Evaluation of the genotype, environment and its interaction on carotenoid and ascorbic acid accumulation in tomato germplasm.

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Abstract

BACKGROUND: Tomatoes are an important source of antioxidants (carotenoids, vitamin C, etc.) due to their high level of consumption. There is a great interest in developing cultivars with increased levels of lycopene, β-carotene or L-ascorbic acid. There is necessary to survey new sources of variation. In this study, the potential of improvement for each character in tomato breeding programs, in a single or joint approach, and the nature of genotype (G), environment (E) and GxE interaction effects in the expression of these characters were investigated.

RESULTS: The content of lycopene, β-carotene and ascorbic acid determined was very high in some phenotypes (up to 281, 35 and 346 mg kg^{-1} respectively). The important differences in the three environments studied (with some stressing conditions in several situations) had a remarkable influence in the phenotypic expression of the functional characters evaluated. Nevertheless, the major contribution came from the genotypic effect along with a considerable GxE interaction.

CONCLUSION: The joint accumulation of lycopene and β-carotene has a high genetic component. It is possible to select elite genotypes with high content of both carotenoids in tomato breeding programs but multi-environment trials are recommended. The improvement of ascorbic acid content is more difficult because the interference of uncontrolled factors mask the real genetic potential. Among the accessions evaluated, there are four accessions with an amazing genetic potential for functional properties that can be used as donor parents in tomato breeding programs or for direct consumption in quality markets.

Keywords: *Solanum* section *Lycopersicon*, genetic resources, functional quality, lycopene, β-carotene, vitamin C, linear mixed models.

INTRODUCTION

In developed country markets, such as in European countries ones, therenis a tendency to evolve from an agriculture focused on yield towards an agriculture focused on quality[1]. In these areas, with high spending power, consumers demand products with higher internal quality which lead to the development of new higher quality products. This is especially true for 'functional foods' which offer an interesting growth opportunity for the food industry[2].

Tomato has moderate nutritional value, but it is consumed all year round. It is one of the most important sources of antioxidants, such as vitamin C or carotenoids, which are protective to degenerative diseases[3,4]. In this context, during the last decade there has been an increasing interest in the development cultivars with increased levels of L-ascorbic acid or the main carotenoids present in tomato: beta-carotene and lycopene. Cultivar such as 'DoubleRich' has twice as much vitamin C content or the 'high pigment' cultivars that are becoming popular in the processing tomato industry[5]. Several mutations have been identified related to the carotenoid content in tomato, but important organoleptic or agricultural deficiencies have limited their use[6,7] and it is necessary to survey new sources of variation.

Although several works have been focused on this objective[i.e.5,8,9], the elevated influence of the agronomic and environmental variables in the expression of characteristics of the functional value of the fruits of tomato[7,10] is yet to be determined. Not only the environment plays an important role in the system. It has been suggested that the GxE interaction would be considerably high[11]. Therefore more studies on the contribution of different environments, genotypes and their interactions to the expression of properties of functional value should be carried out in order to select elite genotypes with more precision that enhances the accumulation of favourable

compounds. Information on the structure and nature of GxE interactions is particularly necessary to determine if it is possible to develop 'high functional value' cultivars with high environmental stability or specific cultivars for specific target environments.

The objective of this study is to perform an evaluation of *Solanum* section *Lycopersicon* germplasm in different environments in order to elucidate the nature and structure of genotype, growing environment and its interaction and to identify the genotypic potential of these materials for direct use or as sources of variability in breeding programs for lycopene, β-carotene and/or ascorbic acid accumulation in tomato fruits.

MATERIALS AND METHODS

Plant material

Five *Solanum lycopersicum* L. accessions, one *S. lycopersicum* var *cerasiforme* L. and four *S. pimpinellifolium* L. representing a wide diversity of fruit shapes and colours were studied (Table 1). Three modern tomato cultivars with normal levels of ascorbic acid and carotenoids and a high pigment line were included as controls: CDP8779 (experimental line developed by COMAV, Valencia, Spain), Cambria (a hybrid commercialized by Seminis Vegetable Seeds, Almería, Spain), Gevora (a processing tomato variety developed by el Centro de Investigación "La Orden-Valdesequera", Badajoz, Spain) and LA1563 (accession provided by TGRC, University of California, Davis, with enhanced carotenoid content[12] due to the Intense Pigment gene).

