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THE ROLE OF TRADITIONAL VARIETIES OF TOMATO AS SOURCES OF NUTRIOTIONAL COMPOUNDS

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RUNNING TITLE

ABSTRACT

BACKGROUND: Traditional varieties of tomato have developed their own niches in quality markets in some European markets associated with their excellent organoleptic quality.

RESULTS: A collection of 126 populations of 16 traditional varieties from the East of Spain (a secondary diversity center for tomato) have been evaluated during two years in order to determine their potential value also as sources of functional compounds, including ascorbic acid, lycopene and β -carotene. Population and population x year interaction significantly affect lycopene and ascorbic acid content, while year effect was also significant for β -carotene. Despite finding some global trends in certain varieties concerning their functional value, high levels of variation have been found in the intra-varietal level. Populations with high levels of the three levels have been found, with different

levels of intra-population and inter-year variation. Maximum mean contents for both years have reached 308 mg kg⁻¹ of ascorbic acid, 130 mg kg⁻¹ of lycopene and 30 mg kg⁻¹ of β-carotene, though it is difficult to identify accessions with joint high values of the three compounds.

CONCLUSION: These results open the possibility to promote traditional materials as sources of functional compounds, thus strengthening their quality niches and consolidating their price-premium. Additionally, these materials could also be used in breeding programs for quality.

HIGHLIGHTS:

- Traditional varieties of tomato have been evaluated for functional compounds.
- Some traditional populations of tomato show high contents in functional compounds.
- Some show limited degree of variability.
- They can supply quality markets with fruits with added functional value.
- They are a source of variation in breeding programs of commercial hybrid varieties.

KEYWORDS:

Lycopene, β-carotene, ascorbic acid, *Solanum lycopersicum*, quality, food composition

INTRODUCTION

Modern consumers are increasingly interested in their personal health and expect the food they eat to be healthy or capable of preventing illnesses (Granato et al., 2010). Among the functional foods that satisfy the requirements of these consumers, those including omega 3 fatty acids, plants sterols or probiotics, have experienced an upward trend in consumer interest (Jones and Jew, 2007). Accordingly, the dairy sector has experienced a boost in the development of this kind of products, though non-dairy matrices are also gaining prominence (Sun-Waterhouse, 2011).

However, the niche market of functional food is not only restricted to processed products. There is also a trend to promote the characteristics of vegetable consumption in the diet. In this sense, dietary antioxidants and components of fruit and vegetable extracts are increasingly suggested to have the capacity to modulate the complex mechanisms involved in maintaining a healthy physiology and reducing early onset of age-dependent diseases (Auroma et al., 2012). It should also be considered that recent evidence suggests the ability of phytochemical components in whole foods is more effective in protectively supporting human health than isolated individual phytochemicals (Vattem et al., 2005). Accordingly, the development and commercialization of new varieties with increased levels of functional phytochemicals has become a new trend in the fruit and vegetable market and breeding industry.

In this context, tomato is included as one of the most studied active plant-based food (Sun-Waterhouse, 2011). In tomato marketing, as in other fruits and vegetables, a high content in active phytochemicals has gained importance, though it is not clear whether consumer interests in healthy products have conditioned the marketing of this kind of produces based on health functionality or vice versa (Goldman, 2011).

Among the different functional compounds present in tomato, vitamin C or ascorbic acid, carotenoids and flavonols have received most part of the attention. In the case of vitamin C the levels found in tomato compared with other species such as orange or broccoli are considerably low (Davey et al., 2000). But the high level of consumption of tomato, reaching 40–50 kg per capita and year in countries such as Spain, Italy or USA (source: FAO databases), makes this fruit one of the main sources for this vitamin. The carotenoid lycopene is in major part responsible for the red colour of ripe tomatoes, which represent the major source for this compound. It does not have provitamin A activity, but it has a physical quenching rate constant with singlet oxygen almost twice as high as that of β -carotene (Shi and Maguer, 2000). β -carotene is the second major carotenoid in tomato fruits, with concentrations around ten times lower than lycopene (Davies and Hobson, 1981).

Several studies have determined an inverse relation between the intake in the diet of lycopene and β -carotene and the development of certain types of cancer (Ziegler, 1989; van Poppel and Goldbohm, 1995; Giovannucci, 1999) and a direct relation with a reduced incidence of heart disease (Palace et al., 1999; Rao, 2002). But in recent years some studies have questioned the relation between carotenoid or vitamin C intake and reduced cancer risk (Kavanaugh et al., 2007; Lin et al., 2009). Nonetheless, it should be considered the difficulty of proving that a specific component of a complex diet prevents the development of a disease. On the other hand, measurement errors in the dietary intake of fruit and vegetables may also attenuate these associations (Aune et al., 2012). In fact, recent publications again suggest moderate evidences between dietary vitamin C and β -carotene and the prevention of coronary heart disease (Mente et al., 2009). At the same time, new large population studies again confirm the existence of a certain relation between carotenoid intake and certain types of cancer, at least in some populations (e.g. Mignone et al., 2009).

Despite the absence of conclusive data for the role of tomato antioxidants in the prevention of a diseases, new varieties with increased levels of lycopene or other antioxidants are being developed (Ilahy et al., 2011). In this context, it should be considered that in successful marketing strategies, functional foods should deliver their health benefits above and beyond the standard perceived quality, including organoleptic quality, of the equivalent conventional food product. (Krystallis et al., 2008).

