

## New opportunities for developing tomato varieties with enhanced carotenoid content

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**ABSTRACT:** The development of varieties with a high content of antioxidant compounds, such as carotenoids, has become a major focus in the marketing of tomato. Several mutants have been used in the development of high pigment varieties, but the significant influence of the environment on carotenoid content and the presence of negative side effects in vegetative growth and yield have limited the success of these variants. Consequently, the identification of alternative sources of variation in the quest for high carotenoid content is ongoing. In this study, 12 accessions of *Solanum lycopersicum* (including the former var *cerasiforme*) and *S. pimpinellifolium* have been evaluated in three different environments: open field and glasshouse cultivation at two sites. Three accessions (BGV6195 of *S. pimpinellifolium*, LA1423 of the former var *cerasiforme* and LA3633 a possible hybrid between *S. pimpinellifolium* and *S. lycopersicum*) showed outstanding and stable lycopene content, that doubled in all three environments the content of the positive control LA3538, with the *high pigment-1* mutation (*hp1*). In addition, accession CATIE14812 would also be interesting as regards improvement of  $\beta$ -carotene content. These materials offer new opportunities in the development of tomato varieties with enriched and reliable carotenoid content and the close taxonomic relationship of these accessions with cultivated tomato will facilitate their use in breeding programs.

**Keywords:** *Solanum lycopersicum*, functional quality, lycopene,  $\beta$ -carotene, genetic resources

### Introduction

The consumer is starting to value the role food can play in the improvement of their health and the reduction of the risk of disease beyond the conventional roles of nutrients or sensorial pleasures (Granato et al., 2010). The increasing cost of healthcare, life expectancy and the desire of older people to improve their life quality have reinforced the importance of this role (Roberfroid, 2007). One of the strategies pursued in the development of functional food is the concentration of bioactive substances in quantities higher than usual (Bigliardi and Galati, 2013).

Although not especially rich in vitamins and antioxidants, tomato has become an interesting source of antioxidants considering its high level of consumption (mean values of food supply in 2011 of 27.6 and 36.2 kg yr<sup>-1</sup> per capita in Europe and North America, respectively; source: FAO databases). As an example, in Spain tomatoes represent the first dietary source of lycopene, the second of vitamin C and  $\beta$ -carotene and the third of vitamin E (García-Closas et al., 2004). In addition, tomato may also represent an important source of flavonols in the diet (Stewart et al., 2000). Consequently, tomato has demonstrated moderately strong evidence of clinical efficacy presumably because of its beneficial constituents (Sun-Waterhouse, 2011). In fact, the latest research highlights the relationship between the consumption of tomato and its products and a reduced risk of various maladies like obesity, hyperglycemic and hypercholesterolemic attributes, cardiovascular disorders, and cancer insurgences (Perveen et al., 2015).

Special attention has been focused on the carotenoid content and its effects on health. Higher lycopene

dietary intake was inversely associated with total prostate cancer and with angiogenic potential, suggesting a reduction of the aggressive potential of prostate cancer (Zu et al., 2014). The use of lycopene extracts as a supplement has been compared to the consumption of lycopene in the form of whole food sources, and it has been concluded that in the case of cardiovascular risk factors, tomato intake was more favorable with the exception of blood pressure management (Burton-Freeman and Sesso, 2014).

It is, therefore, of great interest to enhance the accumulation of carotenoids in tomatoes. Several mutants have been identified for this purpose, but the most efficient ones (high pigment mutations) have deleterious effects on agronomic performance (reviewed by Cebolla-Cornejo et al., 2013). In this context, the aim of this study was to evaluate germplasm in order to identify sources of variation with high carotenoid content.

### Materials and Methods

#### Plant materials

Five *Solanum lycopersicum* L. cultivated accessions and ten accessions collected in the wild and belonging to the tomato wild ancestor *Solanum pimpinellifolium* L. and to the former variety *cerasiforme* of the cultivated species *S. lycopersicum* were studied (Table 1). Accession LA3633, despite being classified as the former *cerasiforme* variety of *S. lycopersicum* and later as possible *S. pimpinellifolium* in the Tomato Genetic Resource Center (TGRC, U.S.) database, showed intermediate traits between *S. lycopersicum* and *S. pimpinellifolium*. The origin of the accessions evaluated is as follows: PI accessions were provided by the Northeast Regional PI Station

Table 1 – Characteristics and origin of the 15 tomato accessions evaluated for  $\beta$ -carotene and lycopene content.