Experimental design and growing conditions

The trials were carried out in 3 growing environments representing common cycles and cultivation techniques. For a precise evaluation of genotype, environment and its interaction effects, clones of all the plants studied were used in each environment. A

randomized complete block design was used with 4 blocks per environment, 14 plots per block (one per accession) and 8 plants per plot. All the blocks of each accession had clones of the same 8 plants in order to have a better estimate of block and environment effects.

Two sites of cultivation were used. Cultivation at Valencia was carried out in two different cultivation cycles (autumn-winter and spring-summer) in a glasshouse with automated climate control. Cultivation at Turis was carried out in spring-summer cycle at the open air. In protected cultivation, heating systems (in autumn-winter cycle) and heat dissipation systems (progressive shadowing and cooling in spring-summer cycle) were used. In all the environments fertirrigation was scheduled daily and plants were staked and pruned properly. In order to have information about climatic parameters influencing plant metabolism and growth, air temperature and photosyntetically active radiation (PAR) were recorded every 10 minutes using WatchDog wheatear stations (Spectrum Technologies Inc., Illinois, USA) equipped with temperature, quantum light PAR sensors and data logger.

Sampling

Uniformly ripe, healthy fruits, at the red-ripe stage were harvested. Accessions with colours other than red were harvested when fruits reached maximum colour intensity. A total of 5 to 20 representative fruits (depending on the species) were collected from each plant only from the first 3 trusses to minimise intra-plant variability. Samples were blended at 4ºC and low light intensity to minimise antioxidant loss. The laboratory homogenizer (Diax 900, Heidolph, Germany) was used with a generator 6G to disrupt tissue to particle sizes <0.4 mm. Samples were stored at -80ºC until analysis.

Ascorbic acid determination

Ascorbic acid was quantified by Capillary Zone Electrophoresis using a P/ACE System MDQ (Beckman Instruments, Fullerton, USA). Two grams of sample were thawed in the dark at 4ºC and centrifuged at 12500 rpm in a refrigerated centrifuge. The supernatant was diluted in 2% metaphosphoric acid to avoid ascorbic acid oxidation[13]. Potassium hydrogen phthalate (100 mg l^{-1}) was used as an internal standard. Sample extracts were filtered through a 0.2 mm filter membrane (Millipore, Bedford, USA) prior to injection. Uncoated fused-silica capillaries (31.2 cm of total length, 21 cm of effective length, 50 μm i.d.) were used (Polymicro Technologies, Phoenix, USA). Hydrodynamic injection of samples was carried out at 0.5 psi during 5 s. The detection wavelength was 254 nm. Separation was performed at -15 kV and 25ºC. Three analytical replicates per sample were made.

Carotenoid determination

Determination was based on a spectrophotometric analysis[9] using a spectrophotometer with double-beam operation (model Lambda-25, Perkin-Elmer, Waltham, USA). The samples were thawed at 4ºC. Carotenoid extractions were performed with 0.1 g of thawed samples, which were shaken for 1 hour using 7 mL of organic solvents (ethanol:hexane, 4:3) . The extractions were conducted in the dark to prevent light-induced carotenoid oxidation. Afterwards, 1 mL of distilled water was added to separate organic solvent layers and 0.5 mL of the upper layer (hexane phase) was recovered and refrigerated at 4ºC to avoid carotenoids loss. A calibration line which relates standards concentrations and absorbance at 510 nm was used to obtain lycopene concentrations. For β-carotene, a calibration plane relating the concentrations from standards and absorbance at 452 nm (positive correlation) and 510 nm (negative correlation) was used. Seven standards with joint concentrations (randomly paired up) of lycopene and β-carotene were used for calibration. Three analytical replicates per sample were made.