Consumer complains on the taste of modern commercial varieties (Bruhn et al., 1991) has fostered the development of niche markets for heirloom, local or traditional varieties. In fact, consumers appreciate the outstanding organoleptic quality of these materials and they are willing to pay a differential up to 4.70 times the price of commercial modern varieties (Cebolla-Cornejo et al., 2007). These varieties have shown high levels of variation in agro-morphological, genetic (Casals et al., 2011) and organoleptic levels (Cebolla-Cornejo, 2011), but little is known on the variation in the concentrations of functional compounds, such as the antioxidants previously described. The

objective of this study is to provide a consistent evaluation of the composition in key antioxidant compounds in traditional varieties of tomato. In this case, Spanish traditional varieties have been selected as a model study to examine their diversity in an inter- and intra-variety scale using a large collection of populations. These findings may be relevant both in the promotion of these materials targeted to quality niche markets combining excellent taste with an added functional value, and in the use of these materials as sources of variation in breeding programs.

MATERIALS AND METHODS

Plant Material

A total of 126 populations belonging to 16 different Spanish traditional varieties of tomato (*Solanum lycopersicum* L.), were studied during two consecutive years, 2009 and 2010 (Table 1). Most of them, including 115 populations, were provided by the Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV, València, Spain) germplasm bank, eight populations were provided by Estación Experimental Agraria de Carcaixent, (EEAC, belonging to the Instituto Valenciano de Investigaciones Agrarias, IVIA, Carcaixent, Spain) and three populations were obtained in local nurseries. A different number of populations were studied per variety, considering the different interest of local markets and the amount of diversity available in the germplasm banks: one population belonged to the variety ‘Amarillo’, one to ‘Centenares’, six to ‘Cuarenteno’, ten to ‘De Colgar’, four to ‘De la Pera’, three to ‘De Pera’, one to ‘Elchero’, two to ‘Flor de Baladre’, four to ‘Gordo Rojo’, 28 to ‘Muchamiel’, one to ‘Negre’, eight to ‘Pimiento’, two to ‘Redondo Rojo’, 12 to ‘Rosa’, one to ‘Tres Cantos’ and 42 to ‘Valenciano’. These varieties represent representing a wide diversity of fruit colours and shapes (Table 1).

Furthermore, four commercial F₁ hybrids, genetically uniform, were kindly provided by *Rijk Zwaan Iberica S.A* (Almería, Spain) and were included and used as a control. The variety ‘Razymo RZ’

presents round, red, medium sized tomatoes, 'Gransol RZ' presents slightly flattened, large sized fruits and 'Piccota RZ' presents round, small sized Cherry type fruits and 'Mariscal RZ' presents flattened, medium to large sized fruits.

Experimental Design and Crop Conduction

The seedbeds were sown in April and were transplanted to field in May in 2009 and 2010. The trial was carried out in a 5800 m² field located in Carcaixent (+39° 6' 37.13", -0° 26' 45.05", València, Spain), surrounded by orange trees. There were no other vegetable crops grown in the proximities. The field was previously cleaned and fertilized with 36,000 kg of sheep manure and 200 kg of potassium sulphate.

A randomized complete block design was used with two blocks, and 2 replicates of 10 plants per population and block. A spacing of 1.2 m x 0.4 m (2.1 plants m⁻²) was applied. The crop was managed using the traditional practices for tomato cultivation in the area, including staking, pruning and drip fertirrigation. In order to control *Tuta absoluta* Meyrick population, pesticide treatments were performed when insect counts suggested the convenience.

Sampling

Healthy and uniformly ripe fruits were harvested at the mature-red stage from second to third truss. Samples were composed of a mix of representative fruits of each of the 10 plants in the replicate. In order to provide a biological mean, equivalent longitudinal portions for each fruit were blended using a blender (KRUPS KB720, Groupe Seb Iberica, Barcelona, Spain), and subsequently homogenized through a laboratory homogenizer (DiAx 900, Heildolph, Germany), which was able to disrupt tissue to particle sizes <0.4 mm. Samples were quickly cryopreserved using liquid nitrogen and were kept frozen at - 80 °C until analysis.

Characterization

All the populations were morphologically characterized to study the correlation between chemical composition and morphological traits. The following descriptors were evaluated: Fruit height (mm), fruit width (mm), fruit width to fruit height ratio, fruit core width (mm), fruit core width to fruit width ratio, pericarp thickness (mm), pericarp thickness to fruit width ratio, number of locules and fruit colour. Fruits colour was analysed using Hunter coordinates through a digital colorimeter (CR 300, Minolta, Japan). Results of each sample were based on the average of three determinations taken on ten representative fruits. The results were reported as L, a, b and a/b rate.

L-ascorbic acid quantification

Ascorbic acid was quantified by capillary zone electrophoresis using a P/ACE System MDQ (Beckman Instruments, Fullerton, CA, USA) using the method described by Galiana-Balaguer et al. (2001). Two grams of sample were thawed in the dark at 4°C and centrifuged at 3500 rpm during 5 minutes. The supernatant was diluted in 2% (w:v) metaphosphoric to avoid oxidation, and potassium hydrogen phthalate was added as internal standard. Samples were filtered, prior to injection, using a 0.22 µm membrane filter (Millipore, Ultrafree-MC, MA, USA).