Accession	Species <sup>1</sup>	Fruit characteristics	Origin
BGV2091	Sl	Medium size, red	Girona, Spain
BGV12627	Slc	Small size, red	Periera, Colombia
CATIE14817	Slc	Small size, red	San Carlos, Guatemala
CATIE18628	Slc	Small size, red	San José, Costa Rica
LA1289	Slc	Small size, red	Junin, Peru
LA1423	Slc	Small size, red	Pichincha, Ecuador
BGV4654	Sp	Small size, red	Connectica, USA
BGV6195	Sp	Small size, red	Cañar, Ecuador
CATIE14812	Sp	Small, size red	San Carlos, Guatemala
PI365959	Sp	Small size, red	Ancash, Peru
LA3633	Slp	Small size, red	Ghana
LA3538	Sl	Medium size, red, high carotenoid ( <i>hp1</i> )	USA
CDP4777	Slc	Small size, red, high $\beta$ -carotene	Chiquimula, Guatemala
BGV5655	Slc	Medium size, red, medium-high lycopene	Valencia, Spain
CDP8779	Sl	Big size, red, low carotenoid content	Spain
FORTUNA-C	Sl	Big size, red, reference in other studies	Spain

<sup>1</sup>Sl: *Solanum lycopersicum*; Slc: *S. lycopersicum* (former var. *cerasiforme*); Sp: *S. pimpinellifolium*; Slp: Possible cross between *S. lycopersicum* and *S. pimpinellifolium*.

(U.S.), LA accessions were provided by the TGRC, CATIE accessions were provided by the Tropical Agricultural Research and Higher Education Center (CATIE, Costa Rica) and CDP and BGV accessions were provided by the Institute of Conservation & Improvement of Valencian Agrodiversity (COMAV, Spain). Two breeding lines FORTUNA-C and CDP8779 (alias NE-1) were also provided by COMAV (Spain), and were included as a reference of standard to low carotenoid content considering results of previous studies. As references for high carotenoid content accession LA-3538, which carries the *high pigment 1*, *hp-1*, gene (Jarret et al., 1984), and accession CDP4777, with high  $\beta$ -carotene content (Roselló et al., 2011, Adalid et al., 2012), were included. Finally, accession BGV5655 was also included as a traditional variety with intermediate lycopene content (Adalid et al., 2007).

#### Experimental design and growing conditions

A randomized complete block design was used with three blocks per environment, 15 plots per block (one per accession) and 3 plants per plot. Plants were cultivated in three different environments in order to check the stability of carotenoid accumulation. In Castellón (39°59' N, 0°2' W 35m asl) plants were cultivated in both a glasshouse and the open field. In Valencia (39°28' N, 0°22' W 15 m asl) plants were cultivated in a glasshouse only. The growing cycle selected for all cases was spring-summer. In protected cultivation, heat dissipation systems (progressive shadowing and cooling) were used. Plants were staked and pruned properly and fertirrigation was applied following the usual regimes for the crop and the area. Air temperature and photosynthetically active radiation (PAR) were recorded using a weather station equipped with temperature, quantum light PAR sensors and data logger (Figure 1A).

#### Sampling

Fruits were harvested at the red-ripe stage (when fruits reached maximum color intensity) from the first three trusses. In small fruited populations at least 10 fruits per plant were sampled; otherwise four fruits were sampled per plant. Samples were blended at low light intensity and stored at -20 °C until analysis.

#### Lycopene and $\beta$ -carotene determination

Samples were thawed in the dark at 4 °C and 100 mg of the homogenate were extracted with 14 mL of a 8:6 v/v, ethanol/hexane solution at 4 °C, for 24 h at 200 rpm using a horizontal shaker. Hexane was complemented with 0.1 % butylated hydroxytoluene, (BHT). Hexane supernatant was separated and concentrated using a SpeedVac to complete dryness, and then re-suspended in 500  $\mu$ L of hexane containing 0.1 % BHT. The sample was then filtered using a hydrophobic filter of 0.20  $\mu$ m.

Lycopene and  $\beta$ -carotene were determined using reverse phase high performance liquid chromatography (HPLC), with the method reported by García-Plazaola and Becerril (1999) with slight modifications. Analyses were carried out on a 1200 series HPLC detector equipped with G1322A vacuum degasser, G1312A quaternary pump, G1329A standard autosampler, G1316 thermostated column compartment and G1315b diode array detector.