Data analysis

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

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

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=GenotypexEnvironment interaction effect with mean 0 and variance V_{GxE}; B=the block effect with mean 0 and variance V_B; e=residual effect with mean 0 and variance V_e. All the factors were considered as random. The MINQUE (1) method[14,15] was used to obtain unbiased variance and covariance components for each trait. Variance and covariance estimates were used to calculate the corresponding correlation coefficients for phenotypic, genotypic, environmental and interaction effects. The random effects were predicted using the adjusted unbiased prediction (AUP) method[14]. Standard errors of the statistics were obtained by the jackknife procedures[14,16] and a two-tail *t*-tests were performed for testing the significance of parameters obtained. The model was also recalculated considering environment as fixed factor for growing season and type of cultivation comparison computing the pairwise mean comparison using the False Discovery Rate (FDR) criterion[17] at $\alpha = 0.05$.

All the data analyses were performed with QTModel (v. 0.7) and QGAStation (v. 1) software (Bioinformatics Institute, Zhejiang University, China).

RESULTS AND DISCUSSION

Phenotypic means of carotenoids and ascorbic acid content for the tomato accessions studied

In general, it could be observed that the phenotypic antioxidant content of tomato largely varied among accessions in each environment (Table 2). Moreover, the phenotypic values of the content of lycopene, β-carotene and ascorbic acid seemed to be very promising for some accessions. For the lycopene content, values up to 281 mg kg^{-1} were observed, which are much higher than the reported average phenotypic value (30 mg kg^{-1})[11,18]. A similar situation occurred for β-carotene content (35 mg kg^{-1} observed in contrast with the 3.9 mg kg^{-1} commonly reported[18]) and for ascorbic acid content (346 mg kg^{-1} versus 200 mg kg^{-1} commonly accepted[19]).

Although it was possible to detect high phenotypic values for all the compounds, important environmental and interactions effects were easily detected, as the values obtained fluctuated with different trends for different accessions and environments (Table 2). In order to obtain a better estimation of the potential of improvement for each character in tomato breeding programs it was necessary to ascertain the relative contribution of the genotype, environment and genotype x environment interactions, variance components. Genetic correlations between traits were also analysed in order to determine if a combined selection for these antioxidant traits would be feasible.

Estimation of variance components and correlations analysis

First, a decomposition of phenotypic variances in genetic, environmental and GxE interaction components was carried out (Table 3). All the estimates of the variance components calculated were significantly different from zero, thus offering reliable information on the relative contribution of each one to the total phenotypic variance. For carotenoid content, the residual variance was around 25% of the total phenotypic variance; hence it can be considered that the model explained well the distribution of the variation with the factors included. However, for ascorbic acid, the residual variance

was two times higher. The model, despite providing useful information, only explained one half of the total phenotypic variance. Nevertheless, it should be considered that ascorbic acid plays a very active and important role in reducing the oxidative damage at cellular level caused by stress conditions[20] and it is very difficult to model the GxE interactions in its accumulation due to uncontrolled factors. For all the traits, the block effect was very small (between 0.41% and 1.28% of the total phenotypic variance) so this effect could be discarded. The more important result to consider was that, for carotenoid accumulation, the genotypic component represented the larger contribution to the phenotypic variance (around the 60%) and the environmental variance was very low, having less contribution to the phenotypic value than the GxE interaction. The GxE interaction represented between 5 to 10 times less variance (for β-carotene and lycopene respectively) than the genotypic component. These results show that the improvement of lycopene and β-carotene is feasible in breeding programmes and that elite carotenoid accumulation cultivars can be commercialised independently of the growing conditions used, to obtain good phenotypic values. In the case of ascorbic acid accumulation, the genetic variance represented a quarter of the total phenotypic variance, and the environmental and GxE variance was around the 10%, indicating that the improvement of the ascorbic acid content in tomato breeding programs will be difficult and that the use of high ascorbic acid cultivars not necessarily implies the production of high ascorbic acid fruits. Therefore this situation may lead to important conflicts in quality controls during commercialisation.