Uncoated fused-silica capillaries with a 31.2 cm total length, 21 cm effective length, 363 µm e.d. and 50 µm i.d. were used (Polymicro Technologies, Phoenix, AZ, USA). Hydrodynamic injection of samples was carried out at 0.5 psi for 5 seconds. Separation was performed at -15 kV and 25°C. The detection wavelength was 254 nm. Two analytical replicates per sample were made. Results were expressed as mg kg⁻¹ fresh weight (fw).

Carotenoid quantification

Samples were thawed in the dark at 4°C and 100 mg of the homogenate were extracted with 7 ml of a 4:3 v/v, ethanol/hexane solution at 4°C, during 1 hour at 200 rpm using an horizontal shaker (Platform Rocker STR6, Viví, Stuart). Hexane was complemented with 0.05% butylated hydroxytoluene, (BHT). Hexane supernatant was separated and concentrated using a SpeedVac

(Termo, RVT 4104) to complete dryness, and then re-suspended in 500 µl of hexane. Sudan I was added as internal standard and the processed sample was then filtered using a hydrophobic filter of 0.20 µm (Millex-FG, Phobic PTFE). During all the process samples were protected from light.

The quantification of the carotenoids lycopene and β-carotene was carried out by reverse phase high performance liquid chromatography (HPLC) using the method reported by García-Plazaola and Becerril (1999) with slight modifications. Analyses were performed on a 1200 series chromatographer (Agilent Technologies, Santa Clara, US) with G1322A vacuum degasser, G1312A quaternary pump, G1329A standard autosampler, G1316 thermostated column compartment and G1315b diode array detector. A reserved phase Tracer Spherisorb ODS-1 (250 x 4.6 mm i.d., 5 µm particle size) column protected by a guard column (20 x 3.9 mm i.d., 4 µm particle size) was used. 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 µl. Sample was then eluted using a lineal gradient from 100% of solvent A to 100% of solvent B during 12 minutes, followed by an isocratic elution of 100% of solvent B during 7 minutes. Then, a lineal gradient was established from 100% of solvent B to 100% of solvent A during 1 minute. Finally, an isocratic elution of 100% of solvent A during 6 minutes was performed to allow the column to re-equilibrate. The integrations of β-carotene and lycopene were performed at 445 nm and 470 nm respectively. Two analytical replicates per sample were made. The results were reported as mg kg⁻¹ fw.

Statistical analysis

Correlations and bilateral signification between variables were calculated. Population and block effects were analysed with an ANOVA. Variety effect was not estimated due to the unbalanced design and the low number of populations in some varieties. Statistical analyses were performed using the statistical package SPSS v.12 (SPSS Inc., Chicago, IL, USA). Bartlett's test was used to check significant differences in the variances of the populations evaluated in each year.

For the analysis of variability, intra-population coefficient of variation (IPCV) was calculated for each year, as well as the mean of IPCV for both years. Inter-year coefficient of variation (IYCV) was calculated with the means of the contents for each year.

RESULTS

The growing cycle in 2009 was almost 15 days shorter in 2009 than in 2010. Despite this difference in the growing speed, environmental conditions were not so much different (figure 1). Photosynthetically active radiation (PAR) was similar in both years (figure 1), though cumulative PAR in the first third of the cycle was higher in 2009. Final cumulative PAR per day was also slightly higher in 2009 ($1838 \mu\text{mol m}^{-2} \text{s}^{-1}$ vs. $1807 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Temperatures, especially maximum ones were slightly higher in most part of the growing cycle during 2009 than in 2010, with mean maximum temperature for 2009 of 30.1°C and 29.5°C for 2010. In both cycles, maximum temperatures higher than 32°C were registered at the end of the cycle for both years (32 days for 2009 and 45 days for 2010). The difference would be that in 2009 higher values were obtained during those days, and in fact mean maximum temperature for those days was 35.7°C for 2009 and 34.6°C for 2010.

Lycopene accumulation was strongly influenced by the population factor ($p < 10^{-6}$) and the interaction between population and year of cultivation ($p = 0.0005$), while the year of cultivation (environment) was not significant ($p = 0.85$). On the contrary, in the case of β -carotene population ($p < 10^{-6}$), year of cultivation ($p = 0.00003$) and their interaction were highly significant ($p < 10^{-6}$). In this case lower contents of β -carotene were obtained with the environmental conditions of year 2010. In the case of ascorbic acid, the year effect was not significant ($p = 0.094$), as the environmental effect affected differently each population, thus a strong year x population interaction was detected ($p = 0.00003$). Following the trend for the other compounds, population effect was highly significant ($p < 10^{-6}$). The

variety effect was not considered in the model, as different numbers of populations were included for each variety, and it was not the purpose of this work to analyse this factor, but rather to detect interesting populations from the functional point of view, no matter from which variety they belonged to.

Moderate significant correlations were obtained between lycopene content and fruit external colour parameters (table 2). For this compound, low significant correlations were found with fruit height (positive) and fruit width and fruit width to fruit height ratio (negative). For β -carotene only low significant negative correlations were found (<0.4) with colour, size and shape parameters and number of locules. In this case the only positive correlation was found with the pericarp thickness to fruit width ratio. In the case of ascorbic acid, only low significant negative correlations were found with colour and size parameters and number of locules. No significant correlation over 0.2 was detected among the contents of the different compounds.

