ± 75.5	25.1 ± 3.2	9.8 ± 1.1	43.4 ± 33.9
CDP8908	OF	n.d.	39.3 ± 1.0	1.4 ± 1.4	9.6 ± 4.9	80.9 ± 50.9	1.8 ± 0.7	4.8 ± 4.8	333.1 ± 153.1	85.7 ± 46.0	7.4 ± 3.1	7.7 ± 4.2	9.5 ± 3.4
CDP8908	SH	n.d.	40.6 ± 5.0	5.7 ± 1.7	5.8 ± 2.6	18.4 ± 12.7	3 ± 1.9	n.d.	759.5 ± 206.4	110.2 ± 35.1	8.6 ± 2.6	11.3 ± 8.2	7.0 ± 1.7
CDP8075	OF	61.3 ± 8.3	957.9 ± 211.5	72.5 ± 26.5	1.8 ± 1.1	86.3 ± 59.2	2.9 ± 1.9	n.d.	145.9 ± 88.8	326.4 ± 34.5	19.9 ± 5.4	7.3 ± 0.7	0.5 ± 0.3
CDP8075	SH	34.4 ± 20	573.3 ± 245.4	58.4 ± 22.4	3.3 ± 1.9	43.8 ± 24.6	3.3 ± 1.1	n.d.	130.5 ± 102.6	260.0 ± 48.9	11.8 ± 2.2	9.6 ± 1.7	n.d.
CDP9944	OF	n.d.	1006.9 ± 0.1	70.5 ± 16.3	14.8 ± 4.9	92.1 ± 37.9	1.3 ± 0.8	n.d.	152.0 ± 66.3	326.3 ± 47.4	20.9 ± 3.6	15.8 ± 7.0	4.3 ± 1.3
CDP9944	SH	n.d.	203.0 ± 0.1	151.3 ± 47.0	6.5 ± 1.7	7.8 ± 7.8	2.9 ± 1.0	n.d.	184.9 ± 93.9	171.4 ± 48.5	13.6 ± 3.2	7.0 ± 2.5	1.2 ± 0.9

[a] Means and standard errors. All compounds expressed in ng g^{-1}. OF, open field; SH, screenhouse; n.d., below detection limits.

Table 6. Determinations of Background Aroma Compounds[a]