After partitioning phenotypic covariance into its genotypic, environmental and GxE components, the corresponding paired correlation coefficients were calculated (Table 4). For Lycopene and β-carotene accumulation an important and highly significant positive genotypic correlation (r_G) was observed. On the contrary, the GxE correlation

coefficient was negative but not significant. Accordingly, the total genetic correlation (r_G+r_{GxE}) indicated that it is possible to select genotypes with high levels of both carotenoids. Nevertheless, it is interesting to point out that a high negative significant environmental correlation was determined and this makes difficult the development of selection trials, as the growing environments that increase the lycopene accumulation seem to reduce the β-carotene content and vice versa. Therefore, multi-environment trials must be implemented in order to obtain a reliable genotype evaluation. In the case of the pair β-carotene and ascorbic acid there was a very high and positive significant total genetic correlation (0.8), mainly due to the genotype component, that allows a practicable joint improvement of these two traits. There also exist a minor significant and negative environmental correlation but this may not represent an important difficulty for the selection.

When analysing the phenotypic correlations for lycopene and ascorbic acid accumulations it seemed that these two characters were independent (very low non-significant positive correlation). This result is similar to others reported in previous single environment trials[21]. Nevertheless, a deeper insight to the components of this correlation showed a more complex relation. There exists an important negative and highly significant environmental correlation. In the case of the total genetic correlation there is a positive significant correlation with opposite contribution of each subcomponent, as the genotypic correlation component is negative, but the GxE correlation component is positive and much more important. Therefore, the growing environments used can highly influence the selection due to their contribution to two opposite effects (the environmental and the interaction) making complicated the joint selection for high genotypic potential of both lycopene and ascorbic acid content. Summarizing, in most breeding programs it will only be realistic the combined

improvement of two characters, lycopene and β-carotene or β-carotene and ascorbic acid. The production of cultivars with increased levels of the three compounds, even if feasible, would be unstable and probably cause commercialization problems.

Prediction of the environmental, genotypic and interaction effects

A general mixed linear model was used for the prediction of the growing environment, genotype and interaction factors on the total phenotypic response, thus enabling a more appropriate and independent analysis of each effect (Fig 1).

For all the studied traits important differences between growing environments were detected (left side of Fig 1). The paired differences between spring-summer and autumn-winter and between open field and glasshouse cultivation were all significant (all P<critical values for FDR test at 0.05). These results could be better understood if the reported influence of climatic conditions in the biosynthesis of the antioxidants is considered together with the combination of temperature and radiation registered in the three environments. In this regard, it has been reported that the lycopene accumulation depends on temperature and seems to take place at a range of average day temperature between 12 and 32ºC[22]-35ºC[23], with the optimal conditions around 22-26ºC[24]. For β-carotene accumulation the range of average day temperature is wider than for lycopene. Its biosynthesis is poorly affected by temperatures lower than 12ºC[25] and with temperatures higher than 35ºC when the lycopene accumulation is inhibited the conversion of lycopene into β-carotene is stimulated[23]. Nevertheless, the optimal temperature for β-carotene accumulation seems to be around 30ºC[23]. The ascorbic acid accumulation in tomato fruits seems to be also directly correlated with temperature[26]. It has been suggested that at relatively high temperatures probably there is a decrease in

the ascorbic acid content due to oxidation[27], however these harmful conditions have not been studied properly. At favourable temperatures, the lycopene, β-carotene and ascorbic acid biosynthesis increase whit the sunlight intensity[24,28] probably due to the increase of photosynthetic rate. These light induced variations are especially important in the case of ascorbic acid accumulation. Normally, open field leads to higher ascorbic acid content than greenhouse cultivation, as well as harvesting at the later summer versus other seasons[29]. The reduction in ascorbic acid accumulation with reduced radiation conditions occurs can be as important as a 70%[10,28]. In the case of lycopene, when a harmful direct radiation level occurs (650 Wm^{-2} for 1.5-4 hours) its synthesis is inhibited. On the other hand, for ascorbic acid synthesis the excessive radiation does not inhibit its synthesis but causes a reduction on its accumulation[30].

