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Evaluation of germplasm in *Solanum* section *Lycopersicon* for tomato taste improvement

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Abstract: The genetic potential of accessions from *Solanum* section *Lycopersicon* (*S. lycopersicum* L., *S. lycopersicum* var. *cerasiforme*, *S pimpinellifolium* L., and *S. habrochaites* Knaap & Spooner) for breeding tomato taste has been studied in three environments with clonal replicates. The environment clearly affected the accumulation and level of variation of sugars and acids and derived variables through a direct effect. It seems that photosynthetically active radiation would exert a major effect on sugar accumulation while in the case of organic acids the effect of temperature might be more important. Even more, important genotype × environment interactions can considerably modify the real value of germplasm, being considerably higher in wild species. The environment affected not only mean contents but also the levels of variation. Thus, the need to develop multienvironmental screening programs is suggested to identify solid sources of variation. An important intraaccession variability was also found in wild germplasm, emphasizing the need to analyze a high number of plants per accession in order to identify sources of variation. Accessions with a significant genotypic contribution to the accumulation of sucrose within the *Lycopersicon* group were identified and may be interesting to analyze the regulation of vacuolar invertase. Accessions with different genotypic contributions to citric, malic, and glutamic acid accumulation have also been identified. These accessions will be valuable for the development of breeding programs considering the acid component of taste. Additionally, these genetic resources will be interesting to study the regulation of the tricarboxylic acid cycle and the gamma-aminobutyric acid shunt.

Key words: Environment, genotype × environment, organic acids, organoleptic quality, sugars

1. Introduction

Concerns regarding the loss of tomato taste became evident in the 1990s (Bruhn et al., 1991), but these complaints are still concerning researchers (Bennett, 2012). The special emphasis placed on high yield during selection had negative side effects on fruit quality, as well as the introgressions from wild species and the use of genes such as *uniform ripening* (*u*), or those related to delayed ripening (*nor*, *rin*), or even the replacement of alleles associated with the biosynthesis of key flavor volatiles (Bertin et al., 2000; Causse et al., 2003; Powell et al., 2012; Tieman et al., 2017). Other common practices by farmers or consumers, such as the harvest of mature green fruits (Kader et al., 1977) or refrigeration after purchase, have also contributed to this degeneration in flavor.

In this context, great interest has been placed in increasing organoleptic quality in tomato. Considering the difficulty existing in the improvement of aroma (with more than 20 important compounds and their relationships and background notes contributing to this trait), most efforts have focused on improving tomato taste. Stevens et al. in 1977 had already revealed that tomato taste is significantly determined by the concentration of sugars, acids, and the relation between them. Among sugars, at the red ripe stage, the concentrations of sucrose are negligible and the most important are fructose and glucose. Nevertheless, as sucrose sweetening power is a common reference it is usual that concentrations of sugars are expressed as sucrose equivalents multiplying the concentrations of each sugar by the relative sweetness compared to sucrose (Koehler and Kays, 1991). In fact, this derived variable is highly correlated with sweetness perception in sensory panels (Baldwin et al., 1998).

Citric and malic acids are the most prominent organic acids in tomato. The concentration of citric acid is usually higher and the balance between both of them is variety-

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dependent (Davies and Hobson, 1981). It is more difficult to determine the relative contribution of each acid to sourness, as it depends on pH, concentrations, and other variables. Nevertheless, at pH close to 4.5 (quite standard for tomato), malic acid may be perceived as sourer (De Bruyn et al., 1971). The amino acid glutamic acid does not seem to play an important role in the perception of sourness, but some researchers have pointed out that a high ratio between sucrose equivalents and glutamic acid may be convenient to improve tomato taste (Bucheli et al., 1999; Fulton et al., 2002). Nevertheless, more insight is required regarding the role of glutamic acid in tomato taste.

Wild species of tomato have been used to improve different traits in tomato ever since the 1930s (Rick, 1986). In the case of organoleptic quality, several wild species have been used in the improvement of fruit quality (Fernie et al., 2006). In fact, important genes altering the carbohydrate metabolism have been identified in wild germplasm. For example, *Solanum chmielewskii* (C. M. Rick, E. Kesicki, J. F. Fobes & M. Holle) D. M. Spooner, G. J. Anderson & R. K. Jansen accumulates sucrose instead of hexoses in the fruit, though side effects such as a reduction in fruit weight and yield of ripe fruits are found (Chetelat et al., 1995). *Solanum habrochaites* Knaap & Spooner also accumulates low levels of hexoses, but with a high ratio of fructose to glucose (>1.5:1), though it does not increase total sugar content in tomato (Schaffer et al., 1999). *Solanum pennellii* Correll has offered better results in increasing total sugar content in tomato. One of the detected mutations in the catalytic site of the apoplastic invertase LIN5 (Fridman et al., 2000) facilitates increased invertase activity in the fruit, leading to a higher capacity to take up sucrose from the phloem and increased levels of hexoses in the ripe tomato fruit (Matsukura, 2016).

Despite these advances, today, the search for sources for variation targeted to the improvement of tomato taste still represents a continuous necessity. The objective of the present study is to evaluate the potential of different accessions from tomato and wild relatives as donors of traits related to tomato taste perception. Previous studies with functional compounds suggested that the genotype × environment (G × E) interactions may interfere with the consistency and reliability of screening programs (Leiva-Brondo et al., 2012, 2016). Therefore, for this work the number of species and accessions was sacrificed to enable the evaluation of the specific contribution of genotype and environment. This information enabled the analysis of the difficulties that arise in this kind of screening programs. Specifically, several landraces of cultivated tomato (*Solanum lycopersicum* L. and *S. lycopersicum* L. var. *cerasiforme*) and accessions from the tomato ancestor species *Solanum pimpinellifolium* L. were included. They were selected as their close phylogenetic relation with tomato would make it easier to exploit their potential in breeding programs. One accession from *S. habrochaites* was also included to represent the more distant green-fruited species.

2. Materials and methods

2.1. Plant material and cultivation

Thirteen accessions from different species were evaluated, including materials from *S. lycopersicum* L., *S. lycopersicum* var. *cerasiforme*, *S. pimpinellifolium*, and *S. habrochaites* (Table 1). Three modern tomato cultivars with normal levels of sugars and acids were included as controls: CDP8779 (experimental line), Cambria (a commercial hybrid), and Gevora (a processing tomato variety).

The accessions were evaluated during 1 year in three different environments at two Spanish sites: Valencia and Turis. Cultivation at Valencia was carried out in autumn-winter and spring-summer cycles in a glasshouse with automated climate control. Cultivation at Turis took place in the spring-summer cycle in the open air.

In protected cultivation, heating systems were used in the autumn-winter cycle when required, while progressive shadowing and cooling was used in the spring-summer cycle. In all cases, fertigation was scheduled daily, and plants were staked and pruned. Air temperature and photosynthetically active radiation (PAR) were recorded every 10 min using WatchDog weather stations (Spectrum Technologies Inc., Aurora, IL, USA) equipped with temperature and quantum light PAR sensors and a data logger. Fertigation was adapted for each cycle considering crop necessities.

As wild accessions may be composed of different genotypes, 3 clones were obtained from each plant and grown in each of the three environments. Consequently, an exact clonal replicate was used in each environment and block, enabling a precise evaluation of the contribution of genotypes and environment. A randomized complete block design was used with 4 blocks per environment, 16 plots per block (one per accession), and 8 plants per plot. Clones from the shoot apex were obtained and propagated in vitro following the procedure described by Cano et al. (1998).

2.2. Sampling and analysis of sugars and acids

Uniformly ripe, healthy fruits at the final-ripe stage (maximum color intensity) were harvested per plant. The number of representative fruits harvested (5–20) varied depending fruit weight. Fruits were collected only from the first three trusses to minimize intraplant variability. The fruits of each plant were ground and blended with a homogenizer (Diax 900, Heidolph, Schwabach, Germany). Thus, each sample represented the biological mean of a single plant, and it was stored at –80 °C until analysis.