The levels of variation detected in the accumulation of functional compounds were population dependant. In fact, Bartlett's test confirmed that the variances were different in 2009 ($p < 10^{-6}$) and in 2010 ($p = 0.0003$) for lycopene, β -carotene ($p < 10^{-6}$ both years) and ascorbic acid ($p < 10^{-6}$ both years). Consequently, variability was also analysed in intra-population and inter-year levels. Ascorbic acid accumulation registered the higher level of intra-population variation, IPCV (table 3), with mean values considering all populations of 0.40 followed by lycopene (0.33) and β -carotene (0.17). A similar pattern was observed for the variation between different years, but with limited differences in the variation of the carotenoids (table 3). In this case, the mean inter-year coefficient of variation, IYCV, was respectively 0.35, 0.24 and 0.28. The level of variation was also affected by environmental conditions. For example, in 2010 the intra-population coefficients of variation for ascorbic acid were lower.

A high level of intra-varietal variation was detected for the three compounds, though for ascorbic acid it was considerably lower (table 4). Among the different varieties, 'De colgar' and 'Rosa'

showed the higher range of variation in their populations. These varieties also showed high levels of mean IPCV. The high level of variation made difficult to assign a certain functional profile for each variety. Nevertheless general trends could be found. For example, the variety 'Amarillo' showed low levels of carotenoids, as expected due to their yellow external colour. Variety 'Centenares' showed in general high values for the three compounds, while 'De pera' and 'Pimiento' stood out for lycopene accumulation and 'De colgar' and 'Redondo rojo' for β -carotene.

Ascorbic acid

In each variety, a wide range of population was found and several populations were identified with outstanding values for each compound. Ten populations showed ascorbic acid contents higher than 200 mg kg⁻¹, and one them higher than 300 mg kg⁻¹ (table 3). In several cases though, inter-year coefficient of variation was considerably high. Therefore, these populations could be considered unstable. Nevertheless, population CDP00142 from 'Valenciano' with 308.1 mg kg⁻¹ and IYCV of 0.44, population CDP01040 from 'De Colgar' with 262.7 mg kg⁻¹ mean content and IYCV of 0.16, population CDP05973 from 'Cuarenteno' with 216.1 mg kg⁻¹ mean content and IYCV of 0.10 cv and population CDP05079 from 'De Colgar' with 253.6 mg kg⁻¹ mean content and IYCV of 0.02 could be interesting from the point of view of ascorbic acid accumulation.

Carotenoids

In the case of lycopene eight populations showed contents higher than 100 mg kg⁻¹ (table 3). The higher mean contents were detected in the populations CDP04303 from 'Rosa' with 151.9 mg kg⁻¹ and CDP06083 from 'Pimiento' with 132.2 mg kg⁻¹. In the case of population CDP04303 the IYCV was considerably high (0.72) and showed high differences in the mean content for each year, but populations CDP06083, or CDP00929 from 'De pera', with mean content of 123.59 mg kg⁻¹, showed medium to high contents with low IYCV (0.21 and 0.13 respectively).

Regarding β -carotene accumulation, 23 populations of traditional varieties of tomato showed β -carotene mean contents higher than 20 mg kg^{-1} and two of them, populations CDP07064 from 'Redondo Rojo' and CDP03774 from 'De colgar' outreached 30 mg kg^{-1} , with medium IYCV (0.31 and 0.32 respectively).

As shown above, it was possible to identify materials with moderate to high contents and low IPCV and IYCV indicating a high degree of stability. It was much more difficult to identify materials with outstanding values for several compounds. In fact, no significant important correlations were found between ascorbic acid and carotenoids nor between lycopene and β -carotene (table 2). Nonetheless, population CDP05729 and CDP00450 belonging to the variety 'Valenciano' combined moderate to high levels of β -carotene and lycopene.

DISCUSSION

Among the functional compounds evaluated, ascorbic acid showed the higher degree of variation at the intra-population and inter-year scales. Intra-population level of variation would be mainly related to genotype and micro-environmental effects, while inter-year variation would be mainly related to macro-environmental effects. The genotype component of intra-population variation is explained by the complex genetic structure of traditional varieties that are usually configured as population varieties, formed by a mixed of different genotypes that may explain high variability in an intra-year intra-population basis. Among the environmental components, micro-environmental effects would be explained by small differences in temperature, soil, fertirrigation etc., while macro-environmental effects would be mainly related with the different climatic conditions of both years of cultivation.

It is difficult to determine which part of intra-population variation is due to genetic differences between the individuals of each population or to micro-environment. Nevertheless, the fact that the F_1 hybrid controls, which are genetically uniform, showed similar values of intra-population

coefficients of variation compared to traditional populations seems to indicate that the main factor explaining this level of variation would be micro-environmental differences in the growing conditions of each plant. It should be considered that the different levels of intra-population variation imply a lack of homoscedasticity. Therefore, despite the robustness of the ANOVA analysis, the results obtained should be considered only as indicative.

Inter-year variation might be explained by the different climatic conditions. These differences would not only affect mean content, but also might buffer the level of variation, as it happened with ascorbic acid in 2010 when the intra-populations coefficients of variation were lower. But, the environmental effect would not be explained by generalized trends, as its effect would be mainly explained by a strong population x year interaction. In fact, previous studies decomposing phenotypic variances have shown that differences in ascorbic acid in promising materials are mostly due to a high GxE interaction rather than to environmental effect (Leiva-Brondo et al., 2012). This interaction might explain the extraordinary levels in several traditional populations during the year 2010, with contents higher than 300 mg kg^{-1} in three populations, as compared to the levels obtained in 2009, when this content was not reached by any population.