accession	growing system	nonanal	(*E*)-2-octenal	guaiacol	eugenol	camphor	naphtalene	R-limonene	*γ*-terpinene	α-pinene	6-methyl-5-hepten-2-ol	(*Z*)-citral	(*E*)-citral	1-hexanol
Bond	OF	16.8 ± 3.0	26.9 ± 7.9	265.5 ± 44.9	44.2 ± 7.7	6.2 ± 1.4	1.2 ± 0.4	27.1 ± 7.9	3.6 ± 3.6	0.5 ± 0.5	n.d.	29.1 ± 10.8	55.2 ± 17.1	2.6 ± 2.6
Bond	SH	13.2 ± 1.3	13.3 ± 6.3	297.1 ± 78.1	45.5 ± 6.2	5.1 ± 0.5	0.8 ± 0.3	27.0 ± 8.1	1.6 ± 1.6	1.9 ± 0.4	2.1 ± 1.4	26.3 ± 4.8	55.4 ± 6.2	15.0 ± 7.6
Cambria	OF	9.0 ± 2.2	8.8 ± 3.2	181.0 ± 130.2	21.3 ± 9.6	2.5 ± 1.2	1.7 ± 0.6	9.9 ± 2.4	n.d.	0.2 ± 0.2	0.2 ± 0.2	23.2 ± 4.4	66.9 ± 9.4	24.6 ± 18.1
Cambria	SH	8.1 ± 0.9	7.4 ± 3.7	95.9 ± 28.0	19.9 ± 6.3	2.5 ± 0.9	1.4 ± 0.6	16.7 ± 7.7	n.d.	0.7 ± 0.5	3.2 ± 3.0	24.8 ± 5.9	59.9 ± 12.8	75.1 ± 36.6
CDP0916	OF	14.1 ± 4.0	24.0 ± 9.8	234.8 ± 31.1	98.6 ± 28.4	3.6 ± 1.0	0.4 ± 0.2	30.5 ± 9.1	n.d.	1.4 ± 0.8	3.9 ± 2.5	15.9 ± 5.1	54.6 ± 18.3	17.6 ± 6.7
CDP0916	SH	19.9 ± 1.5	16.2 ± 2.3	350.7 ± 15.8	79.5 ± 12.9	5.4 ± 0.3	0.2 ± 0.2	20.9 ± 3.7	n.d.	1.0 ± 0.6	0.2 ± 0.2	13.5 ± 1.6	67.8 ± 7.5	50.9 ± 39.9
CDP8908	OF	11.9 ± 3.2	10.7 ± 3.4	141.5 ± 54.5	92.5 ± 35.0	2.3 ± 1.3	0.8 ± 0.6	8.3 ± 2.4	n.d.	0.2 ± 0.2	n.d.	6.5 ± 3.3	19.2 ± 11.3	36.7 ± 28.8
CDP8908	SH	19.2 ± 2.3	13.0 ± 4.3	297.6 ± 68.8	176.8 ± 51.4	11.0 ± 4.2	1.0 ± 0.8	15.4 ± 6.4	8.2 ± 5.4	0.1 ± 0.1	n.d.	1.7 ± 1.7	29.0 ± 13.5	n.d.
CDP8075	OF	21.7 ± 9.9	6.3 ± 3.7	383.9 ± 163.0	56.9 ± 21.3	2.3 ± 1.4	0.7 ± 0.5	13.2 ± 1.4	n.d.	n.d.	n.d.	18.7 ± 6.7	64.0 ± 10.7	20.5 ± 20.5
CDP8075	SH	13.7 ± 5.4	15.0 ± 5.0	191.4 ± 54.0	39.2 ± 4.1	3.7 ± 1.3	0.1 ± 0.1	20.6 ± 10.1	n.d.	n.d.	n.d.	14.0 ± 8.1	63.2 ± 10.5	n.d.
CDP9944	OF	9.0 ± 2.7	12.3 ± 7.0	197.1 ± 67.6	61.2 ± 18.1	4.4 ± 1.1	1.3 ± 0.4	27.3 ± 10.7	n.d.	1.1 ± 0.5	n.d.	58.3 ± 32.2	67.3 ± 4.6	42.4 ± 22.4
CDP9944	SH	10.5 ± 2.0	16.2 ± 5.1	189.6 ± 59.6	92.8 ± 28.7	11.5 ± 7.4	1.0 ± 0.3	11.7 ± 2.9	n.d.	1.7 ± 0.6	n.d.	11.8 ± 5.4	24.0 ± 8.5	2.7 ± 2.7

[a] Means and standard errors. All compounds expressed in ng g^{-1}. OF, open field; SH, screenhouse; n.d., below detection limits.

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Table 7. Effect of Environment, Genotype, and Their Interaction on Main and Background Aroma Compounds[a]

main aroma compounds (p values on concentration)				aroma background compounds (p values on logodor units)			
	E	G	G × E		E	G	G × E
β-ionona	0.865	0.014	0.984	α-pinene	0.186	0.018	0.507
hexanal	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	camphor	0.067	0.280	0.313
(*E*)-2-heptenal	0.320	$<10^{-5}$	0.775	(*E*)-2-octenal	0.536	0.169	0.418
(*E*)-2-hexenal	0.089	10^{-5}	0.419	(*E*)-citral	0.501	0.014	0.228
geranyl acetone	0.743	$<10^{-3}$	0.948	eugenol	0.322	$<10^{-4}$	0.226
methyl salicilate	0.042	$<10^{-3}$	0.157	γ-terpinene	0.433	0.210	0.186
phenylacetaldehyde	0.529	0.216	0.653	guaiacol	0.866	0.236	0.275
isobutylthiazole	0.073	$<10^{-5}$	0.591	naphtalene	0.339	0.086	0.973
2-phenylethanol	0.276	$<10^{-5}$	0.339	nonanal	0.810	0.037	0.259
6-methyl-5-hepten-2-one	0.204	0.058	0.078	R-limonene	0.868	0.137	0.442
(*Z*)-3-hexen-1-ol	0.157	0.017	0.079	1-hexanol	0.839	0.366	0.171
(*Z*)-3-hexenal	0.343	$<10^{-5}$	0.276	6-methyl-5-hepten-2-ol	0.632	0.484	0.177
				(*Z*)-citral	0.136	0.095	0.234

[a] Environment (E), genotype (G), and genotype × environment (G × E). p values of ANOVAs obtained in mixed linear models.