For lycopene accumulation, in the spring-summer cycle in Turis favourable day average temperature conditions during the cultivation were recorded, especially in the harvest period when they were near to the optimum interval and did not exceed the thermal stress threshold (Fig 2). On the contrary, regarding the radiation conditions in the first half of the cycle the PAR radiation increased, reaching the maximum photosynthetic capacity and a high growing performance, but for the harvest period the amount of radiation surpassed the harmful threshold. To see it, the 650 W m^{-2} of total sun radiation was be expressed in the PAR scale. We considered that the proportion of PAR radiation vs. direct total radiation in our latitude for spring-summer cycle is 78.77% (information provided by National Meteorology Agency at Valencia) and the expression W $m^{-2}*4.57$ $^{=}\mu molm^{-2}$ s^{-1} for sun and sky daylight[31] led to obtain the harmful radiation threshold of 2340 $umolm^{-2}s^{-1}$. So, in this part of the growing cycle, the fruits would be exposed to excessive solar radiation that could lead to an arrest of lycopene biosynthesis and reduce the final level of lycopene accumulation

In the case of Valencia in spring-summer cycle in glasshouse, the day average temperature was slightly higher than in Turis but the heat dissipation systems were able to maintain it inside the favourable, though not optimal, temperature range almost all the days of cultivation. Regarding the radiation, due to the use of a shadowing system as part of the heat dissipation management, its level inside the protection was reduced and, in general, no radiation stress occurred.

In Valencia during the autumn-winter cycle the use of heating system maintained the temperature lightly under the lower limit of the optimal interval but inside the favourable temperature range of lycopene biosynthesis. Obviously for this cycle the radiation was not high and the lycopene accumulation was relatively good but not optimal.

Regarding β-carotene accumulation the worst growing environment was spring-summer cycle in Valencia, coinciding with the better growing conditions for lycopene accumulation. This is in agreement with the regulation proposed for the major biosynthesis pathway of both carotenoids in tomato: phytoene → phytofluene → ζ-carotene → neurosporene → lycopene → γ-carotene → β-carotene in which the enhanced flux of carotene in the pathway is arrested at lycopene in no stressing conditions[32]. On the contrary, in Turis in spring-summer cycle and in Valencia at autumn-winter cycle there were some stressing conditions that limited the lycopene accumulation but not the β-carotene. In Turis, the temperature range was better for the β-carotene biosynthesis than for lycopene, but as the radiation conditions led to an arrest in lycopene biosynthesis, its subsequent accumulation was important but not as high as it would be in this season with no radiation stress. This could explain that the β-carotene accumulation in Turis in the open air were similar to the levels accounted in a growing cycle (Valencia, autumn-winter) less favourable for carotenoid accumulation.

With respect to the ascorbic acid accumulation, the better combination of temperature and radiation occurred at Turis in open air during the spring-summer cycle, probably not being affected by the high radiation level as it may have been the case of lycopene. The worst condition for ascorbic acid accumulation was the autumn-winter cycle at Valencia, as the temperature and radiation in this cycle were lower than in the others.

The genetic merit of the accessions tested must be evaluated on both genotype main effect and GxE interaction (Fig 1), being compared with the genetic merit of the controls for reference.

For the lycopene accumulation the controls of fresh market type (CDP8779 and Cambria) had shown a genotypic main effect (black bars of Fig 1 a) that diminished the general mean (105.07 mg kg^{-1}) in 24.22 and 14.20 mg kg^{-1} respectively leading to a predicted lycopene content due to the genotypic effect of 80.85 and 90.87 mg kg^{-1} respectively. These genotypic potential of lycopene expression can be considered in the higher segment of modern commercial fresh market cultivars, as even considering the worst growing environment and interaction effects the predicted lycopene content for these two controls would be 66.15 and 71.96 mg kg^{-1} respectively, values higher than the best phenotypic value reported for cvs broadly grown in Spain (65 mg kg^{-1}, represented graphically in Fig 1a by the horizontal continuous line)[33].