Table 1. Characteristics of the accessions evaluated.

Code	Accession	Species	Fruit characteristics	Origin
1	CDP8779	1	Large, light red	Valencia, Spain, X
2	Cambria	1	Medium-sized, red	Almería, Spain (Seminis Vegetables Seeds), X
3	Gevora	1	Medium-sized, red	Badajoz, Spain, X
4	LA3538	1	Medium-sized, intense red, high lycopene	University of California, Z
5	LA1563	1	Large, red, high SSC	University of California, Z
6	CDP2178	1	Medium-sized, red	Piura, Peru, X
7	CDP7632	1	Medium-sized, red	Loja, Ecuador, X
8	CDP2087	1	Large, red	Gran Canaria, Spain, X
9	CDP6957/A	1	Small, yellow	Alicante, Spain, X
10	CDP6957/R	1	Small, red	Alicante, Spain, X
11	CDP4777	2	Small, orange-brownish	Ipala, Guatemala, X
12	CDP7090	3	Very small, dark red	Piura, Peru, X
13	CDP1568	3	Very small, dark red	Piura, Peru, X
14	CDP9822	3	Very small, dark red	Piura, Peru, X
15	CDP9999	3	Very small, yellow	Lambayeque, Peru, X
16	CDP4941	4	Very small, green	Loja, Ecuador, X

1, *Solanum lycopersicum*; 2, *S. lycopersicum* var. *cerasiforme*; 3, *S. pimpinellifolium*; 4, *S. habrochaites*. X, Supplied by COMAV, Spain; Z, Supplied by Tomato Genetics Resource Center, USA.

The sugars (fructose, glucose, and sucrose) and the organic acids (malic, citric, and glutamic) were quantified by capillary zone electrophoresis following the method described by Cebolla-Cornejo et al. (2012). Capillary electrophoresis was performed with an Agilent 7100 capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany).

2.3. Statistical analysis

Sucrose equivalents, a variable highly correlated with sweetness perception (Baldwin et al., 1998) were calculated by adding up glucose, fructose, and sucrose contents multiplied by 0.74, 1.73, and 1, respectively. Following the same approximation, acid equivalents were calculated considering the relative sourness of each compound, summing up the contents of citric acid and malic acid multiplied by 1 and 1.14, respectively (Stevens et al., 1977). Several authors pointed out the importance of the ratios of sugar to acid (Stevens et al., 1977; Baldwin et al., 1998). Consequently, the ratios of sucrose equivalents to citric acid, glutamic acid, and acid equivalents were also calculated.

To obtain deeper insight into the contribution of the growing environment (E), genotype (G), and their interaction (GE) to the phenotypic expression (Y) of each taste-related component, we used the following mixed linear model considering the i genotype, j environment, and k block (B) inside each environment:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + B_{k(j)} + e_{ijk}$$

All the factors were considered as random and predicted using the adjusted unbiased prediction method. Standard errors of the statistics were obtained by jackknife procedures and two-tailed t-tests were performed for testing the significance of parameters obtained. All the data analyses were performed with QGAStation (v. 2) software (Bioinformatics Institute, Zhejiang University, China).

MANOVA biplots were used to study the level of variation within accessions in each environment, providing the results of Turis spring-summer as an example. Univariate Bonferroni confidence circles were added to the group markers. Significance of the difference between two accessions-environments can be inferred if the projections on a variable vector do not overlap. MultBiplot, a software free-licensed by Professor Vicente Villardón (http://biplot.usal.es/ClassicalBiplot/index.html), was used for these calculations. Coefficients of variation for each analyte and environment were also calculated.

3. Results

3.1. Environmental effects

Only the ratio of malic to citric acid showed null general environmental contributions. In this case, the environment affected the phenotypic value mainly via G × E interactions (Figure 1).

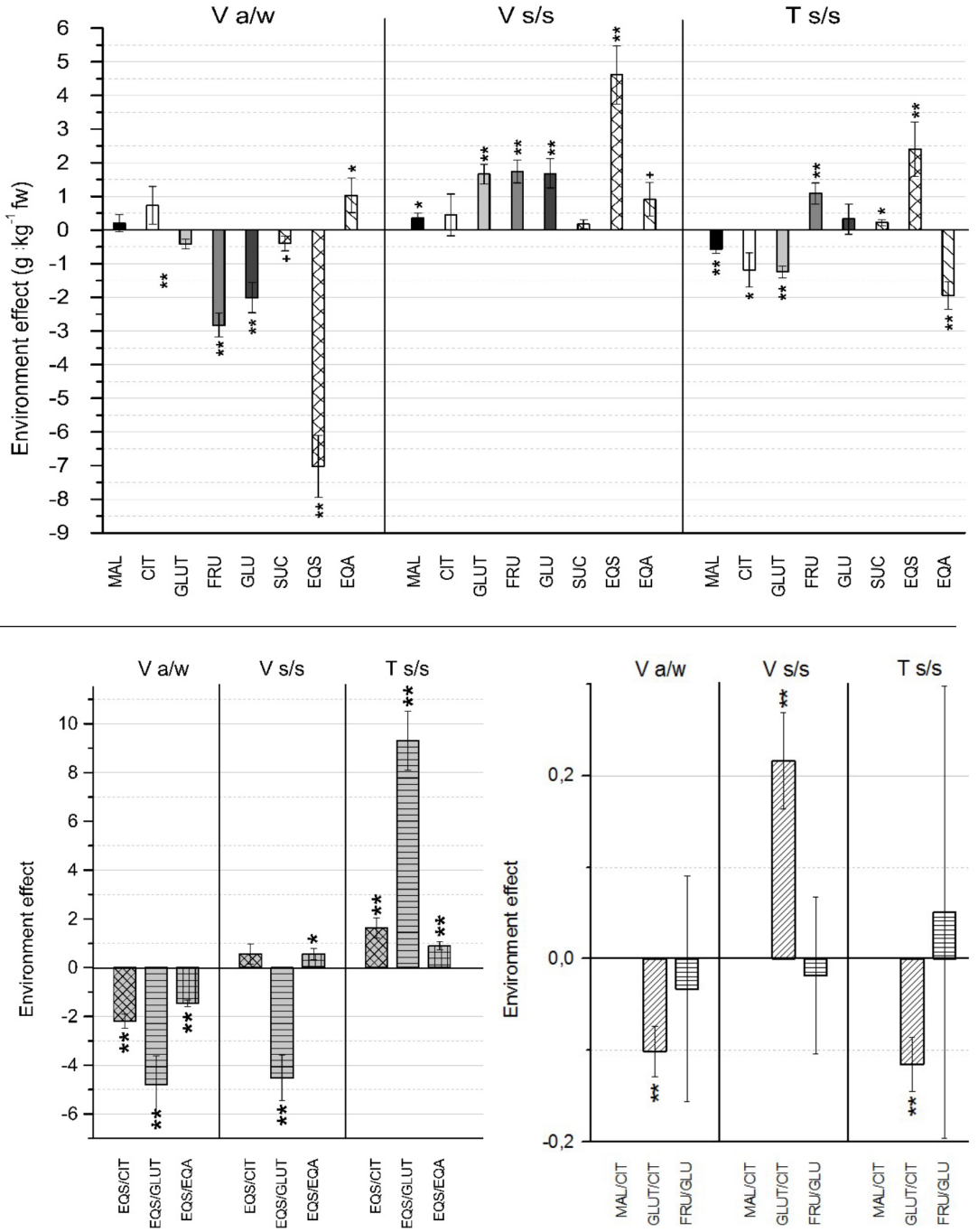

Figure 1. Estimated contribution of the environment to the phenotypic accumulation of sugars and acids and to derived variables. MAL: malic acid; CIT: citric acid; GLUT: glutamic acid; FRU: fructose; GLU: glucose; SUC: sucrose; EQS: sucrose equivalents; EQA: acid equivalents. V a/w: Valencia autumn/winter; V s/s: Valencia spring/summer; T s/s: Turis spring/summer.*: Significant effect, P = 0.05. **: Significant effect, P = 0.01.