The chromatography was conducted in a column of reverse phase Zorbax ODS (250  $\times$  4.6 mm i.d., 5  $\mu$ m, particle size) protected by a guard column (12.5  $\times$  4.6 mm i.d., 5  $\mu$ m particle size). The mobile phase consisted of two components: solvent A with 84:9:7 v/v/v, acetonitrile/methanol/water and solvent B with 68:32 v/v, methanol/ethyl acetate. The injection volume was 40  $\mu$ L. The sample was then eluted using a lineal gradient from 100 % of solvent A to 100 % of solvent B for 12 min, followed by an isocratic elution of 100 % of solvent B for 7

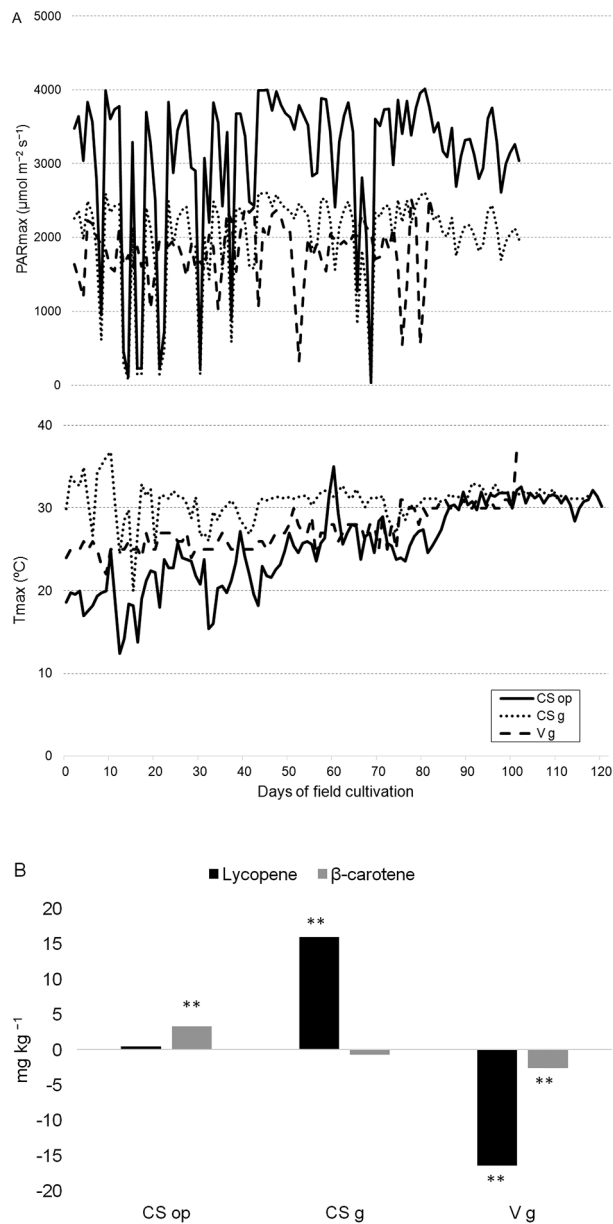


Figure 1 – (A) Maximum temperature and maximum photosynthetically active radiation (PAR) registered in the environments used for tomato cultivation. (B) Mean environmental predicted effect for lycopene and β-carotene accumulation in each of the environments tested. CS op: Castellón open field; CS g: Castellón glasshouse; V g: Valencia glasshouse. \*\*Significant environmental effect (t-test;  $p < 0.01$ ).

min. Then, a lineal gradient was established from 100 % of solvent B to 100 % of solvent A for 1 min. Finally, an isocratic elution of 100 % of solvent A was administered for 6 min to allow the column to re-equilibrate. The integration of the lycopene and β-carotene peaks was conducted at 470 and 445 nm respectively. The concentration of each item was computed using linear regressions

using five solutions with variable concentrations of each component. The determination coefficient was higher than 0.99 in all cases. Each sample was analyzed twice. The results were reported as mg kg<sup>-1</sup> of fresh weight.