Regarding the interaction effect, CDP8779 control showed a very stable performance with no significant and negligible predicted values in the three growing environments studied. Cambria had a similar performance but with a small instability (two significant GxE predicted effects). The processing tomato control (Gevora) showed a very high genotypic effect (increased in 45.38 mg kg^{-1} the general mean leading a lycopene accumulation of 150.45 mg kg^{-1}). It should be pointed out that Gevora is a cv adapted to

open field cultivation in spring-summer cycle in hot Spanish regions, and it showed negative interactions with growing environments differencing to this conditions that diminished its lycopene accumulation. The control accession LA1563, with the Intense Pigment (IP) gene, that has been reported to have an increased carotenoid accumulation around 60%[12], showed a genotypic potential between the other controls. Only in protected cultivation with climatic control in spring summer cycle its total genetic potential (G+GxE) would be higher than the processing tomato control $(7.19+40.99=48.18$ mg kg^{-1}). Anyway, the genotypic value of Gevora was chosen as the high threshold criterion to select interesting accessions.

Following this comparison criteria (represented graphically in Fig 1a by the horizontal dashed line), the best predicted genotypic values for lycopene accumulation were detected in accessions CDP1568, CDP7090 and CDP9822, all of them belonging to *S. pimpinellifolium*, with respectively 1.9, 1.71 and 1.68 times the genotypic potential of the industry control and 6, 5.47 and 5.36 times the genotypic potential of the commercial hybrid for fresh consumption. These accessions would be very interesting as donor parents in breeding programs for developing new cultivars. Due to its wild origin, these three accessions showed better adaptation to open field and spring-summer growing conditions (no significant GxE interaction in this environment). Accession CDP1568 showed small negative interactions with protected environment, especially in autumn-winter cycle that slightly diminished its total genetic potential $(86.54-23.59=62.95$ mg kg^{-1}). Nevertheless, it was the most stable accession of the selected three. Accession CDP7090 showed an important negative interaction in the growing environment with higher temperatures, which would decrease its total genetic potential $(77.71-41.21=36.5$ mg kg^{-1}) and hinder the selection of its descendants. On the contrary, accession CDP9822 showed an important and highly significant interaction in the

growing environment with higher temperatures. If this accession is selected to derive cultivars targeted to specific environments with these growing conditions, the total genetic potential would be very high (76.2+51.23=127.43 mg kg^{-1}). However, it should be considered that this accession is highly unstable and in other environments with lower temperatures and radiation its total genetic potential to accumulate lycopene would be dramatically diminished (76.2-57.45=18.75 mg kg^{-1}). Accessions CDP6957/R and CDP7632 (traditional varieties with interesting organoleptic quality) despite having a negative genotype subcomponent prediction, offered a total genetic potential for lycopene accumulation of 23.99 and 19.64 mg kg^{-1} respectively, twice as much as the fresh market reference control.

For β-carotene accumulation (Fig 1b), controls showed a genotypic potential and stability opposite to that observed for lycopene accumulation. These controls had shown, in the worst conditions (V s/s), a phenotypic β-carotene content that is 1.5 times the reported average content in tomato[18], so they could be considered good references. The best accession for β-carotene accumulation was CDP4777 from *S. lycopersicum* var *cerasiforme*. This accession showed more than twenty times the genotypic potential of the best control, the high carotenoid IP genotype, LA1563, and a high stability. Therefore, it will be very useful for both its use as donor parent in breeding programs and for direct consumption in gourmet uses, as it is a cherry tomato. Other accessions interesting for its use as donor parents in breeding programs for β-carotene accumulation were the three *S. pimpinellifolium* previously selected for their high lycopene content. In this sense, accessions CDP9822 and CDP1568 showed a genotypic value for β-carotene accumulation approximately ten times higher than the best control. However, these two accessions should be used in specific environments in order avoid negative GxE interaction. Accession CDP9822 should be targeted to protected

cultivation in spring-summer cycle and CDP1568 accession to open field cultivation. Accession CDP7090 showed a genotypic potential five times higher than the best control and as the other two selected wild accessions should be targeted to a specific environment (protected cultivation in autumn-winter cycle) to escape from negative interactions (note that lycopene and β-carotene interactions are opposite, thus for joint improvement of both compounds the condition showing negligible interaction is preferred). The same applies to the traditional variety CDP6957/R, but in this case the growing environment adequate for selection is open field cultivation in spring-summer cycle.