Of the three environments, Valencia with an autumn-winter growing cycle in a glasshouse had a clearly negative contribution to the accumulation of both fructose and glucose. Only accessions with small fruits presented quantifiable amounts of sucrose (Tables 2–4).

In this environment, the environmental contribution to the accumulation of citric acid was positive, resulting in a positive contribution to acid equivalents. As result, the contributions to the ratios between sucrose equivalents and the different acids were negative, as well as the

environmental contributions to the ratios of glutamic to citric acid and fructose to glucose.

Comparatively, this environment was characterized by a much lower irradiance and lower temperatures during the second half of the growing cycle (Figure 2). Between both variables, the effect of irradiance was more accentuated, as when accumulated PARmax and temperatures were considered the difference between irradiance is much higher than in terms of temperature (e.g., differences in accumulated temperature during the last part of the cultivation period with open-air cultivation in Turis were limited).

The environment of Valencia with cultivation in a glasshouse during the spring-summer cycle had a positive contribution to the accumulation of acids and sugars, especially of the latter (Figure 1). In the case of organic acids, the contribution to the accumulation of glutamic acid was much higher than to citric or malic acid, and a positive contribution to the ratio glutamic to citric acid was detected. Consequently, there was a positive contribution to the ratios of sucrose equivalents to citric acid and a negative one to the ratio sucrose equivalents to glutamic acid. On the contrary, a small negative contribution to the ratio fructose to glucose was observed. This environment was characterized by a higher irradiance compared to the autumn-winter cycle, much higher temperatures, and higher temperature ranges (Figure 2).

The environment of Turis, with higher irradiance levels, lower temperatures, and intermediate temperature ranges (Figure 2), had a negative contribution to the accumulation of acids and a positive contribution to the accumulation of sugars. In this last case, the contribution to glucose contents was lower than the corresponding one to fructose and lower than the one detected in Valencia in the same growing cycle. This led to a positive contribution to the fructose to glucose ratio. The imbalance between the contributions to the accumulation of sugars and acids resulted in positive contributions to the ratio between sugars and acids. This environment had a negative contribution to the glutamic to citric acid ratio, similar to the one detected in Valencia during the autumn-winter cycle.

The analysis of coefficients of variation revealed that the environment presented a significant effect on the level

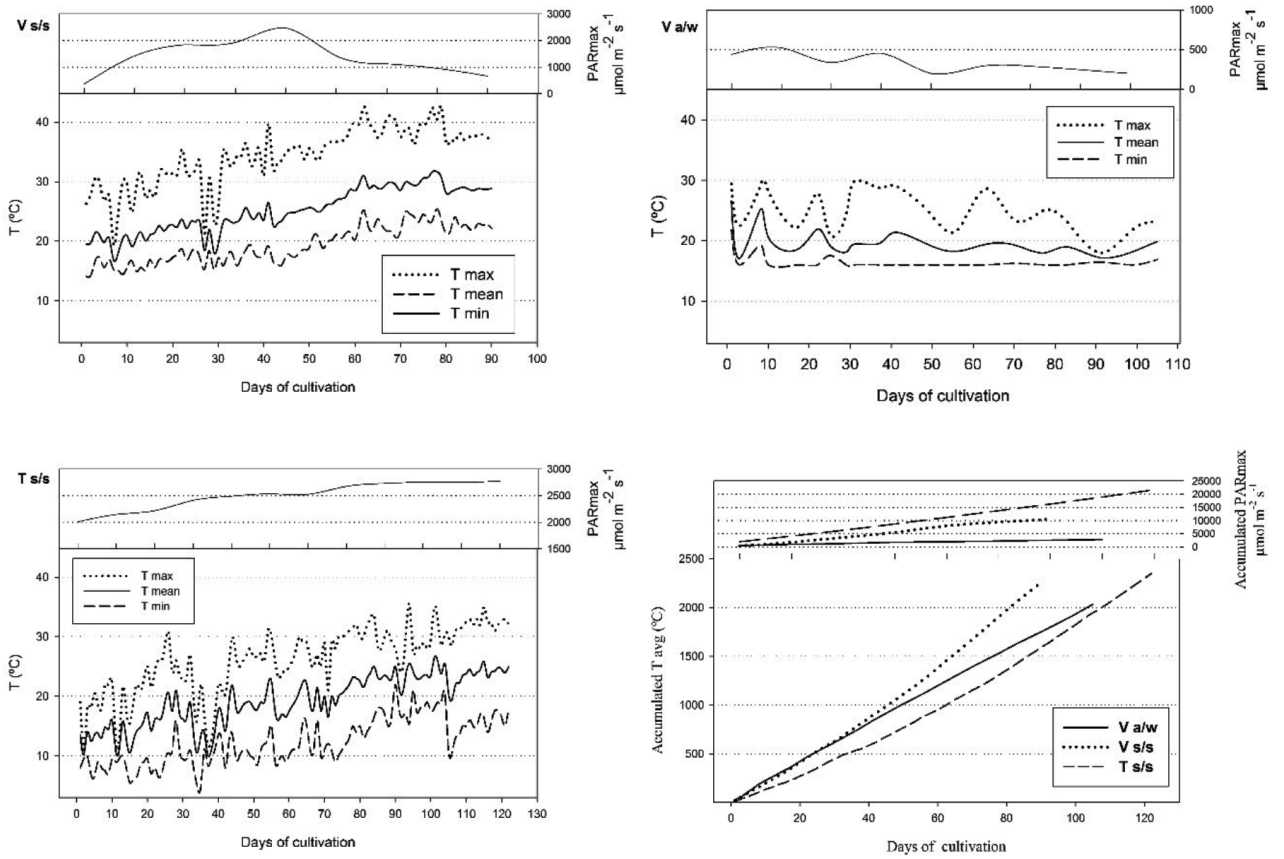

Figure 2. Climatic data recorded during the study. V a/w: Valencia autumn/winter; V s/s: Valencia spring/summer; T s/s: Turis spring/summer.

of variation in the accumulation of all the compounds, except for sucrose (Tables 2–4). The lowest coefficients of variation were detected in Valencia in autumn-winter followed at a distance by Turis in spring-summer.

3.2. Genotypic and G × E interaction effects

The specific contributions of genotype and G × E interactions were analyzed to identify the accessions with higher genotypic contributions (Figures 3 and 4). Accessions CDP4941 and CDP7090 showed the highest genotypic contribution to the accumulation of citric acid. For CDP7090 the contribution in Valencia in autumn-winter would be higher, while for CDP4941 Turis spring-summer would have a higher effect. This last accession also stood out for malic acid accumulation, showing positive interactions with the environments of Valencia.

The two accessions with the highest contribution to citric acid accumulation showed a distinct acid accumulation profile. CDP4941 showed a significant positive correlation between citric and malic acids (R = 0.41), while the relationship between citric and glutamic and malic and glutamic was negative or null (R = –0.36 and R = 0.03, respectively). In the case of CDP7090 only malic and glutamic acids were significantly correlated (0.54).

Regarding the malic to citric acid ratio, two different profiles were identified. One was formed by accessions with smaller genotypic contributions, similar to the controls Cambria and CDP87779, and another was formed by CDP9822, CDP9999, CDP6957/R, CDP2178, and CDP4941, with a profile similar to Gevora. The interaction contribution was especially noticeable in the accessions with high genotypic contribution. Among them, the performance was different, as CDP9822 and CDP9999 showed a considerably negative contribution in Turis while CDP2178 and Gevora showed a considerable positive interaction in the same environment.