### Data analysis

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

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

where  $Y$  = phenotypic value (population mean  $\mu$ ; variance  $V_p$ );  $G$  = genotype effect (mean = 0; variance =  $V_G$ );  $E$  = environment effect (mean = 0; variance =  $V_E$ );  $GE$  = Genotype  $\times$  Environment interaction effect (mean = 0; variance =  $V_{G \times E}$ );  $B$  = the block effect (mean = 0; variance =  $V_B$ );  $e$  = residual effect (mean = 0; variance =  $V_e$ ). All the factors were considered as random and the adjusted unbiased prediction (AUP) method (Zhu and Weir, 1996) was used to predict them. Standard errors of the statistics were obtained by the jackknife procedures (Miller, 1974; Zhu and Weir, 1996) and a two-tail t-test was performed for testing the significance of parameters obtained. The model was also recalculated considering the genotype as a fixed factor for mean performance accession comparison computing using the LSD method where  $\alpha = 0.05$ . All the data analyses were performed with QGASStation (version 1) software.

## Results

A wide range of lycopene and β-carotene accumulation was observed among the accessions and environments tested and even within the accession itself (Table 2). For both the accumulation of lycopene and β-carotene the block effect was not significant ( $p > 0.7$ ) and this effect was discarded in the remaining statistical analysis. The observed phenotypic variation and the different trends of variation across environments indicated that the genotype and the genotype  $\times$  environment interaction would be important in the accumulation of carotenoids. Consequently, a deeper study of the effects of environment, genotype and genotype  $\times$  environment interaction was performed, as the breeding value of the germplasm depends on the genetic potential.

### Environmental effect

Contribution of the main environmental effect on phenotypic variance in lycopene ( $10 \% \pm 1 \%$ ) and β-carotene ( $9 \% \pm 1 \%$  accumulation) was estimated as significant ( $p < 0.001$ ). This environmental effect was mainly attributed to temperature and photosynthetically active radiation (PAR) differences between the three environments evaluated (Figure 1). Shadowing used in the glasshouse as part of the heat dissipation systems almost halved the radiation compared with the open field (Figure 1A). Differences in PAR measured in the two glass-

Table 2 – Phenotypic content (mean  $\pm$  standard error) in  $\beta$ -carotene and lycopene ( $\text{mg kg}^{-1}$ ) of 15 tomato accessions grown in three environments in Spain. The first five accessions are references for high (+), intermediate (+/-) or low (-) carotenoid content or have been studied for the accumulation of other bioactive compounds (R).

Accession	$\beta$ -carotene ( $\text{mg kg}^{-1}$ )			Lycopene ( $\text{mg kg}^{-1}$ )		
	Castellón Open-field	Castellón Glasshouse	Valencia Glasshouse	Castellón Open-field	Castellón Glasshouse	Valencia Glasshouse
CDP8779 (-)	16.64 $\pm$ 2.85	9.31 $\pm$ 1.83	8.15 $\pm$ 2.35	46.27 $\pm$ 6.59	45.70 $\pm$ 11.21	37.43 $\pm$ 10.80
FORTUNA-C (R)	10.17 $\pm$ 1.31	9.61 $\pm$ 1.64	7.33 $\pm$ 2.11	54.69 $\pm$ 6.81	85.10 $\pm$ 17.56	45.64 $\pm$ 13.18
LA3538 (+)	37.52 $\pm$ 3.19	25.57 $\pm$ 5.30	25.18 $\pm$ 7.27	71.31 $\pm$ 12.00	107.06 $\pm$ 17.84	55.18 $\pm$ 15.93
CDP4777 (+)	23.28 $\pm$ 2.63	21.68 $\pm$ 3.01	25.32 $\pm$ 7.31	48.30 $\pm$ 4.97	89.33 $\pm$ 13.71	66.08 $\pm$ 19.07
BGV5655 (+/-)	10.51 $\pm$ 1.14	7.23 $\pm$ 1.40	9.26 $\pm$ 2.67	67.80 $\pm$ 7.03	69.15 $\pm$ 13.38	43.46 $\pm$ 12.55
PI365959	24.30 $\pm$ 3.39	21.88 $\pm$ 3.69	14.38 $\pm$ 4.15	46.68 $\pm$ 16.32	82.98 $\pm$ 20.03	26.34 $\pm$ 7.60
BGV12627	10.36 $\pm$ 2.06	8.84 $\pm$ 1.59	11.29 $\pm$ 3.26	69.35 $\pm$ 12.55	65.34 $\pm$ 10.87	67.14 $\pm$ 19.38
CATIE18628	22.69 $\pm$ 1.80	22.09 $\pm$ 4.32	17.53 $\pm$ 5.06	58.81 $\pm$ 5.47	91.87 $\pm$ 14.55	71.46 $\pm$ 20.63
CATIE14812	29.57 $\pm$ 4.53	32.97 $\pm$ 4.82	25.42 $\pm$ 7.34	40.36 $\pm$ 6.18	92.76 $\pm$ 12.55	40.75 $\pm$ 11.76
BGV6195	22.52 $\pm$ 2.96	14.83 $\pm$ 1.93	8.31 $\pm$ 2.40	154.89 $\pm$ 15.25	164.92 $\pm$ 22.91	118.27 $\pm$ 34.14
LA1289	14.05 $\pm$ 1.90	11.60 $\pm$ 1.80	10.85 $\pm$ 3.13	70.64 $\pm$ 9.01	106.04 $\pm$ 14.61	43.55 $\pm$ 12.57
LA3633	26.37 $\pm$ 2.32	9.11 $\pm$ 1.88	12.35 $\pm$ 3.56	161.89 $\pm$ 19.30	119.72 $\pm$ 20.41	123.06 $\pm$ 35.53
LA1423	21.88 $\pm$ 2.34	13.16 $\pm$ 1.83	12.08 $\pm$ 3.49	147.64 $\pm$ 16.33	162.99 $\pm$ 39.92	121.47 $\pm$ 35.07
CATIE14817	25.69 $\pm$ 3.29	18.80 $\pm$ 1.35	17.05 $\pm$ 5.14	73.42 $\pm$ 10.95	72.40 $\pm$ 11.68	43.34 $\pm$ 13.07
BGV2091	19.46 $\pm$ 4.13	21.70 $\pm$ 2.48	15.68 $\pm$ 4.53	83.64 $\pm$ 10.82	68.96 $\pm$ 17.85	14.45 $\pm$ 4.17