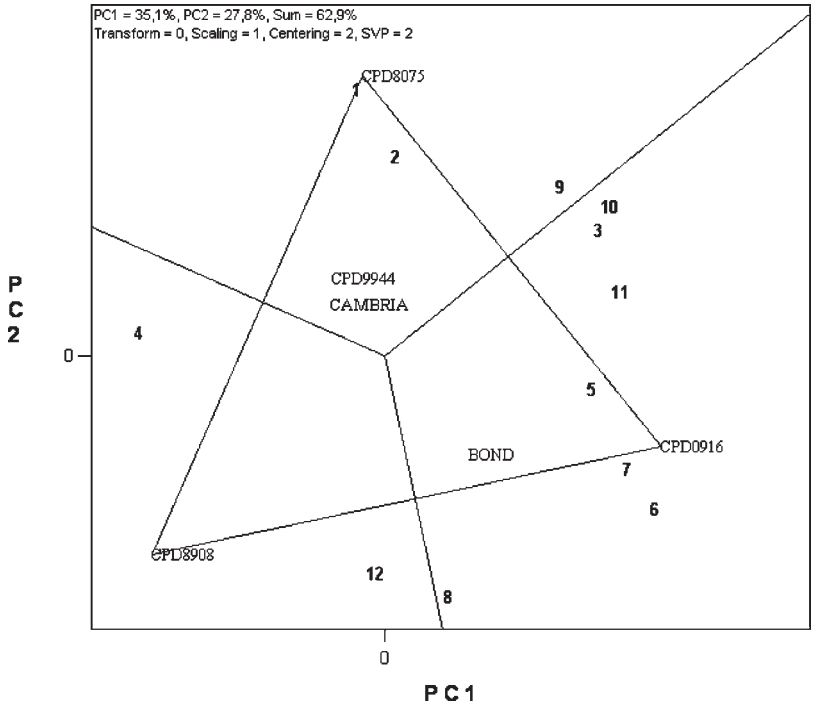

Figure 1. GGE biplot analysis of main aroma volatiles showing the "which is best for what" display with genotypic averages: (1) (*Z*)-3-hexenal; (2) hexanal/capronaldehyde; (3) (*E*)-2-hexenal; (4) (*E*)-2-heptenal; (5) (*Z*)-3-hexen-1-ol; (6) phenylacetaldehyde; (7) 2-phenylethanol; (8) methylsalicylate; (9) 6-methyl-5-hepten-2-one; (10) geranylacetone; (11) β-ionone; (12) 2-isobutylthiazole.

sucrose equivalents to glutamic acid obtained in the analysis model with the protected cultivation reinforces the idea that screenhouse cultivation, though providing lower incidence of pests and viral diseases, reduces organoleptic quality. This negative effect on quality-related variables is more obvious if the actual values are reviewed without considering the resampling carried out in the model.

Aroma Components. The evaluation of genotype and environment effects on tomato aroma is much more complex. In some species, single compounds prevail in the determination of the aroma, as it is the case of 3-methylbutyl acetate in bananas. However, in tomatoes no single compound dominates and 16 volatiles have been described as having positive logodor units.[12] Nevertheless, compounds with negative logodor units should not be neglected, as they may still contribute to the overall flavor as background notes,[12] and it has recently been proved that some of them, such as eugenol, may have an impact on tomato aroma upon release from their glycosidic conjugates.[27] Therefore, according to their relative importance in aroma configuration, the volatiles analyzed were grouped in two sets, main aromas (Table 5) and background notes (Table 6).

In the group of main aroma notes, the environment was only significant for hexanal and methyl-salicilate (Table 7). Despite the low number of compounds being significantly affected, it