Something similar happened with the glutamic to citric acid ratio. This time LA1563, CDP2078, CDP6957/R, and CDP9822 showed high and similar genotypic contributions, like the control Gevora, generally doubling those of the rest of accessions. Interestingly, those were not exactly the same accessions as in the case of the malic to citric acid ratio.

The genotypic contribution of the studied accessions to glucose and fructose accumulation was not remarkable. Only accession CDP9999 showed high genotypic contributions, but only when it was cultivated in the open field. On the other hand, an important genotypic

Table 2. Phenotypic content (mean ± SD, g kg^{-1} fresh weight) of sugars and organic acids and mean coefficient of variation (CV) from accessions evaluated in Valencia autumn-winter. Different letters for CV means represent significant differences (Tukey test, P = 0.05).

Accession	Citric acid	Malic acid	Glutamic acid	Fructose	Glucose	Sucrose
CDP8779	6.7 ± 1.2	1.8 ± 0.5	1.9 ± 0.9	11.7 ± 1.7	10.9 ± 2.5	n.d.
Cambria	8.3 ± 0.9	1.7 ± 0.8	2.3 ± 0.7	15.4 ± 2.1	15.4 ± 2.2	n.d.
Gevora	4.8 ± 1.7	2.0 ± 0.5	3.4 ± 1.5	10.4 ± 2.7	9.0 ± 2.9	n.d.
LA3538	7.6 ± 1.2	2.2 ± 0.4	1.2 ± 0.6	12.1 ± 2.3	11.5 ± 2.0	n.d.
LA1563	7.0 ± 1.9	1.6 ± 0.5	2.9 ± 1.7	15.6 ± 3.8	15.8 ± 3.3	n.d.
CDP2178	5.0 ± 1.3	1.8 ± 0.6	3.8 ± 1.2	14.1 ± 2.1	12.8 ± 1.4	n.d.
CDP7632	7.7 ± 1.5	1.7 ± 0.3	1.9 ± 0.8	12.6 ± 1.5	13.0 ± 2.9	n.d.
CDP2087	6.8 ± 1.5	1.8 ± 0.5	1.8 ± 0.6	12.6 ± 2.0	12.8 ± 2.0	n.d.
CDP6957/A	10.3 ± 2.2	2.6 ± 1.2	3.3 ± 0.8	16.2 ± 2.6	13.7 ± 2.5	1.3 ± 0.8
CDP6957/R	7.4 ± 1.1	3.1 ± 0.8	4.6 ± 1.6	15.4 ± 3.1	14.8 ± 2.8	1.1 ± 1.8
CDP4777	14.6 ± 4.3	2.0 ± 0.7	1.7 ± 0.7	11.1 ± 2.8	9.7 ± 2.8	2.3 ± 0.8
CDP7090	24.8 ± 1.8	2.2 ± 0.8	4.9 ± 1.9	5.2 ± 1.9	3.5 ± 1.3	0.7 ± 0.3
CDP1568	14.2 ± 2.2	2.4 ± 0.5	5.5 ± 1.8	4.7 ± 2.4	4.5 ± 1.4	2.1 ± 1.5
CDP9822	9.7 ± 1.8	2.5 ± 0.8	4.9 ± 1.3	10.6 ± 3.2	13.1 ± 1.4	1.0 ± 0.5
CDP9999	13.0 ± 3.4	5.1 ± 2.9	0.8 ± 0.3	13.7 ± 5.4	9.8 ± 4.1	1.4 ± 1.6
CDP4941	19.3 ± 4.7	7.8 ± 2.1	2.2 ± 1.7	6.2 ± 2.2	3.6 ± 1.7	20.2 ± 12.4
Cv environment	19.8[a]	31.7[a]	39.8[a]	23.8[a]	23.8[a]	69.2[a]
n.d.: Not detected.						

Table 3. Phenotypic content (mean ± SD, g kg^{-1} fresh weight) of sugars and organic acids and mean coefficient of variation (CV) from accessions evaluated in Valencia spring-summer. Different letters for CV means represent significant differences (Tukey test, P = 0.05).

Accession	Citric acid	Malic acid	Glutamic acid	Fructose	Glucose	Sucrose
CDP8779	5.4 ± 2.6	1.4 ± 0.4	2.7 ± 1.8	15.5 ± 5.0	14.5 ± 6.6	n.d.
Cambria	8.1 ± 5.0	1.5 ± 1.1	3.2 ± 2.9	19.3 ± 6.2	18.2 ± 6.9	n.d.
Gevora	7.8 ± 1.0	1.6 ± 0.7	3.3 ± 2.2	16.0 ± 5.1	14.6 ± 5.6	n.d.
LA3538	6.4 ± 2.5	1.8 ± 0.6	2.0 ± 1.3	17.5 ± 5.0	17.1 ± 5.7	n.d.
LA1563	4.8 ± 3.8	1.2 ± 0.5	3.8 ± 2.4	20.0 ± 4.6	18.8 ± 5.6	n.d.
CDP2178	4.9 ± 2.1	1.4 ± 0.6	2.9 ± 1.9	18.9 ± 5.3	17.1 ± 5.2	n.d.
CDP7632	7.2 ± 3.3	1.3 ± 0.6	2.7 ± 2.0	17.1 ± 5.1	15.7 ± 5.4	n.d.
CDP2087	7.5 ± 3.3	1.6 ± 0.7	2.2 ± 1.1	19.8 ± 5.8	18.6 ± 5.7	n.d.
CDP6957/A	8.1 ± 4.2	1.9 ± 1.0	5.6 ± 4.4	20.3 ± 5.0	17.7 ± 5.1	1.8 ± 1.5
CDP6957/R	9.6 ± 6.0	3.5 ± 1.8	7.65 ± 4.9	22.2 ± 8.1	20.8 ± 8.4	1.7 ± 1.3
CDP4777	11.7 ± 4.6	1.3 ± 1.0	3.1 ± 2.4	16.2 ± 4.0	13.5 ± 3.9	3.5 ± 1.5
CDP7090	14.2 ± 4.0	5.8 ± 2.2	7.1 ± 2.4	15.8 ± 5.8	12.8 ± 4.8	4.8 ± 2.8
CDP1568	14.0 ± 5.5	2.8 ± 1.9	5.9 ± 2.7	8.2 ± 4.8	6.1 ± 3.9	2.7 ± 1.3
CDP9822	11.6 ± 4.0	6.1 ± 2.8	10.5 ± 5.1	12.2 ± 4.7	11.6 ± 5.1	2.7 ± 2.7
CDP9999	15.0 ± 3.6	4.4 ± 2.5	1.2 ± 0.4	16.5 ± 4.6	14.5 ± 4.7	1.9 ± 1.6
CDP4941	17.0 ± 3.7	6.5 ± 2.7	3.9 ± 2.2	6.7 ± 3.6	5.6 ± 3.2	24.5 ± 14.2
Cv environment	41.9[b]	48.7[b]	59.7[b]	33.2[b]	38.1[b]	64.5[a]
n.d.: Not detected.						

Table 4. Phenotypic content (mean ± SD, g kg^{-1} fresh weight) of sugars and organic acids and mean coefficient of variation (CV) from accessions evaluated in Turis spring-summer. Different letters for CV means represent significant differences (Tukey test, P = 0.05).