In the case of ascorbic acid, Dumas (2003) reported higher effects of solar radiation than temperature. In our case, the PAR registered in the middle part of the growing cycle was similar for those years, while 2009 registered slightly higher maximum and minimum temperatures. Considering that during this period maximum temperatures exceeded 30°C , these conditions could have led to stressing situation that might have led to a consumption of ascorbic acid, thus explaining lower levels obtained in several populations in this year. Nevertheless, it should be considered that other studies, even though recognizing a probable influence of radiation and temperature in ascorbic accumulation, did not find clear correlations with climatic parameters (Raffo et al., 2006). In the same sense, Hamner et al. (1945) found that environmental effects were higher than genotypic effects, but found it difficult to ascribe the differences to a specific cause.

As the variation in carotenoid contents is concerned, the lower levels of intra-year variation in β -carotene content than those for ascorbic acid and lycopene have been already observed in previous studies where different materials have been screened as sources of variation in breeding programs for these compounds (Roselló et al., 2011).

Regarding the environmental effect on carotenoids, the differences in the significance of the environmental factor between lycopene and β -carotene could be related to the environmental regulation of the biosynthetic pathway. As commented for ascorbic acid, maximum temperatures were higher in the middle part of the growing cycle during 2009, and higher than 30°C. Under these conditions, lycopene biosynthesis is restrained, while its conversion to β -carotene continues, and this might explain higher levels of this compound in those years with higher maximum temperatures.

The high degree of variation in intra-population and inter-year scale indicates that the use of high contents in functional compounds as a marketing strategy in tomato should be carefully addressed. Multi-environmental assays are essential to clearly identify the minimum levels of accumulation; otherwise consumer might be deceived if the materials are cultivated in worse environmental conditions. These assays would also be valuable to identify which growing conditions promote the accumulation of each of the materials considered, due to the high genotype x environment interaction.

Ascorbic acid

But before that, it should be considered if there is really an opportunity in the traditional varieties of tomato in the market of functional foods. The mean ascorbic content in most of the populations analysed represented, in general, conventional values of tomato cultivars. Gould (1992) established 200 mg kg⁻¹ as the normal content in commercial varieties, while George et al. (2004) found in a more recent study a range between 92 and 324 mg kg⁻¹ in tomato pulp, though the highest values corresponded to cherry tomatoes. And in this case, as ascorbic acid can be found at higher

concentrations in the jelly and skin than in the pericarp, small fruited varieties tend to have higher contents than standard varieties (Stevens et al., 2007). In our case, the population CDP08734 of the variety 'Centenares' (cherry type) and the cherry control 'Piccota RZ' gave high contents of ascorbic acid, but not the highest ones. Nevertheless, low negative correlations were found with size parameters and number of locules. As it has been indicated, ascorbic acid content is higher in the locular matrix. As usually a higher number of locules represents lower size of locules, this would explain the negative correlation found between this parameter and ascorbic content.

Apart from that small fruited population, our results here also show that it is possible to identify materials such as the medium sized 'Valenciano' population CDP00142 with contents as high as 308.1 mg kg⁻¹. Bhatt et al. (2001) identified heterotic effects studying diallel crosses with maximum ascorbic contents in the F₁ populations of 341.3 mg kg⁻¹, a content difficult to find in standard varieties. Consequently, the contents found in the collection of traditional populations would not only be interesting to promote the consumption of these materials, but make these populations useful in breeding programs.

But, would really these materials be interesting in breeding programs? In this context, wild species, with up to 5 times the content of ascorbic acid of commercial varieties, have been used for this purpose, and some of the QTL controlling the underlying genetic control have been identified (Stevens et al., 2007). In these programs the final content is not as high as expected considering the donor parent. For example in studies analysing the introgression from the wild species *Solanum pennellii* Correl, the wild parent may accumulate up to 710 mg kg⁻¹, but when it is crossed with the cultivated species, the contents in introgression lines of tomato range up to 273.3 mg kg⁻¹ fresh weight (Stevens et al., 2007). Nevertheless, it is true that ascorbic acid is highly influenced by the environment (Toor et al., 2006), and probably it would be possible to obtain higher amounts of ascorbic acid in breeding lines derived from high ascorbic wild species.

But in any case, our results show again that it is possible to identify high contents in traditional varieties that equal or even exceed the contents of elite breeding lines bred with this purpose, avoiding the negative side effects of the use of wild species. In this sense, it should be remembered the case of “*Double Rich*” cultivars. They were developed through interespecific crosses between cultivated tomato and *Solanum peruvianum* L. accessions and their ascorbic acid contents (500 mg kg⁻¹) doubled the normal contents, however, their small fruit size and their poor production limited their use on a commercial level (Stevens and Rick, 1986). Moreover, “*Double Rich*” cultivars showed lower contents (318 mg kg⁻¹) in other trials (Watada *et al.*, 1976), in a range similar to that of population CDP00142 in this study.

The high levels of ascorbic acid found in some populations would not only result in improved nutritive or functional value of plants, but also may have a side effect on the plant response against different stresses. In fact, ascorbic acid biosynthesis and recycling have been related with plant health and development and in particular to abiotic stress tolerance (Gallie, 2013).