houses were also observed, radiation in Castellon-glasshouse being 20 % higher than in Valencia-glasshouse. The maximum temperature in Castellon-glasshouse was considerably higher during the bulk of the cultivation cycle (Figure 1A). Between Valencia-glasshouse and Castellón-open field, maximum temperatures were higher in the former during the first third of the cultivation cycle.

The general mixed linear model used provided an estimation of the individual contribution of each of the three environments tested to the phenotypic expression of lycopene and  $\beta$ -carotene content (Figure 1B). The environmental conditions in Castellón-glasshouse (an average of maximum PAR radiation near 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and average maximum temperature of 31 °C) were significantly the best for potentiating lycopene accumulation, while the conditions in Castellón-open field (an average of maximum PAR radiation near 3000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and an average maximum temperature of 24 °C) significantly favored  $\beta$ -carotene accumulation. A negative effect was detected in the environmental conditions of Valencia-glasshouse (an average of maximum PAR radiation near 1150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and average maximum temperature of 27 °C) for the accumulation of both carotenoids. The maximum environmental effect was higher in lycopene than in  $\beta$ -carotene (Figure 1), though the differences in relative values (considering the total mean value) were lower, 20 % in the case of lycopene and 15 % in the case of  $\beta$ -carotene.

#### Genotype and genotype $\times$ environment interaction effects

Both the genotype's main effect ( $p < 0.001$ ) and the genotype  $\times$  environment (G  $\times$  E) interaction ( $p < 0.001$ ) made a significant contribution to phenotypic variance in lycopene and  $\beta$ -carotene accumulation. The main con-

tribution was due to the genotype's main effect (41 %  $\pm$  1 % for lycopene and 39 %  $\pm$  1 % for  $\beta$ -carotene). Being considerably lower, the contribution of the genotype  $\times$  environment interaction was not negligible (5 %  $\pm$  1 % both for lycopene and  $\beta$ -carotene). Consequently, the genetic merit of the accessions should be considered including both the genotype's main effect and the G  $\times$  E interaction.

For lycopene accumulation, the general linear mixed model provided an estimation of the significant contributions of total genotype ( $\mu + G$ ), and each interaction (Figure 2). CDP8779, a negative control with usually standard to low lycopene values showed, as expected, a low genotypic contribution. FORTUNA-C, used as a control in the analysis of other bioactive compounds, showed an intermediate total genotypic contribution. Positive controls CDP4777 and LA3538, which had previously shown medium to high lycopene content, showed total genotypic values compatible with the expectations. Considering the total genotypic contribution of the best and worst control, a range of variation was established in order to identify promising accessions (horizontal dashed lines in Figure 2).