Accession	Citric acid	Malic acid	Glutamic acid	Fructose	Glucose	Sucrose
CDP8779	4.5 ± 1.1	1.4 ± 0.4	0.9 ± 0.5	13.8 ± 2.1	12.4 ± 2.6	n.d.
Cambria	4.8 ± 1.4	1.7 ± 1.2	1.7 ± 0.3	16.0 ± 2.3	14.2 ± 2.1	n.d.
Gevora	4.0 ± 1.5	1.8 ± 0.8	1.9 ± 0.8	14.6 ± 4.2	12.9 ± 3.8	n.d.
LA3538	4.8 ± 3.0	2.6 ± 0.9	1.0 ± 0.5	17.4 ± 9.0	13.1 ± 5.8	n.d.
LA1563	3.2 ± 1.1	1.5 ± 0.7	2.1 ± 0.6	17.4 ± 3.9	14.4 ± 4.3	n.d.
CDP2178	3.1 ± 1.3	1.6 ± 0.8	1.5 ± 0.5	13.2 ± 2.4	12.0 ± 2.9	n.d.
CDP7632	4.3 ± 1.1	1.4 ± 0.4	1.1 ± 0.4	14.7 ± 2.6	13.1 ± 3.2	n.d.
CDP2087	4.9 ± 4.0	1.6 ± 1.1	1.2 ± 0.7	17.5 ± 8.1	15.9 ± 7.6	n.d.
CDP6957/A	5.4 ± 1.4	2.1 ± 1.2	2.2 ± 1.2	19.9 ± 5.0	15.2 ± 5.0	0.3 ± 0.5
CDP6957/R	3.8 ± 1.0	1.9 ± 0.8	2.2 ± 0.8	15.7 ± 3.4	14.9 ± 6.8	0.1 ± 0.3
CDP4777	8.2 ± 2.0	1.1 ± 0.9	1.4 ± 0.8	16.2 ± 4.6	13.5 ± 2.7	1.1 ± 0.7
CDP7090	15.5 ± 3.8	2.0 ± 0.7	3.6 ± 1.9	18.5 ± 6.6	15.3 ± 5.6	2.5 ± 0.9
CDP1568	13.5 ± 2.1	1.3 ± 0.4	2.5 ± 1.3	13.6 ± 3.1	10.8 ± 2.4	2.6 ± 0.9
CDP9822	11.4 ± 1.9	1.7 ± 0.4	3.6 ± 0.5	21.9 ± 5.1	19.2 ± 5.0	2.4 ± 0.9
CDP9999	12.7 ± 4.1	3.0 ± 2.3	1.0 ± 0.3	26.7 ± 6.2	23.2 ± 5.5	1.5 ± 0.5
CDP4941	22.0 ± 5.0	4.6 ± 1.0	1.5 ± 1.1	7.7 ± 3.5	5.7 ± 3.5	34.5 ± 14.1
Cv environment	32,7[b]	45.7[b]	41.4[a]	27.7[ab]	31.3[ab]	73.0[a]
n.d.: Not detected.						

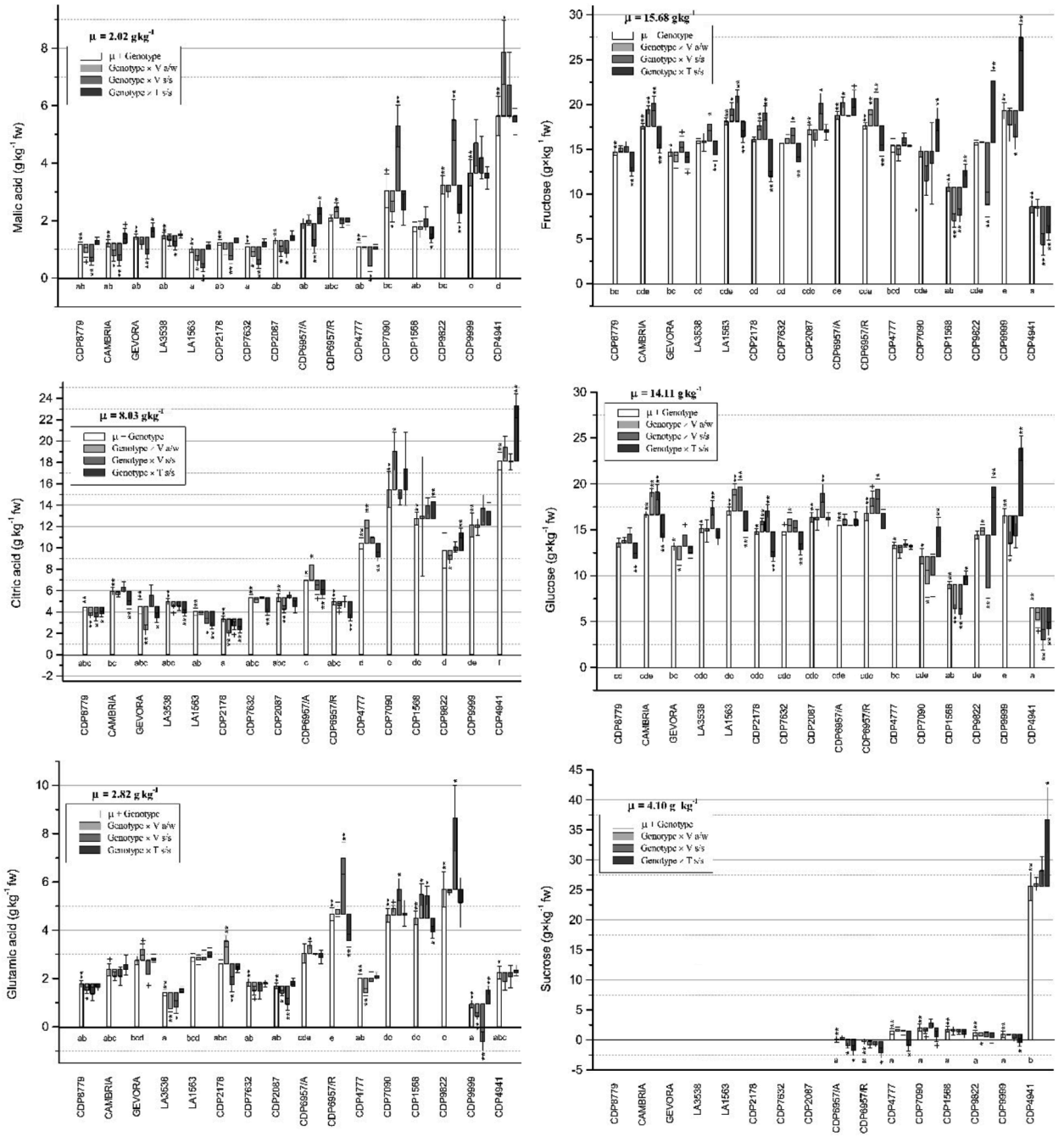

Figure 3. Predicted total genotypic (μ + G) and interaction (G × E) effects for organic acids and sugars in three environments. V a/w: Valencia autumn/winter, V s/s: Valencia spring/summer, T s/s: Turis spring/summer. The first three accessions are controls. Significant departures from zero (t-test) are indicated with + ($P < 0.1$), * ($P < 0.05$), and ** ($P < 0.01$).

contribution to sucrose accumulation was only observed in CDP4941. However, these levels were obtained at the expense of hexoses accumulation and the genotypic contribution to sucrose equivalents was rather standard. Much lower variation was found regarding the genotypic contribution to the fructose to glucose ratio, where only CDP4941 (*S. habrochaites*) showed higher values. CDP4941 in certain environments showed a sum of genotypic and G × E interaction leading to ratios close to 1.75.