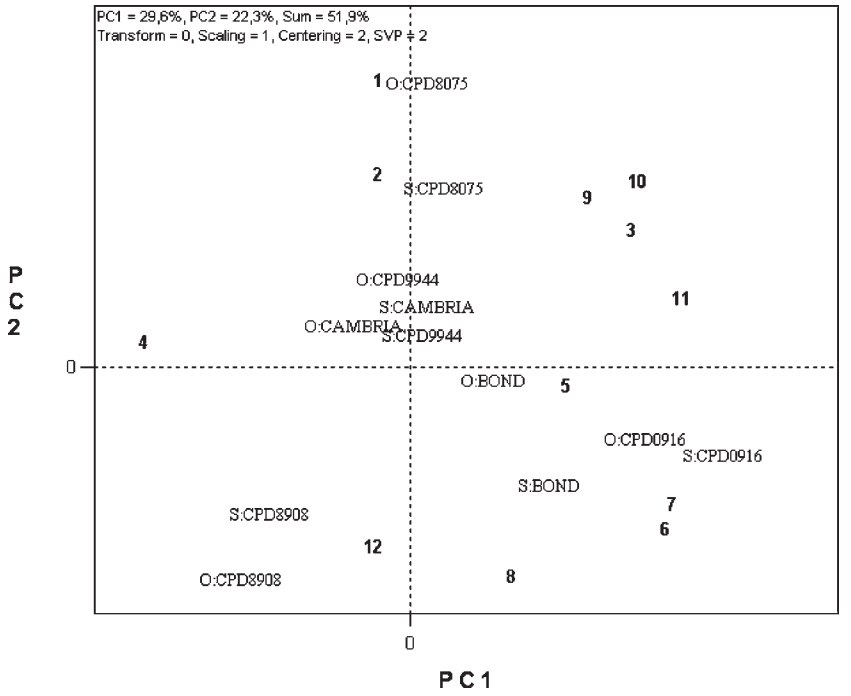

Figure 2. GGE Biplot analysis of main aroma volatiles using environmental averages. "O" as prefix indicates open field cultivation, "S" as prefix indicates screenhouse cultivation: (1) (*Z*)-3-hexenal; (2) hexanal/capronaldehyde; (3) (*E*)-2-hexenal; (4) (*E*)-2-heptenal; (5) (*Z*)-3-hexen-1-ol; (6) phenylacetaldehyde; (7) 2-phenylethanol; (8) methylsalicylate; (9) 6-methyl-5-hepten-2-one; (10) geranylacetone; (11) β-ionone; (12) 2-isobutylthiazole.

should be pointed out that hexanal is precisely one of most important volatiles contributing to tomato aroma. Mixed model analysis showed significantly higher logodor units estimates in open field conditions in the case of hexanal, whereas the opposite occurred in the case of methyl-salicylate. Dalal et al.[28] detected a different trend when comparing field and glasshouse grown fruits, as they found higher levels of all the volatile compounds in the open field cultivation except for hexanal, hexanol, and isobutanol.

The effect of genotype was much more important. It was significant for almost all the compounds, excluding phenylacetaldehide (Table 7) and 6-methyl-5-hepten-2-one; though in the last case the probability (p = 0.058) was very close to the significance level. A genotype × growing system interaction was only detected for hexanal.

Regarding the group of background notes, the effect of environment was not significant for any of the volatiles, and the genotype had significant effects only in 4 out of the 12 compounds determined: α-pinene, (*E*)-citral, eugenol, and nonanal. This result strengthens the importance of main aroma volatiles beyond their concentration over the perception threshold, as environmental and genotypic effects in background notes are much less important.

The interpretation of the aroma results becomes easier observing the GGE biplots. Considering that the genotype was highly significant in the case of main aroma compounds, a biplot with this set of variables expressed as logodor units was obtained, explaining 62.9% of the variance (Figure 1). The biplot display of the "which is best for what" showed that accession CDP8908 had the higher values of 2-isobutylthiazole and (*E*)-2-heptenal. CDP8075 stood out for (*Z*)-3 hexenal, hexanal, and 6-methyl-5-hepten-2-one, and CDP6618 had globally higher levels of methyl-salicilate, 2-phenyl-ethanol, phenyl-acetaldheide, β-ionone, (*E*)-2-hexenal, and geranyl-acetone. The same analysis showed that Cambria and CDP9944 had similar aroma profiles (Figure 1).