CDP8779 and CDP4777 proved to be the most unstable controls showing important and significant contributions of the G  $\times$  E interaction in two of the three environments tested (Figure 2). High lycopene LA3538, and intermediate lycopene CDP5665 were the most stable controls and showed no significant G  $\times$  E interactions. In the case of FORTUNA-C only one significant contribution was found in Castellon open field.

Most of the accessions showed total genotypic contribution values for lycopene content in the range defined by the extreme positive and negative controls (Figure 2). Two of them, CATIE18628 and LA1289,



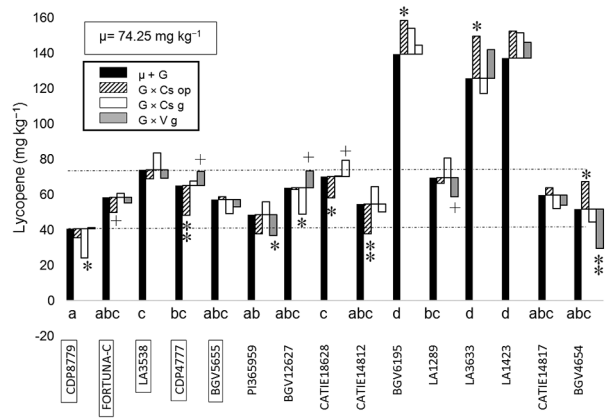


Figure 2 – Predicted total genotypic ( $\mu + G$ ) and interaction ( $G \times E$ ) effects for lycopene accumulation in three environments: Cs op: Castellón open field, CS g: Castellón glasshouse, V g: Valencia glasshouse. First five accessions are controls. Reference line: higher genotypic value of controls (upper dashed line), lower genotypic value of controls (lower dashed line). Different letters indicate significant differences in total genotypic effect between accessions (LSD test,  $p > 0.05$ ). Significant departures from zero (t-test) are indicated with + ( $p < 0.1$ ), \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ ).

showed a total genotypic effect similar to those of the high lycopene control. Accessions BGV6195, LA3633 and LA1423 showed a similar total genotypic effect that nearly doubled the value of the high lycopene control LA3538. Among these accessions, LA1423 showed higher stability, as it had no significant  $G \times E$  interactions. BGV6195 and LA3633 showed a significant positive  $G \times E$  interaction in Castellón open field that enhanced their total genotypic potential (Figure 2).

Regarding  $\beta$ -carotene accumulation, a wider range of variation was found between the control with the highest total genotypic contribution, LA3538, and the one with the lowest, FORTUNA-C (Figure 3). As in the case of lycopene accumulation, the relative contributions of interactions were considerably lower than the total genotypic contributions. The  $G \times E$  interactions tended to decrease  $\beta$ -carotene in the case of the controls with lower accumulation and increase it in the controls with higher accumulation.

Among the accessions, only CATIE14812 showed a total genotypic contribution similar to the high carotenoid control LA3538 and significantly higher than the high  $\beta$ -carotene control CDP4777. Cultivation in Castellón glasshouse offered a significant  $G \times E$  interaction that increased the potential  $\beta$ -carotene accumulation of CATIE14812.

## Discussion

Some authors consider that lycopene content in tomato accession ranges between 78.3 and 181.2  $\text{mg kg}^{-1}$ , for low-content and high-content varieties respectively

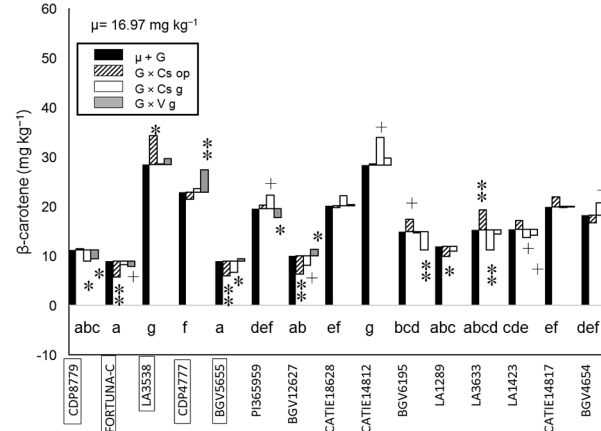


Figure 3 – Predicted total genotypic ( $\mu + G$ ) and interaction ( $G \times E$ ) effects for  $\beta$ -carotene accumulation in three environments: Cs op: Castellón open field, Cs g: Castellón glasshouse, V g: Valencia glasshouse. First five accessions are controls. Reference line: higher genotypic value of controls (upper dashed line), lower genotypic value of controls (lower dashed line). Different letters indicate significant differences in total genotypic effect between accessions (LSD test,  $p > 0.05$ ). Significant departures from zero (t-test) are indicated with + ( $p < 0.1$ ), \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ ).