Carotenoids

Regarding the results for lycopene accumulation, the amounts registered in some populations of traditional varieties in this work demonstrate that there is an alternative for the improvement of its accumulation besides the use of wild species. Two populations displayed lycopene contents higher than 130 mg kg⁻¹ which can be placed at the lower range of variation of “high pigment” cultivars, especially considering the big size of these materials. In this sense, the distribution of major carotenoids in the ripe fruit is irregular, with more than 2-fold amounts of lycopene in the pericarp than in the locules and 4-fold higher amounts of β-carotene in the locules than in the pericarp (Davies and Hobson, 1981). That would mean that those populations with smaller size would have more probabilities to show high lycopene contents, as the relation surface to volume would be higher. Despite this relation in our study the commercial cherry control ‘Piccola RZ’ showed a standard lycopene content while ‘Centenares’ had a moderately high content (table 3).

It should also be considered that the values obtained here could be surpassed in other environments. Temperatures over 32°C inhibit lycopene biosynthesis (Dumas et al., 2003) and during the last part of the cycle of the two years, maximum temperatures overreached that limit. Therefore, lycopene accumulation in the materials evaluated should not be at their maximum potential.

Holden et al. (1999) reported that the standard lycopene content in red ripe raw tomatoes in the United States would be 30.25 mg kg⁻¹. Considering environmental and genotypic effects, it is reasonable that different studies in other parts of the world could be higher. In fact, Martinez-Valverde (2002) analysing different varieties and growing cycles in Spain found lycopene contents in winter growing cycle up to 64.98 mg kg⁻¹. These standard values have been considerably improved in “high pigment” tomato varieties, with contents up to 254 mg kg⁻¹, though strong environmental effects led to a variation of up to 25 in different growing seasons (Ilahy et al., 2011). These varieties carry any of the “*high pigment*” mutations (*hp-1*, *hp2*...) that result in increased carotenoid content, especially lycopene. These mutations are related with light signal transduction machinery and result in increased light response and a consequent increase in the levels of carotenoids and other compounds including flavonoids and ascorbic acid (Bino et al., 2005). These genes and modifiers have been used in breeding programs targeted to the development of new functional varieties (Levin et al., 2004). Nonetheless, these mutations have secondary side effects resulting in worse agronomic performance and consequently those genes, such as *ogc*, involved in single steps of the biosynthetic pathway have retained interest in breeding programs (Sacks and Francis, 2001).

In the case of β-carotene, the content in standard varieties rounds 3.93 mg kg⁻¹ (Holden et al., 1999). Several high β-carotene varieties have been developed using wild species as donor parents. As an example, the cultivar “CaroRed” offered contents of β-carotene up to 44.2 mg kg⁻¹ (Tomes and Queackebush, 1958). Usually these materials show orange colour, as the increase in the content of β-carotene is due to the cyclisation of lycopene, and therefore at its expense. Despite offering high levels of pro-vitamin A, consumers have not shown a considerable interest in these materials due to

the orange external colour, and it is preferred to combine in the same materials high β -carotene with normal to high content in lycopene, resulting in red coloured tomatoes. Following this target, it has been possible to identify wild cherry accessions with similar β -carotene contents to those above mentioned with normal lycopene contents (Adalid et al., 2012). In this work, it has been possible to identify several populations of traditional varieties of tomato with β -carotene mean contents higher than 20 mg kg^{-1} , and some higher than 30 mg kg^{-1} . Consequently, the levels obtained can be compared with those found in the best high pigment tomato cultivars that range up to 20 mg kg^{-1} fresh weight in open field conditions (Lenucci et al., 2006).

CONCLUSION

The variation present in traditional populations of tomato enables the identification of materials with moderate to high contents in functional compounds and limited degree of variability. These materials could be then targeted to supply quality markets demanding not only produces with high organoleptic characteristics but also an added functional value. For this purpose it would be convenient to develop intra-varietal complementary crosses in order to develop new populations with high levels of joint accumulations of the three compounds. This strategy would be very valuable to explore the possibilities of obtaining a price premium as a result of the added functional value. In other functional food markets these price premia has been ranged between 30%-50% (Menrad, 2003). And this difference would be useful to compensate the usually lower yields of traditional varieties. Thus, it will contribute to offer an *in situ* conservation promoting the recovery of these materials by farmer. In addition, Spanish growing condition seem not only to contribute to high levels of carotenoids in tomato, but also promote their bioaccessibility (Aherne et al., 2009), thus strengthening the added value of these materials. Additionally, these genetic resources could also be used as sources of variation in breeding programs of new commercial hybrid varieties. Considering that the high levels detected are already present in medium-large sized fruits and that the contents detected are even

higher than previously found sources of variation in small fruited accessions (Adalid et al., 2010), these materials seem to be promising alternatives for this purpose.

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Table 1. Description of the varieties and origin of the populations evaluated (varietal code in parentheses).