A single compound might have a positive or negative effect on aroma perception depending on its concentration.[10] Nevertheless, some authors have given general trends to be pursued. In this sense, Tandon et al.[29] suggested that increased levels of compounds contributing to floral (6-methyl-5-hepten-2-one and β-ionone) and fruity ((*Z*)-3-hexenal and geranylacetone) notes and reduced levels in compounds contributing to stale (hexanal, (*E*)-2-hexenal), pungent (2-isobutylthiazole), and alcohol (2-phenylethanol) notes would likely be beneficial to tomato flavor. Baldwin et al.[8] also found a positive correlation between (*Z*)-3-hexenol and overall acceptability. Therefore, genotypes CDP 0916 and CDP8075 would have high interest in this sense, even though they also show high levels of negative compounds, as it can be deduced from the biplot (Figure 1). Though previously published studies on chemical and sensory correlations are helpful to guess probable outcomes, it should considered, as it has been previously stated, that the best way to ascertain the effect on aroma perception is the use of sensory panels.[6]

In order to highlight the influence of the growing environments on aroma contents in each accession, the Phenotype x trait view of GGEbiplot was calculated using environment averages of genotypes (Figure 2). In this case the biplot explained 51.7% of the variance. In the biplot, the means from the same genotype but from different environments clustered together and generally apart from the rest of genotypes. This was especially evident for CDP0916, Bond CDP8075, and CDP8908, while Cambria and CDP9944 were grouped together. This arrangement reinforces the results obtained from the predictions of the mixed model analysis, in the sense that though the environment had an effect on the aroma profile, it was much lower than the genotypic effect. Ruiz et al.[30] studying similar traditional varieties also suggested that genotype differences might play a more important role than previously thought.

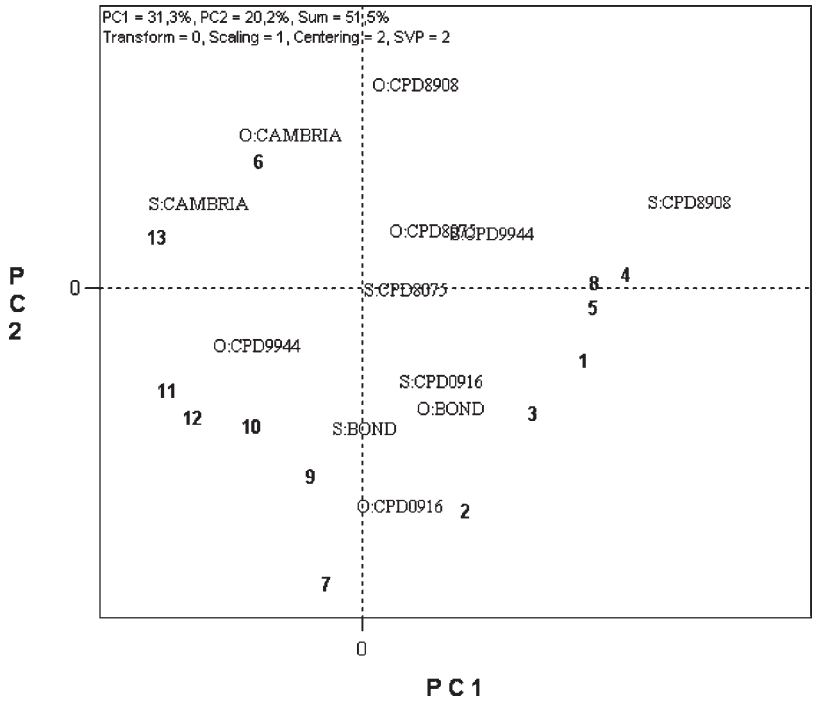

Figure 3. GGE Biplot analysis of background aroma volatiles using environmental averages. "O" as a prefix indicates open field cultivation, "S" as a prefix indicates screenhouse cultivation: (1) nonanal; (2) (*E*)-2-octenal; (3) guaiacol; (4) eugenol; (5) camphor; (6) naphtalene; (7) R-limonene; (8) γ-terpinene; (9) α-pinene; (10) 6-methyl-5-hepten-2-ol; (11) (*Z*)-citral; (12) (*E*)-citral; (13) 1-hexanol.

Additionally, the effect of the genotype × environment interaction could also be identified in the biplot. Although for all the genotypes higher values of the first component were obtained in the screenhouse, changes on the second component (direction and magnitude) were genotype-dependent. It is difficult to interpret the limited effect on the first component, as despite being related to higher contents of "positive" compounds such as 6-methyl-5-hepten-2-one, geranylacetone, and β-ionone, it was also related to higher contents of "negative" compounds including (*E*)-2-hexenal and 2-phenylethanol.