The intraaccession variation was analyzed to evaluate the genotypic differences between plants of one accession, using a MANOVA biplot to summarize the results. For

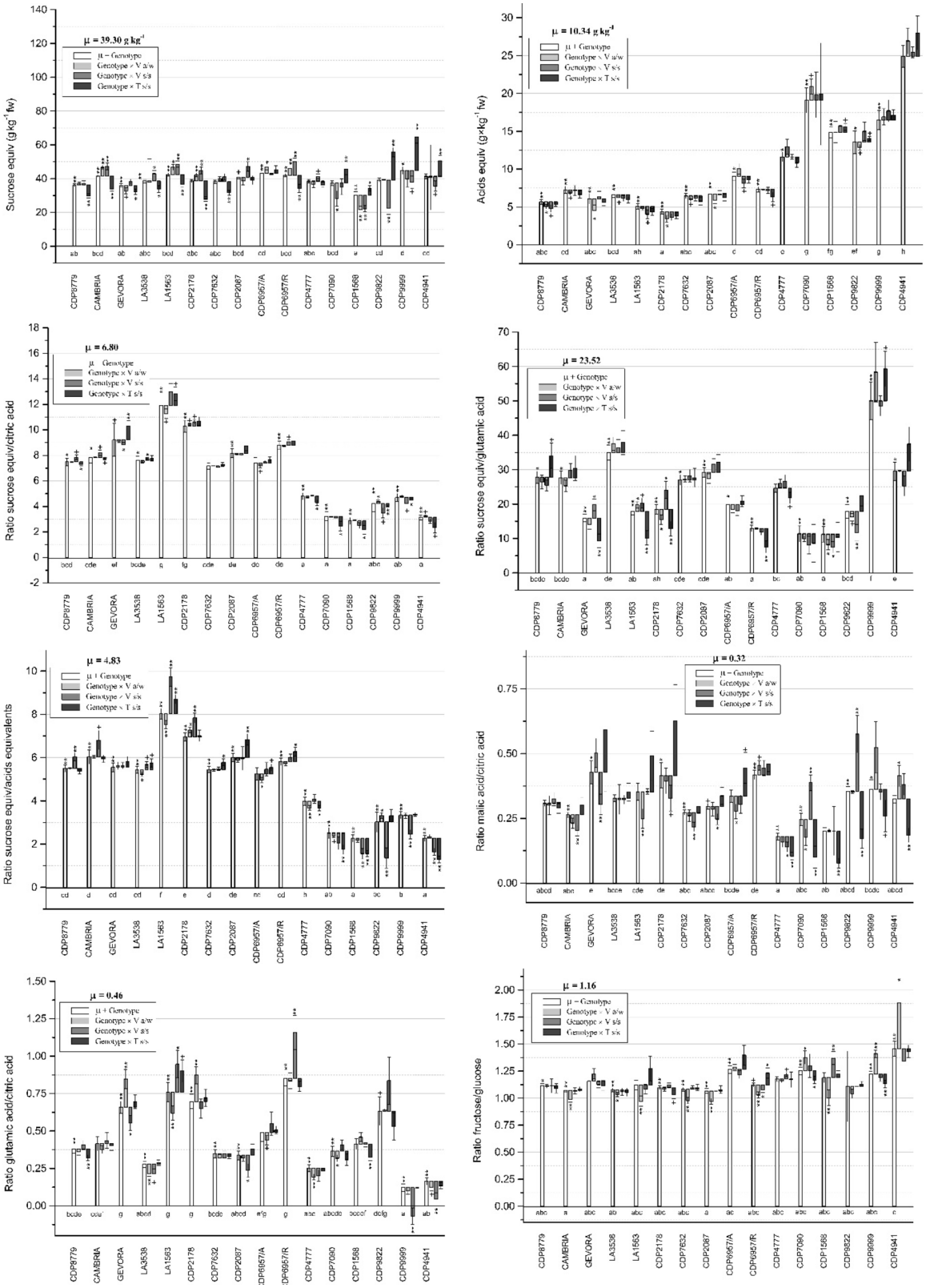

Figure 4. Predicted total genotypic (μ + G) and interaction (G × E) effects for derived variables in three environments. V a/w: Valencia autumn/winter, V s/s: Valencia spring/summer, T s/s: Turis spring/summer. The first three accessions are controls. Significant departures from zero (t-test) are indicated with + ($P < 0.1$), * ($P < 0.05$) and ** ($P < 0.01$).

this purpose, only data for Turis are presented, though similar conclusions would be extracted from the rest of the environments. The F1 hybrid Cambria showed a considerable level of variation, suggesting an important level of variation due to microenvironmental differences. In general, a higher level of variation was detected in wild accessions, especially in those with the highest accumulation of certain compounds, such as CDP4941 (Figure 5, genotype code 16) or CDP7090 (Figure 5, genotype code 12).

4. Discussion

The environment had an important effect on the accumulation of sugars and acids and derived ratios and variables, both via its main effect and the G × E interaction. Regarding the main effect, the accumulation of sugars seemed to depend to a larger extent on the PAR rather than on the temperature, whereas in the case of the acids, the temperature seemed to exert a major effect. A major effect of PAR on sugar accumulation was expected, considering that it may limit the photosynthetic activity of the plant. Consequently, during the autumn-winter cycle, the lower PAR radiation would have a major impact on sugar accumulation. Several studies have analyzed the effect of shading or season on the accumulation of taste-related compounds. Regarding shading studies, Gautier et al. (2008), comparing fruits grown under light or shaded conditions at different temperatures, also observed lower reducing sugars content in shaded fruits and higher titratable acidity. Comparing mesh-protected cultivation to open air (26.7% lower PAR), we previously found a decrease in fructose and glucose contents and similar levels of organic acids (Cebolla-Cornejo et al., 2011). On the other hand, in greenhouse conditions, comparing 100% radiation with 50% and 75% shading, Riga et al. (2008) found a higher correlation between cumulative mean temperature and soluble solids, suggesting that the effects of temperature on fruit quality were higher than those of PAR. In this case, a moderate correlation between titratable acidity and cumulative temperature (r = 0.38) was found.

Regarding seasonal fluctuations, Toor et al. (2006), evaluating soluble solids content and titratable acidity throughout the year, found that irradiance and temperature did not have a significant effect on titratable acidity, while soluble solids were higher in samples collected during summer than during spring. Hernández et al. (2008) found for several cultivars that in the autumn-winter cycle less malic and higher citric acid contents were found compared to the spring-summer cycle, whereas higher soluble solids were found in the latter. In our case, the environmental effect of the winter campaign was negative, reducing the accumulation of sugars, suggesting a prominent effect of PAR. On the other hand, the environmental contribution