Finally, regarding ascorbic acid accumulation (Fig 1c), the controls showed phenotypic values lower than the commonly accepted average content of ascorbic acid in tomato (200 mg kg^{-1})[19]. Cambria showed the best performance of all the controls but due to the E and GxE effects. The best accession for use as donor parent in breeding programs was CDP4777 from *S. lycopersicum* var *cerasiforme* which also is the best donor parent for β-carotene content. CDP4777 had a genotypic value for ascorbic acid accumulation more than fifty times greater than the best control. It is also highly stable because the significant GXE interaction effects are small. Nevertheless, it should be noted that, as in the case of β-carotene accumulation, its performance is better in the open field. CDP9822 was other very interesting donor parent for breeding programs because, in specific environments (protected cultivation in spring-summer cycle) it has shown a very high GxE interaction effect, especially for ascorbic acid accumulation, that increased considerably its total genetic potential, and enables the improvement of the three functional traits studied. Finally, the traditional variety accession CDP7632 is also interesting for direct use for its high ascorbic acid genotypic potential (fifteen times greater than the best control) and stability.

CONCLUSIONS

Our results indicate that, in general, the high genetic component responsible of the accumulation of lycopene and β-carotene makes possible the selection of elite genotypes with high content of both carotenoids in tomato breeding programs. The high ratio of genotypic to environmental variance decomposition seems to indicate that high accumulation cultivars with wide adaptation might be successful despite the important environmental effects on carotenoid biosynthesis. Although there is a high genotypic correlation between the carotenoids studied, to perform a joint selection for both carotenoids it is mandatory to conduct multi-environment trials due to the existence of a considerably high negative environmental correlation. The improvement of the content of ascorbic acid is in most cases more difficult because the interference of uncontrolled factors masks the real genetic potential. Nevertheless, it would be possible to make a joint selection with β-carotene but renouncing to improve lycopene content.

Four accessions with an amazing genetic potential for functional traits have been identified. Three of them belong to *S. pimpinellifolium* (CDP1568, CDP7090 and CDP9822) and are especially interesting for their use as donor parents in the improvement of lycopene and β-carotene content. CDP1568 showed the best genotypic potential (1.9 times greater than the processing control and 6 times higher than the commercial hybrid control) and the most stable expression across all the environments tested. CDP9822 is interesting to derive hybrids with high carotenoid and ascorbic acid accumulation for specific target environments (protected cultivation in spring-summer cycle) due to the importance of the GxE interaction. CDP4777 from *S. lycopersicum* var *cerasiforme*, showed a very high genotypic potential to accumulate β-carotene and ascorbic acid (more than twenty and fifty times respectively than the fresh consumption

controls) and a high stability in their expression. This accession is a cherry local cultivar and might be used either as donor parent in breeding programs and for direct consumption in quality markets.

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Table 1. Characteristics of accessions evaluated.

Accession	Sp	Fruit characteristics	Origin
CDP8779	1	Large, light red	Valencia, Spain
CAMBRIA	1	Medium-size, red	Almeria, Spain
GEVORA	1	Medium-size, red	Badajoz, Spain
LA1563	1	Large, red	University of California
CDP2178	1	Medium-size, red	Piura, Perú
CDP7632	1	Medium-size, red	Loja, Ecuador
CDP2087	1	Large, red	Gran Canaria, Spain
CDP6957/A	1	Small, yellow	Alicante, Spain
CDP6957/R	1	Small, red	Alicante, Spain
CDP4777	2	Small, orange-brownish	Ipala, Guatemala
CDP7090	3	Very small, dark red	Piura, Perú
CDP1568	3	Very small, dark red	Piura, Perú
CDP9822	3	Very small, dark red	Piura, Perú
CDP9999	3	Very small, yellow	Lambayeque, Perú

Sp=Specie; 1=*Solanum lycopersicum*; 2= *S. lycopersicum* var. *cerasiforme*; 3=*S. pimpinellifolium*

Table 2. Phenotypic content (mean ± standard deviation, in mg kg^{-1} fresh weight) of lycopene (LYC), β-carotene (βCAR) and ascorbic acid (AsA) of accessions evaluated.