The Phenotype x trait GGEbiplot was also calculated for background notes using concentrations, and it explained 51.5% of the variance. In this case both environmental averages were closely plotted in the case of Bond, CDP0916, cv. Cambria, and CDP8075 (Figure 3). In the case of CDP8908, though the environmental averages were plotted at a certain distance, they were relatively distant from other genotype averages. Despite the proximity of environmental averages, the genotypes were not as clearly separated as in the case of the main aroma notes, though a certain trend could be identified. Again, this result seems to confirm the previous mixed model analysis, as the effect of genotype and environment is more diffuse in the case of background notes than for main aroma notes.

In conclusion screenhouse cultivation tends to reduce organoleptic quality and increases the level of variation in taste related variables. Even commercial hybrids showed considerable levels of variation due to microenvironmental conditions. Nevertheless, it is possible to identify genotypes with higher levels of environmental variability, a priority in the development of elite materials targeted to quality markets. These results strengthen the necessity to carry out multienvironmental trials using genetic clones of genotypes within populations in order to perform genotype selection efficiently. The crucial role of genotype in the definition of main aroma notes has been established. It seems that it would be possible to identify variety dependent aroma profiles and therefore clear targets for breeding programs. Nevertheless, it also seems that selection for positive compounds might be interfered with by negative ones.

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REFERENCES

(1) Bruhn, C. M.; Feldman, N.; Garlitz, J. H.; Ivans, E.; Marshall, M.; Riley, A.; Thurber, D.; Williamson, E. Consumer perception of quality: apricots, cantaloupes, peaches, pears, strawberries, and tomatoes. *J. Food Qual.* **1991**, *14*, 187–195.

(2) Maul, F.; Sargent, S. A.; Balaban, M. O.; Baldwin, E. A.; Huber, D. J.; Sims, C. A. Aroma volatile profiles from ripe tomatoes are influenced by physiological maturity at harvest: An application for electronic nose technology. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 1094–1101.

(3) Bertin, N.; Guichard, C.; Leonardi, J. J.; Longuenesse, D.; Langlois, D.; Navez, B. Seasonal evolution of the quality of fresh glasshouse tomatoes under Mediterranean conditions, as affected by air vapour pressure deficit and plant fruit load. *Ann. Bot.* **2000**, *85*, 741–750.

(4) McGlasson, W. B.; Last, J. H.; Shaw, K. J.; Meldrum, S. K. Influence of the non- ripening mutants rin and nor on the aroma of tomato fruit. *HortScience* **1987**, *22*, 632–634.

(5) Baldwin, E. A.; Nisperos-Carriedo, M. O.; Scott, J. W. Tomato flavor volatile profiles: cultivar and harvest maturity effects. *Proc. Tomato Quality Workshop* **1992**, 18–29.

(6) Causse, M.; Saliba-Colombani, V.; Buret, M.; Lesschaeve, I.; Issanchou, S. Genetic analysis of organoleptic quality in fresh market

tomato 2. Mapping QTLs for sensory attributes. *Theor. Appl. Genet.* **2001**, *102*, 273–283.

(7) Stevens, M. A.; Kader, A. A.; Albright-Holton, M.; Algazi, M. Genotypic variation for flavor and composition in fresh market tomatoes. *J. Am. Soc. Hortic. Sci.* **1977**, *102*, 680–689.

(8) Baldwin, E. A.; Scott, J. W.; Einstein, M. A.; Malundo, T. M. M.; Carr, B. T.; Shewfelt, R. L.; Tandon, K. S. Relationship between sensory and instrumental analysis for tomato flavor. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 906–915.

(9) Bucheli, P.; Voirol, E.; Torre, R. R.; Lopez, J.; Rytz, A.; Tanksley, S. D.; Petiard, V.; de la Torre, R. Definition of nonvolatile markers for flavor of tomato (Lycopersicon esculentum Mill.) as tools in selection and breeding. *J. Agric. Food Chem.* **1999**, *47*, 659–664.

(10) Petro-Turza, M. Flavor of tomato and tomato products. *Food Rev. Int.* **1987**, *2*, 309–351.

(11) Buttery, R. G. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits In *Flavor Science: Sensible Principles and Techniques*; Acree, T. E., Teranishi, R., Eds.; American Chemical Society: Washington, DC, 1993; pp 259−283.

(12) Baldwin, E. A.; Scott, J.; Shewmaker, C. K.; Schuh, W. Flavor trivia and tomato aroma: biochemistry and possible mechanism for control of important aroma components. *HortScience* **2000**, *35*, 1013–1022.