Variety	Fruit description/populations (code, town, province)
'Amarillo' (1)	Description: Large size. Flattened and ribbed yellow tomatoes with a high number of locules (even higher than 20). These tomatoes are typical of interior areas and dry farming. Populations: CDP01733 (Casas Altas, VC).
'Centenares' (2)	Very small size. Deep red and round shape without ribs. Used for salads and decoration considering their small size and sweet taste. Populations: CDP08734 (Racó d'Ademús, VC).
'Cuarenteno' (3)	Description: Intermediate size. Flattened and ribbed fruits with green persistent shoulders. Early production. Populations: CDP07243 (Chelva,VC), CDP07457 (Aldaya,VC), CDP05973 (Torrente,VC), CDP04955 (Rojales,AL), CDP08237 (Massamagrell,VC), CDP07843 (VC),.
'De colgar' (4)	Description: Small size. Round or oblong fruits with two or three locules, transparent or yellow skin and thick pericarp. With delayed ripening (<i>alc</i>), they are harvested and conserved hanging in fresh and aerated places. Populations: CDP05385 (Torrebaja,VC), CDP05079 (Liria,VC), CDP05542 (Alginet,VC), CDP06914 (Náquera,VC), CDP01507 (Benicarló,CS), CDP01040 (Camporrobles,VC), CDP04259 (Xàtiva,VC), CDP03190 (La PLana,CS), CDP02554 (Montroui,VC), CDP01972 (Xàbia,AL).
'De la pera' (5)	Description: Intermediate size. Pear shaped fruits with two or three locules separated by thick septa, green persistent shoulders, small peduncular scar and thick pericarp. Hollow fruits are usual. Populations: CDP02614 (Chelva,VC), CDP00210 (Novelda,AL), CDP00906 (La Aparecida,AL), CDP07874 (Orihuela,AL),.
'De pera' (6)	Description: Small size. Round or oblong fruits without green shoulders. Usually used for cooking. Populations: CDP04299 (Hostalet de Benasal,CS), CDP01280 (Ademuz,VC), CDP00929 (PobleNou.Valencia,VC).
'Elchero' (7)	Description: Mid sized red tomatoes with round shape and angular section, 2 or 3 locules, slight ribbed. Populations: CDP07339 (Muchamiel,AL).
'Flor de baladre' (8)	Description: Large size (200-250 g). Slightly flattened and ribbed fruits with pink color. Populations: CDP04235 (Elche,AL), CDP07166 (Valencia, VC)
'Gordo rojo' (9)	Description: Very large size. Flat and ribbed fruits. Typical of interior areas and dry farming. Populations: CDP02826 (Millares,VC), CDP08786 (Arañuel,CS), CDP06270 (Carcaixent,VC), CDP06009 (Valencia, VC),.
'Muchamiel' (10)	Description: Large size. Flat and strong ribbed fruits, numerous locules, big peduncular scar with an important corky area, hard and heavy pericarp and moderate radial cracking. Green shoulders, orange-red ripe color. It is a late variety. Populations: CDP05658 (Novelda,AL), CDP04133 (La Aparecida,AL), CDP05229 (Viver,CS), CDP00155 (Elche,AL), CDP07582 (San Juan,AL), CDP08091 (Campello,AL), CDP00700 (Muchamiel,AL), CDP01469 (San Juan,AL), CDP07889 (Buñol,VC), CDP08014 (San Juan,AL), CDP01988 (Muchamiel,AL), CDP09344 (Muchamiel,AL), CDP02195 (Muchamiel,AL), CDP08780 (Muchamiel,AL), CDP08427 (Muchamiel,AL), CDP07052 (Muchamiel,AL), CDP00386 (Muchamiel,AL), CDP04512 (Muchamiel,AL), CDP05422 (Muchamiel,AL), CDP00604 (Muchamiel,AL), CDP03096 (Orihuela,AL), CDP01971 (San Juan,AL), CDP05938 (Alboraya,VC), CDP01138 (San Juan,AL), CDP01746 (Orihuela,AL), CDP08999 (San Juan de Alicante,AL), CDP08761 (Catarroja,VC), CDP09432 (Lliria,VC).
'Negre' (11)	Description: Large size. Intense purple coloration. Fruits are flat and slightly ribbed. Populations: CDP02095 (Torrent,VC)
'Pimiento' (12)	Description: Intermediate size. Elongated fruits (similar to "Italian" peppers), green persistent shoulders, two to four locules and moderate radial cracking. Used for cooking, deep red flesh and reduced number of seeds. Populations: CDP06083 (Venta del Moro,VC), CDP04079 (Jérica,CS), CDP06446 (Fontaneres,VC), CDP05734 (Villahermosa del Río,CS), CDP01712 (Alborache,VC), CDP04056 (Catarroja,VC), CDP02391 (Moncada,VC), CDP08320 (Massamagrell,VC).
'Redondo rojo' (13)	Description: Intermediate - large size. Includes rounded, smooth red colored tomatoes. Populations: CDP07064 (Sueca,VC), CDP04562 (Carcaixent,VC).
'Rosa' (14)	Description: Very large size. Flat or slightly flat fruits with intense ribbing. Transparent skin, and pink colour. Typical of interior areas and dry farming. Populations: CDP08690 (Fontaneres,VC), CDP05702 (Castillo de Villamalefa,CS), CDP04903 (Onda,CS), CDP00764 (Aras del Alpuente,VC), CDP01302 (Rincón de Ademuz,VC), CDP09459 (Yátova,VC), CDP04904 (Alboraya,VC), CDP05992 (Requena,VC), CDP07661 (Todolella,CS), CDP03526 (Sellent,VC), CDP04303 (Valencia, VC), CDP04008 (Valencia, VC).
'Tres cantos' (15)	Description: Large size. Round shape and angular section. Red and smooth fruits, without low number of locules considering their size. Populations: CDP06491 (Carcaixent,VC).
'Valenciano' (16)	Description: Intermediate size. This varietal type show two different subtypes: "Masclat" and "Blanca" subtypes. "Masclat" (conventional "Valenciano" type) show a smaller size, hearted shape. "Blanca" show a bigger size, slightly flattened and hearted shape, and paler color in the immature fruits. Both with green shoulders, orange-red ripe color and numerous locules. Usually moderate radial and circular cracking is present. Populations: CDP07303 (Valencia,VC), CDP01509 (SieteAguas,VC), CDP04333 (Picassent,VC), CDP01090 (Segorbe,CS), CDP00616 (Liria,VC), CDP02722 (Segorbe,CS), CDP05254 (Alboraya,VC), CDP06161 (Villena,AL), CDP05260 (Turís,VC), CDP07291 (Valencia,VC), CDP08276 (Sueca,VC), CDP01343 (Foios,VC), CDP04640 (Vinalesa,VC), CDP00623 (Moncada,VC), CDP04486 (L'Alcudia de Crespins,VC), CDP04829 (Pobla de Vallbona,VC), CDP09978 (Valencia, VC), CDP08151 (Valencia, VC), CDP02310 (Morella,CS), CDP00450 (PobleNou.Valencia,VC), CDP07489 (Segorbe,CS), CDP00960 (Villargordo del Cabriel,VC), CDP04423 (Museros,VC), CDP04372(SieteAguas,VC), CDP02109 (VC), CDP05333 (Almenara,CS), CDP05691 (Vinaroz,CS), CDP00142 (Valencia, VC), CDP05729 (El Pereió,VC), CDP01649 (Picassent,VC), CDP01949 (Valencia, VC), CDP08595 (Meliana,VC), CDP02589 (Murod'Alcoi,AL), CDP04915 (Carcaixent,VC), CDP02182 (ViverosTaxes,LN), CDP04052 (ViverosCucala,LN), CDP06753 (ViverosPeris,LN), , CDP01197 (Valencia, VC), CDP01646 (Valencia, VC), CDP05266 (Valencia, VC), CDP03596 (Valencia, VC), CDP07231 (Valencia, VC),.