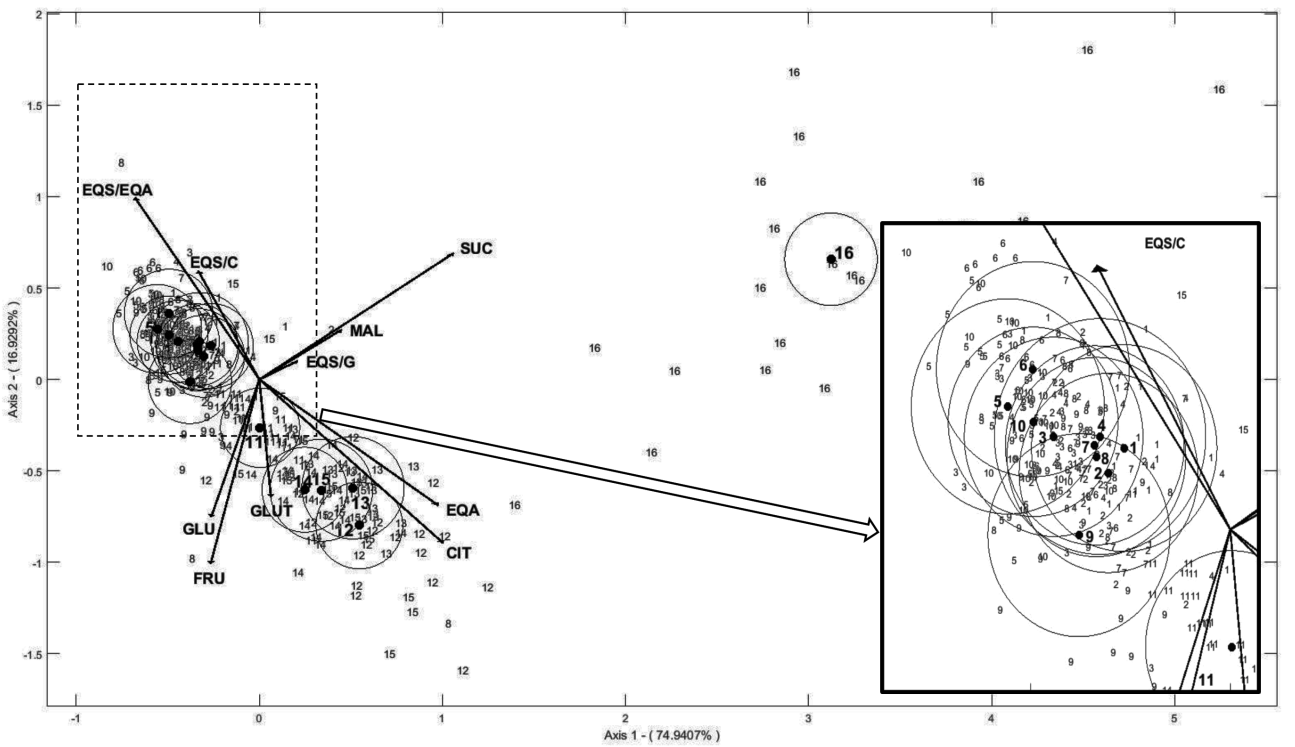

Figure 5. MANOVA biplot of the data from Turis with open-air cultivation (genotype codes in Table 1).

to acid accumulation was negative in Turis open-air cultivation, with higher PAR but lower cumulative temperature. In this case, the differences between spring-summer and autumn-winter cultivation in Valencia were limited, while differences in PAR were considerable. It should be pointed out though that the high positive effect on the accumulation of glutamic acid found in Valencia would suggest that for this amino acid the impact of PAR would be important. Nevertheless, an increase of temperature would be required to increase the levels of glutamic acid, as increasing only irradiance (Turis spring-summer) would limit this effect. The differential behavior of glutamic acid would explain the high environmental effects on the ratio between this and other acids and between sucrose equivalents and glutamic acid.

Interestingly, the environment affected not only the accumulation of taste-related compounds but also the level of variation. It seems that the diffuse irradiance typical of autumn-winter conditions would limit shading effects within the greenhouse. On the other hand, during the spring-summer season the lower variation found in the open air would probably be related to the lack of shading effects of the structure and cover of the greenhouse.

The environment also exerted its influence via G × E interactions. These can be considerably high in the accumulation of both sugars and acids. The importance of the interactions has been emphasized in tomato regarding the accumulation of functional compounds, especially vitamin C (Leiva-Brondo et al., 2012), and to a lower extent carotenoids (Leiva-Brondo et al., 2016). This differential behavior may lead to overestimating or underestimating the value of an accession and consequently emphasizes the need to perform screening studies in multienvironmental designs. The identification of consistent genotypic contributions would save time, avoiding studies of heritability and genetic control studies in materials extremely sensitive to environmental conditions.

Regarding these designs, the number of plants per accession should also be reconsidered, as an important intraaccession variation has been found. Consequently, we should not refer to interesting accessions, but to interesting plants within accessions. These screening programs are still necessary. It is true that huge advances have been obtained through the evaluation of several introgression lines that have been developed from accessions of wild species related to tomato, including red-fruited species belonging to the *Lycopersicon* group *S. pimpinellifolium* or green-fruited species belonging to the *Neolycopersicon*, *Eriopersicon*, or *Arcanum* groups (mainly *S. pennellii* and *S. habrochaites*) (Fernie et al., 2006). However, the production of introgression lines is expensive and time-consuming, and therefore only one or a few accessions have been used in each case as parents in the program, limiting the full exploitation of the potential of the section *Lycopersicon* of the genus *Solanum*. Nevertheless, the considerable drop in the genotyping costs of introgression lines makes it easier and cheaper to continue to exploit the information provided by these materials (Barrantes et al., 2016).

Regarding sugar accumulation, none of the accessions tested offered an important genotypic contribution, although CDP4941 from *S. habrochaites* had a considerable genotypic contribution to the accumulation of sucrose. This behavior has already been described in *S. habrochaites*, and also in *S. chmielewskii*, from which the gene *sucr* was identified and introgressed in tomato, although as stated in the introduction negative side effects were detected. Moreover, when the genotypic contribution to the level of sucrose equivalents is considered, the interest of this accession decreases compared to other accessions from the *Lycopersicon* group that do accumulate sucrose, such as CDP9999. Kortstee et al. (2007) concluded that sucrose synthase and soluble invertase enzyme activities were higher in the domesticated crop and distributed throughout the whole fruit, explaining partially the lack of sucrose accumulation in *S. lycopersicum*. Thus, these accessions from the *Lycopersicon* group accumulating sucrose may be useful to study the regulation of vacuolar invertase.

Accession CDP4941 also offered an important genotypic contribution to the ratio of fructose to glucose. This ratio is important, considering that fructose has a considerably higher sweetening power (Baldwin et al., 1998), and its increase is interesting for example for the development of lines targeted to ketchup production. In this case, this effect is probably due to the presence of the *Fgr* gene, encoding fruktokinase2, which has been described in this species (Levin et al., 2004). More interestingly, accessions CDP6957/A and CDP7090 also offered high genotypic contributions and they belong to the *Lycopersicon* group. These materials are more readily usable, as the complications derived from linkage drag, which is quite important in tomato (Haggard et al., 2013), due to the low recombination rate in wide areas of the genome (Sato et al., 2012), are less important.

Regarding the potential use for increasing acid accumulation, Kortstee et al. (2007) concluded that fruits of wild tomato plants with the exception of *S. pimpinellifolium* had higher contents of citric and malic acid. Our results confirm the higher contents in *S. habrochaites*, although we have found high contents of citric acid also in several *S. pimpinellifolium* accessions. This result emphasizes the need to continue screening new sources of variation, as the metabolic profile of a species is not generalizable. Thus, it is still possible to find interesting sources of variation.

Apart from differences in the accumulation potential of acids, different accumulations of citric, malic, and glutamic acids were identified in the accessions evaluated. These

different profiles will be useful to deepen the knowledge of the regulation of the tricarboxylic acid (TCA) cycle and the gamma-aminobutyric acid (GABA) shunt in tomato. As an example, CDP6957/R showed low ratios between the phenotypic values of citric and malic acid and citric and glutamic acid that were consistent among the three environments tested. This behavior would be compatible with an increased accumulation of glutamic acid at the expense of citric acid, maybe due to an upregulation of the GABA shunt (Etienne et al., 2013).

On the other hand, CDP9999 and CDP4941 showed clearly higher ratios between the phenotypic values of malic and glutamic acid. This behavior was not species-dependent, as CDP9999 is the only accession of *S. pimpinellifolium* showing a ratio so high (around 5-fold the ratio of the other accessions). In the case of CDP4941, this was the only accession form of *S. habrochaites* evaluated. In both accessions, especially in the case of CDP9999, this high ratio derives from a low accumulation of glutamic acid and an increased accumulation of malic acid. These accessions also showed a clearly higher ratio of citric to glutamic acid in the three environments. This result may suggest a downregulation of the GABA shunt resulting in a decrease of the accumulation of glutamic acid and an accumulation of citrate (Etienne et al., 2013).