(Davies and Hobson, 1981; Klee and Giovannoni, 2011). However, other authors have reported that the most common average lycopene content of raw tomatoes is around 30  $\text{mg kg}^{-1}$  (Holden et al., 1999; Kuti and Konuru, 2005). Evidently, an important environmental effect is expected and considerable differences in lycopene accumulation have been identified in tomato cultivars grown in different countries (reviewed in Dumas et al., 2003). In Spain, widely grown hybrid cultivars such as 'Rambo', 'Daniella' and 'Durina' have shown contents ranging between 32, 36 and 65  $\text{mg kg}^{-1}$  (Martínez-Valverde et al., 2002).

In this context, while screening for sources of variation of carotenoid content it is essential to include several controls in order to be able to compare results. The phenotypic average lycopene values obtained in previous trials for the low content control line, CDP8779, reached 90  $\text{mg kg}^{-1}$  (Roselló et al., 2011), whereas in this work, the different environmental conditions contributed to diminish its lycopene content by 50 %. A similar reduction applied to the control with intermediate contents, BGV5655, which in the same publication reached up to 120  $\text{mg kg}^{-1}$ . In the case of the control CDP4777, a lower reduction in lycopene content was observed (around 25 %) compared with previous trials where it reached 104  $\text{mg kg}^{-1}$  (Roselló et al., 2011). The control LA3538 had previously shown 94  $\text{mg kg}^{-1}$  (Adalid et al., 2007); glasshouse cultivation in Valencia represented an important reduction compared with this value, though the differences in the other environments were lower. These results confirmed that the environment has indeed an important influence in lycopene accumulation,

though the influence of different analytical procedures (spectrophotometry vs. HPLC) cannot be discarded. Additionally, it should be considered that all environments included in this study were sub-optimal and better performance is expected for the promising accessions identified.

Among the different environmental determinants for carotenoid accumulation, it has been described that lycopene content increases with temperature (Krumbein et al., 2006) and sun radiation (Dumas et al., 2003) until limiting conditions are reached (Hamauzu et al., 1998). In this sense, it has been reported that the rate of lycopene synthesis in tomato plants declines drastically with temperatures above 30-35 °C (Baqar and Lee, 1978), and is completely inhibited over 32 °C in excised fruit (Tomes, 1963; Dumas et al., 2003). In this study, the maximum temperature was lower than the 32 °C threshold continuously through to the end of the cycle. Considering that average temperature in our conditions was lower than in previous studies (Roselló et al., 2011), sub-optimal temperatures could have resulted in lower lycopene accumulation. Accordingly, a considerable effect of temperature would explain higher lycopene accumulation in Castellón glasshouse, as this environment showed the highest temperatures, below the limit for lycopene synthesis. The worst performance observed in Valencia compared to Castellón open field would not be explained by a temperature effect, but rather by a radiation effect, as PAR in this environment was lower during the entire growing cycle. In this sense, Gautier et al. (2005) found that the content in lycopene and  $\beta$ -carotene increased with exposure to photosynthetic radiation, and Jarquín-Enríquez et al. (2013) observed that tomato fruit which received greater amounts of light accumulated more lycopene. It should be considered though, that Kläring and Krumbein (2013) found no effect of shading in the content of lycopene in tomatoes grown in greenhouses.

Although the effect of environment was quite important (maximum relative contribution compared to total mean contribution around 20 % for lycopene and 15 % for  $\beta$ -carotene), the effect of G  $\times$  E interactions was not negligible and in some cases (eg. BGV0991 Castellón glasshouse) it reached more than 30 % of the total mean contribution. The considerable effects of both environment and G  $\times$  E interaction reinforce the need to develop multi-environmental trials when screening for sources of variation of functional compounds. This important effect was also observed for the accumulation of vitamin C in which the effect of G  $\times$  E interaction was higher than the effect of environment (Leiva-Brondo et al., 2012). The importance of G  $\times$  E interaction was also highlighted in screening trials for vitamin C, carotenoids and polyphenols in traditional varieties of tomato (Cortés-Olmos et al., 2014).