VC: Valencia; CS: Castellón; AL: Alicante; LN: local nurseries.

Table 2. Correlations between functional compounds and morphological traits (only significant correlations higher than 0.2 are shown; p-values in parentheses).

	β -carotene	Lycopene	Ascorbic acid
β -carotene			
Lycopene			
Ascorbic acid			
Hunter a	-0.26(0.004)	0.47 (0.000)	-0.24 (0.006)
Hunter b		-0.30 (0.000)	
Hunter a/b	-0.28 (0.003)	0.53 (0.00)	
Hunter L		-0.55 (0.000)	
Fruit height	-0.27 (0.003)	0.25 (0.006)	-0.25 (0.004)
Fruit width/height ratio		-0.39 (0.000)	
Fruit core width/Fruit width ratio	-0.28 (0.001)		
Fruit width	-0.28 (0.002)	-0.28 (0.001)	
Fruit core width	-0.36 (0.000)		-0.23 (0.012)
Pericarp thickness			
Pericarp thickness/Fruit width ratio	0.21 (0.02)		
Number of locules	-0.31 (0.000)		-0.21 (0.017)
Fruit weight	-0.26 (0.004)		-0.20 (0.023)

Table 4. Varietal mean contents and levels of variation.

VARIETY	No.of populations	Ascorbic acid (mg kg ⁻¹)			β-carotene (mg kg ⁻¹)			Lycopene (mg kg ⁻¹)		
		Mean content (2009/2010)	Mean mIPCV ¹	IVCV ²	Mean content (2009/2010)	Mean mIPCV ¹	IVCV ²	Mean content (2009/2010)	Mean mIPCV ¹	IVCV ²
'Amarillo'	1	149.54	0.12	0.00	10.73	0.09	0.00	12.40	0.14	0.00
'Centenares'	1	221.27	0.42	0.00	19.81	0.11	0.00	100.76	0.28	0.00
'Cuarenteno'	6	161.93	0.34	0.36	16.58	0.13	0.09	65.90	0.30	0.21
'De golgar'	10	184.84	0.61	0.48	23.59	0.22	0.25	48.31	0.41	0.43
'De la pera'	4	122.85	0.36	0.28	17.68	0.16	0.19	74.28	0.35	0.33
'De pera'	3	136.99	0.27	0.22	13.84	0.18	0.12	99.31	0.28	0.22
'Elchero'	1	181.24	0.33	0.00	18.67	0.20	0.00	67.31	0.38	0.00
'Flor de baladre'	2	156.12	0.29	0.48	19.26	0.18	0.15	68.24	0.36	0.01
'Gordo rojo'	4	126.04	0.57	0.30	17.07	0.18	0.13	77.16	0.30	0.34
'Muchamiel'	28	130.66	0.37	0.36	17.31	0.16	0.17	52.07	0.32	0.30
'Negre'	1	194.05	0.30	0.00	14.62	0.15	0.00	47.97	0.22	0.00
'Pimiento'	8	126.83	0.54	0.37	15.53	0.19	0.10	92.16	0.38	0.26
'Redondo rojo'	2	119.84	0.49	0.12	25.09	0.22	0.38	82.47	0.36	0.23
'Rosa'	12	120.10	0.56	0.51	14.82	0.13	0.12	69.55	0.39	0.53
'Tres cantos'	1	51.33	0.38	0.00	15.92	0.14	0.00	51.33	0.38	0.00
'Valenciano'	42	73.83	0.33	0.29	18.13	0.20	0.21	73.83	0.33	0.29

¹Mean mIPCV: Mean inter-population coefficient of variation for both years. ²IVCV: Intra-varietal coefficient of variation.

Fig. 1. Climatic conditions of cultivation, including maximum temperature, minimum temperature and photosynthetically active radiation. Arrows indicate the date of start and end of harvest (continuous: 2009; dotted: 2010).