(13) Cebolla-Cornejo, J.; Roselló, S.; Galiana-Balaguer, L.; Nuez, F. Evaluación de la calidad organoléptica de variedades tradicionales de tomate y del efecto del cultivo bajo malla. *Actas Portuguesas Hortic.* **2005**, *8*, 302–308.

(14) Rosello, S.; Galiana-Balaguer, L.; Herrero-MArtinez, J. M.; Maquieira, A.; Nuez, F. Simultaneous quantification of the main organic acids and carbohydrates involved in tomato flavour using capillary zone electrophoresis. *J. Sci. Food Agric.* **2002**, 10–1101.

(15) Beltran, J.; Serrano, E.; López, F. J.; Valcárcel, M.; Roselló, S. Comparison of two quantitative GC-MS methods for analysis of tomato aroma based on purge-and-trap and on solid-phase microextraction. *Anal. Bioanal. Chem.* **2006**, *385*, 1255–1264.

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

(17) Zhu, J.; Weir, B. S. Diallel analysis for sex-linked and maternal effects. *Theor. Appl. Genet.* **1996**, *92*, 1–9.

(18) Yan, W.; Kang, M. S. *GGE Biplot Analysis. A Graphical Tool for Breeders, Geneticist and Agronomist*; CRC Press: Boca Raton, FL, 2003; p 288.

(19) Desmarais, G.; Ratti, C.; Raghavan, G. S. V. Heat transfer modelling of screenhouses. *Solar Energy* **1999**, *65*, 271–284.

(20) Davies, J. N.; Hobson, G. E. The constituents of tomato fruits: the influence of environment, nutrition and genotype. *Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 205–280.

(21) Dorais, M.; Papadopulus, A. P.; Gosselin, A. Greenhouse tomato fruit quality. *Hortic. Rev.* **2001**, *26*, 239–319.

(22) Ho, L. C.; Hewitt, J. D. Fruit development. In *The Tomato Crop*; Atherton, J. G., Rudich, J., Eds.; Chapman and Hall: London, 1986; pp 201−240.

(23) Yamanaka, H.; Chachin, K.; Ogata, K. Studies on the metabolism of free amino acids during maturation and ripening of tomato fruits. I. Changes of free amino acid contents during ripening of tomato fruits and in parthenocarpic tomato fruits induced by auxins. *J. Jpn. Soc. Hortic. Sci.* **1971**, *40*, 81.

(24) Teitel, M. The effect of screens on the microclimate of greenhouses and screenhouses − A review. ISHS Acta Horticulturae 719: International Symposium on Greenhouse Cooling, 2006; pp 575−586.

(25) Fulton, T. M.; Bucheli, P.; Voiol, E.; Lopez, J.; Petiard, V.; Tanksley, S. D. Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical porperties possibly contrinuting to flavor, identified in four advanced backcross populations of tomato. *Euphytica* **2002**, *127*, 163–167.

(26) Gardner, W. H. *Food Acidulants*; Allied Chemical Corporation: New York, 1966.

(27) Ortiz-Serrano, P.; Gil, J. V. Quantitative Comparison of Free and Bound Volatiles of Two Commercial Tomato Cultivars (Solanum lycopersicum L.) during Ripening. *J. Agric. Food Chem.* **2010**, *58*, 1106–1114.

(28) Dalal, K. B.; Olson, L. E.; Yu, M. H.; Salunkhe, D. K. Gas chromatography of the field-, glass- Greenhouse-grown, and artificially ripened tomatoes: Lycopersicon esculentum mill. *Phytochemistry* **1967**, *6*, 155–157.

(29) Tandon, K. S.; Abegaz, E.; Shewfelt, R. L.; Baldwin, E. A.; Scott, J. W. Interrelationship of sensory descriptors and chemical composition as affected by harvest maturity and season on fresh tomato flavor. *Proc. Florida State Hortic. Soc.* **2000**, *113*,

(30) Ruiz, J. J.; Alonso, A.; García-Martínez, S.; Valero, M.; Blasco, P.; Ruiz-Bevia, F. Quantitative analysis of flavour volatiles detects differences among closely related traditional cultivars of tomato. *J. Sci. Food Agric.* **2005**, *85*, 54–60.