Most of the accessions tested showed genotypic values for lycopene accumulation between those obtained with low and high accumulation control lines.

But outstanding values regarding lycopene contents were identified in three accessions (BGV6195, LA1423 and LA3633). The three accessions nearly doubled (1.9, 1.9 and 1.7 times respectively) the genotypic value of the positive control LA3538 that carries the *high pigment-1*, *hp-1*, mutation (Jarret et al., 1984) and nearly 2.5-fold the value of the low accumulation controls.

Several circumstances point to a different genetic control of increased lycopene accumulation identified in the promising lines compared to the high pigment control. The mutation *hp-1* originated in 1917 in an undetermined *S. lycopersicum* variety (Kerr, 1965) and one of these lines, BGV6195 belongs to a different species: *S. pimpinellifolium* and LA3633, a possible crossing between the cultivated species and *S. pimpinellifolium*. Additionally, high pigment mutations result in increased content in  $\beta$ -carotene (Long et al., 2006), while the total genotypic contribution of these three accessions for  $\beta$ -carotene accumulation was considerably lower than in LA3538. It does not seem either that the high genotypic potential of the lines depends on the size of the fruit. It has been described that carotenoids accumulate differentially in the fruit, with more than 2-fold amounts of lycopene in the pericarp than in the locules and 4-fold higher amounts of  $\beta$ -carotene in the locules than in the pericarp (Davies and Hobson, 1981). Thus, accessions with reduced fruit size may benefit in lycopene accumulation considering the high ratios' fruit surface to volume. Although the three accessions identified with high lycopene content have reduced fruit size, as expected considering their taxonomy, they offer higher content than other accessions with similar sizes; thus the justification of high content should be different.

Accession LA1423 would be the more stable of these promising materials with no significant G  $\times$  E interactions, though the only significant interaction in both LA3633 and BGV6195 would lead to higher lycopene content in the open field. LA1423 and LA3633 previously showed a high potential for vitamin C accumulation, though in that case LA1423 proved to be quite unstable (Leiva-Brondo et al., 2012).

As regards  $\beta$ -carotene accumulation, the average content of raw tomatoes has been reported to be in the range of 1.4 to 11.9 mg kg<sup>-1</sup> (Davies and Hobson, 1981; Holden et al., 1999; Klee and Giovannoni, 2011). The average values of the low control lines obtained in this study were 6 and 11 mg kg<sup>-1</sup> that are within this range, and the values are similar to those obtained in other assays under our conditions (Adalid et al., 2010; Roselló et al., 2011).

Among the evaluated accessions only CATIE14812 of *S. pimpinellifolium* showed a similar total genotypic contribution similar to the best control, LA3538 (28.4 vs. 28.51 mg kg<sup>-1</sup>). Again, as it belongs to a different species and does not accumulate high levels of lycopene, this seems to indicate that the control for such accumulation should be different. What's more, it did not have an orange color. This phenotype would indi-

cate an effect similar to the *Beta* mutant, which entails a mutation in the promoter of the CYC-B  $\beta$ -cyclase, increasing the level of transcription and resulting in increased levels of  $\beta$ -carotene at the expense of lycopene (reviewed by Cebolla-Cornejo et al., 2013). CATIE14812 also reached a  $\beta$ -carotene content higher than CDP4777 which was previously described as a good material for the improvement of this carotenoid in tomato (Adalid et al., 2012).

## Conclusion

Lycopene and  $\beta$ -carotene content varies widely according to the environment. Despite the instability, accessions BGV6195 of *S. pimpinellifolium*, LA1423 of *S. lycopersicon* var *cerasiforme* and LA3633, a possible hybrid between *S. pimpinellifolium* and *S. lycopersicon* var *cerasiforme*, have shown high genotypic potential for the improvement of lycopene content in new varieties of tomato targeted to quality markets appreciating the functional value of foods. The close relationship of these materials with cultivated tomato will enable a more efficient and rapid exploitation of their potential in breeding programs. Furthermore, the accession CATIE14812 of *S. pimpinellifolium*, will also have interest for the improvement of  $\beta$ -carotene.

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