Accession CDP4777 from the former var. *cerasiforme* of the cultivated species also had high citric to glutamic and citric to malic ratios, but the malic to glutamic ratios were not so high. In this case, a relatively high accumulation of citric acid was observed, accompanied by a relatively low accumulation of malic acid, and this profile was consistent in the three environments. It seems that the cause may be an increased accumulation of citric acid at the expense of malic acid, although as the contents of glutamic acid are not so high, a small contribution via a downregulation of the GABA shunt might be collaborating. In addition, accession CDP7090 from *S. pimpinellifolium* could be upregulating the whole accumulation of the three acids. In this case, the GABA shunt may not be not downregulated, and the TCA cycle may be upregulated, resulting in an increased accumulation of organic acids and derived amino acids. This behavior was consistent in the three environments, resulting in high genetic contributions to accumulation of these compounds.

In conclusion, screening studies are still necessary to identify potentially useful genetic resources for breeding programs. In the case of taste-related compounds, important environmental and interaction effects have been detected, suggesting the need to perform multienvironmental studies before placing the focus on a certain accession. A high level of intraaccession variation also reinforces the need to perform individual selection rather than directly working with an accession. By doing so, different accessions with potential to accumulate certain levels of sucrose, to change the ratio of fructose to glucose, or to improve the contents and profiles of acids have been identified. These materials will also be valuable to study the regulation of the TCA cycle and the GABA shunt.

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References

Baldwin EA, Scott JW, Einstein M, Malundo TMM, Carr BT, Shewfelt RL, Tandon KS (1998). Relationship between sensory and instrumental analysis for tomato flavor. J Amer Soc Hort Sci 123: 906-915.

Barrantes W, López-Casado G, García-Martínez S, Alonso A, Rubio F, Ruiz JJ, Fernández-Muñoz R, Granell A, Monforte AJ (2016). Exploring new alleles involved in tomato fruit quality in an introgression line library of *Solanum pimpinellifolium*. Front Plant Sci 7: 1172.

Bennett AB (2012). Taste: Unraveling tomato flavor. Curr Biol 22: R443-R444.

Bertin N, Guichard S, Leonardi C, Longuenesse JJ, Langlois D, Navez B (2000). 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 85: 741-750.

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

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

Cano EA, Pérez-Alfocea F, Moreno V, Caro M, Bolarín MC (1998). Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. Plant Cell Tissue Organ Cult 53: 19-26.

Causse M, Buret M, Robini K, Verschave P (2003). Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. J Food Sci 60: 2342-2350.

Cebolla-Cornejo J, Rosell S, Valc M, Serrano E, Beltr J, Nuez F, Valencia D (2011). Evaluation of genotype and environment effects on taste and aroma flavor components of Spanish fresh tomato varieties. J Agric Food Chem 59: 2440-2450.

Cebolla-Cornejo J, Valcárcel M, Herrero-Martínez JM, Roselló S, Nuez F (2012). High efficiency joint CZE determination of sugars and acids in vegetables and fruits. Electrophoresis 33: 2416-2423.

Chetelat RT, DeVerna JW, Bennett AB (1995). Effects of the *Lycopersicon chmielewskii* sucrose accumulator gene (*sucr*) on fruit yield and quality parameters following introgression into tomato. Theor Appl Genet 91: 334-339.

Davies JN, Hobson GE (1981). The constituents of tomato fruit — the influence of environment, nutrition, and genotype. C R C Crit Rev Food Sci Nutr 15: 205-280.

De Bruyn JW, Garretsen F, Kooistra E (1971). Variation in taste and chemical composition of the tomato (*Lycopersicon esculentum* Mill.). Euphytica 20: 214-227.

Etienne A, Génard M, Lobit P, Mbeguié-A-Mbéguié D, Bugaud C (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. J Exp Bot 64: 1451-1469.

Fernie AR, Tadmor Y, Zamir D (2006). Natural genetic variation for improving crop quality. Curr Opin Plant Biol 9: 196-202.

Fridman E, Pleban T, Zamir D (2000). A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. P Natl Acad Sci USA 97: 4718-4723.

Fulton TM, Bucheli P, Voiol E, Lopez J, Petiard V, Tanksley SD (2002). Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica 127: 163-167.

Gautier H, Diakou-Verdin V, Bénard C, Reich M, Buret M, Bourgaud F, Poëssel JL, Caris-Veyrat C, Génard M (2008). How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? J Agric Food Chem 56: 1241-1250.

Haggard JE, Johnson EB, St. Clair DA (2013). Linkage relationships among multiple QTL for horticultural traits and late blight (*P. infestans*) resistance on chromosome 5 introgressed from wild tomato *Solanum habrochaites*. G3-Genes Genom Genet 3: 2131-2146.

Hernández M, Rodríguez E, Díaz C (2008). Analysis of organic acid content in cultivars of tomato harvested in Tenerife. Eur Food Res Technol 226: 423–435.

Kader AA, Stevens MA, Albright-Holton M, Morris LL, Algazi M (1977). Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. J Am Soc Hortic Sci 102: 724-731.

Koehler PE, Kays SJ (1991). Sweet potato flavor: quantitative and qualitative assessment of optimum sweetness. J Food Qual 14: 241–249.

Kortstee AJ, Appeldoorn NJG, Oortwijn MEP, Visser RGF (2007). Differences in regulation of carbohydrate metabolism during early fruit development between domesticated tomato and two wild relatives. Planta 226: 929-939.

Leiva-Brondo M, Valcárcel M, Cortés-Olmos C, Roselló S, Cebolla-Cornejo J, Nuez F (2012). Exploring alternative germplasm for the development of stable high vitamin C content in tomato varieties. Sci Hortic (Amsterdam) 133: 84-88.

Leiva-Brondo M, Valcarcel M, Martí R, Roselló S, Cebolla-Cornejo J (2016). New opportunities for developing tomato varieties with enhanced carotenoid content. Sci Agric 73: 512-519.

Levin I, Lalazar A, Bar M, Schaffer AA (2004). Non GMO fruit factories: strategies for modulating metabolic pathways in the tomato fruit. Ind Crops Prod 20: 29-36.

Matsukura C (2016). Sugar accumulation in tomato fruit and its modification using molecular breeding techniques. In: Ezura H, Ariizumi T, Garcia-Mas J, Rose J, editors. Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops. Berlin, Germany: Springer, pp. 141-154.

Powell ALT, Nguyen CV, Hill T, Cheng KL, Figueroa-Balderas R, Aktas H, Ashrafi H, Pons C, Fernandez-Munoz R, Vicente A et al (2012). Uniform ripening encodes a Golden 2-like transcription factor regulating tomato fruit chloroplast development. Science 336: 1711-1715.

Rick CM (1986). Germplasm resources in the wild tomato. Acta Hortic 190: 39-48.

Riga P, Anza M, Garbisu C (2008). Tomato quality is more dependent on temperature than on photosynthetically active radiation. J Sci Food Agric 88: 158-166.

Sato S, Tabata S, Hirakawa H, Asamizu E, Shirasawa K, Isobe S, Kaneko T, Nakamura Y, Shibata D, Aoki K et al (2012). The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485: 635-641.

Schaffer AA, Petreikov M, Miron D, Fogelman M, Spiegelman M, Bnei-Moshe Z, Shen S, Granot D, Hadas R, Dai N et al (1999). Modification of carbohydrate content in developing tomato fruit. HortScience 34: 1024-1027.

Stevens MA, Kader AA, Albright-Holton M, Algazi M (1977). Genotypic variation for flavor and composition in fresh market tomatoes. J Am Soc Hort Sci 102: 680-689.

Tieman D, Zhu G, Resende MFR, Lin T, Nguyen C, Bies D, Rambla JL, Ortiz KS, Taylor M, Zhang B et al (2017). A chemical genetic roadmap to improved tomato flavor. Plant Sci 355: 391-394.

Toor RK, Savage GP, Lister CE (2006). Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. J Food Compos Anal 19: 1-10.