Location / Accession	Turis spring/summer			Valencia spring/summer			Valencia autumn/winter		
	LYC	βCAR	AsA	LYC	βCAR	AsA	LYC	βCAR	AsA
CDP8779	77±40	17±4	150±53	90±31	14±4	121±46	71±29	14±3	67±30
CAMBRIA	85±23	13±5	178±62	103±41	10±4	161±52	84±19	14±2	92±32
GEVORA	124±39	7±2	148±45	191±59	7±2	137±34	136±42	10±2	56±32
LA1563	113±40	16±5	116±52	123±43	11±3	137±64	101±34	18±4	71±35
CDP2178	102±32	9±2	135±38	143±51	8±2	142±70	115±33	12±4	52±28
CDP7632	70±25	17±4	261±96	95±37	11±3	193±40	89±29	14±3	136±39
CDP2087	93±36	12±3	138±79	104±38	8±2	113±48	86±32	13±4	59±26
CDP6957/A	1±2	8±2	206±116	2±3	7±2	109±78	1±1	4±1	36±32
CDP6957/R	56±22	20±3	194±91	107±41	18±3	143±66	94±35	19±5	118±57
CDP4777	65±16	32±6	346±108	82±26	29±6	250±80	104±28	35±7	331±122
CDP7090	225±80	18±7	162±96	227±96	17±5	139±93	75±4	18±2	6±5
CDP1568	173±35	28±5	113±58	227±95	16±5	136±94	185±73	24±5	57±29
CDP9822	139±41	24±6	214±139	169±75	22±7	191±104	281±2	22±0	296±30
CDP9999	2±3	15±4	229±253	3±5	11±3	156±125	2±1	15±5	10±7

Table 3. Estimated value and SE of variance components (and its percentage from total phenotypic variance) for lycopene, β-carotene, and ascorbic acid content of tomato fruits.

Parameter†	Lycopene	β-carotene	Ascorbic acid
V_G	3273.94±198.13**	46.47±1.69**	2747.62±242.41**
	(58.05%)	(65.25%)	(23.56%)
V_E	90.23±33.99*	3.27±0.50**	1517.92±215.08**
	(1.60%)	(4.60%)	(13.01%)
$V_{G×E}$	663.46±158.14**	3.82±0.61**	1015.12±241.63**
	(11.76%)	(5.37%)	(8.70%)
$V_{B(E)}$	71.97±17.77**	0.28±0.03**	105.59±28.14**
	(1.28%)	(0.41%)	(0.91%)
V_e	1539.96	17.35	6278.28
	(27.31%)	(24.37%)	(53.82%)
V_P	5639.58	71.22	11664.53

†V_G=genotypic main variance, V_E=environment main variance, V_{GxE}= genotype x environment variance, $V_{B(E)}$= block in growing environment variance, V_e=residual variance, V_P=phenotypic variance.
*·** Significantly different from zero (t-test) at the 0.05 and 0.01 levels of probability respectively.

Table 4. Phenotypic, genotypic, environmental and interaction paired correlations (estimated value ± SE) for the functional characters studied in tomato fruits.

Correlation†	Lycopene vs β-carotene	β-carotene vs Ascorbic acid	Lycopene vs Ascorbic acid
r_P	0.20±0.01**	0.32±0.01**	0.01±0.01NS
r_G	0.36±0.01**	0.77±0.02**	-0.14±0.02**
r_E	-1.00±0.05**	-0.07±0.03*	-0.23±0.06**
r_{GxE}	-0.13±2.47 NS	0.03±0.01**	0.63±0.02**

† r_P = phenotypic correlation, r_G = genotypic correlation, r_E = environmental correlation, r_{GxE} = genotype x environment interaction correlation.

*,** Significantly different from zero (t-test) at P = 0.05and 0.01 level respectively. NS = non-significant