Journal of Food Composition and Analysis 23 (2010) 613–618

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Article

Evaluation and selection of tomato accessions (*Solanum* section *Lycopersicon*) for content of lycopene, β-carotene and ascorbic acid

Ana María Adalid [a], Salvador Roselló [b], Fernando Nuez [a,*]

[a] *COMAV, Polytechnic University of Valencia, 46022 Valencia, Spain*
[b] *Department of Agrarian Sciences and Natural Environment, Universitat Jaume I, 12071 Castellón, Spain*

ARTICLE INFO

Article history:
Received 5 March 2009
Received in revised form 16 February 2010
Accepted 15 March 2010

Keywords:
Agrobiodiversity
Tomato germplasm
Underutilized cultivars
Lycopene
β-Carotene
Ascorbic acid
GGE biplot
Ideal index
Biodiversity and horticulture
Food analysis
Food composition

ABSTRACT

Tomato has been identified as a food of great interest given its nutritional and bioactive components (mainly lycopene, β-carotene and ascorbic acid) and its high consumption rate all year round. Previous works have indicated that some local tomato cultivars and accessions of related species could have great potential, and even as nutraceutical foods. Nevertheless, most local cultivars have disappeared from fields because they have been replaced by hybrids and modern cultivars which produce higher yields and are more disease-resistant. In this work, 49 accessions of underutilized tomato or related species are evaluated in order to recover their use (directly in fields or as variability sources to obtain new cultivars) and increase agrobiodiversity. Fourteen accessions of the cherry type and two of the common tomato type were selected for their high and balanced nutritional properties, causing them to be of great interest for direct human consumption (especially BGV008057, BGV006863 and BGV008060). Furthermore, BGV008365 and BGV012627 (cherry types with over 1.5 times the normal average ascorbic acid content) as well as BGV008166 (*Solanum pimpinellifolium* accession which presented more than nine times the normal average lycopene content) would be of interest as donor parents for breeding programmes to increase the nutrition properties of commercial varieties.

1. Introduction

In recent times, Nutrition Science has moved on from the classical concept of avoiding nutrient deficiencies to the concept of 'optimal' nutrition. The research focus has shifted more to the identification of biologically active components in foods that have the potential to optimise physical well-being and which may also reduce the risk of disease. Many traditional food products, including fruits and vegetables, have been found to contain components with potential health benefits. Within this group, tomato has been identified as a functional and "nutraceutical" food (Canene-Adams et al., 2005; Jack, 1995). A nutraceutical is any substance considered a food, or part of a food, that provides medical or health benefits, including disease prevention and treatment (Jack, 1995).

In the case of tomato, its high consumption all year round makes it one of the main sources of minerals, vitamins and antioxidants in many countries (Esquinas-Alcázar and Nuez, 1995). Ascorbic acid may play a key role in delaying the pathogenesis of a variety of degenerative diseases, such as cardiovascular disease, certain cancers, cataracts and it also prevents DNA mutation induced by oxidative stress (Byers and Guerrero, 1995; Lutsenko et al., 2002; Marchioli et al., 2001). Lycopene and β-carotene are the tomato carotenes which present the highest nutritional value. Specifically, lycopene reduces several cancer types and the risk of heart attack (Canene-Adams et al., 2005; Kun et al., 2006; Omoni and Aluko, 2005). β-carotene is a provitamin A carotenoid and its deficiency can cause xerophthalmia, blindness and premature death (Mayne, 1996).

All of these benefits to human health demonstrate the importance of tomatoes and, as a result, this vegetable is in increasing demand. In recent decades, however, agricultural industrialisation has led to a reduction in the number of cultivars used, which has resulted in a decline in the broad diversity of organoleptic and nutritional quality characteristics. Furthermore, most of the traditional varieties are disappearing worldwide because they are being replaced by modern cultivars. In this sense, the Food and Agriculture Organization of the United Nations (FAO)

* Corresponding author at: Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Ciudad Politécnica de la Innovación, Edificio 8E, Escalera J, 3ª planta, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Tel.: +34 96 387 74 21; fax: +34 96 387 94 22.
E-mail address: fnuez@btc.upv.es (F. Nuez).

0889-1575/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2010.03.001

has considered the planet's genetic resources to be important to agriculture, health, environment and trade. FAO's nutrition work has always included elements of biodiversity in its field and normative operations, and with its compilations of wild, neglected and underutilized genetic resources used for food.

In recent years, FAO and Bioversity International (formerly the International Plant Genetic Resources Institute) are leading a new international initiative on biodiversity for food. The overall aim is to promote the sustainable use of biodiversity in programmes contributing to food security and human nutrition, and to thereby raise awareness of the importance of this link for sustainable development (Toledo and Burlingame, 2006). Following these guidelines, this study will contribute to bring new insight in the research field of biodiversity, nutrition and food composition, evaluating a collection of tomato cultivars or related accessions to identify those that will have the potential to become conventional foods of the future-useful parents in breeding programs, convenient sources of income, and the vehicles for improved nutrition and increased food supply.

2. Materials and methods

2.1. Plant material

A total of 49 accessions of tomato germplasm from 24 countries on 4 continents, provided by the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) genebank, were evaluated. Fourteen accessions of common tomato types (*Solanum lycopersicum* L.), 28 cherry type tomatoes (*S. lycopersicum* var. *cerasiforme* L.) and 7 accessions of small fruit tomato related species (*Solanum pimpinellifolium* L.), which represent a wide diversity of fruit shapes and colours, were studied (Table 1). Two

Table 1
Characteristics and lycopene, β-carotene and ascorbic acid (AsA) contents (mean ± standard deviation, $n = 36$) of the *Solanum* accessions evaluated.

Accession	Sp	Origin	Fruit colour and size[a]	AsA (mg L^{-1})	Lycopene (mg kg^{-1})	β-Carotene (mg kg^{-1})	Ideal index[b]
CAMBRIA	1	Spain (control)	Red, 4	85 ± 29	29 ± 9	6.7 ± 0.7	40
BGV012406	1	Spain (control)	Light red, 5	91 ± 41	49 ± 13	10 ± 1	25
BGV003095	1	Spain	Orange, 6	104 ± 48	0.5 ± 0.5	12 ± 15	27
BGV004209	1	Czech Republic	Yellow, 5	118 ± 30	0.4 ± 0.1	0.79 ± 0.02	49
BGV007022	1	Ecuador	Pink, 5	183 ± 12	79 ± 1	8.60 ± 0.01	13
BGV008097	1	Peru	Pink, 3	95 ± 31	22.85 ± 0.03	1.7 ± 0.1	47
BGV009514	1	Iran	Pink, 6	143 ± 55	63 ± 15	6.4 ± 0.3	29
BGV009515	1	Cuba	Red, 4	115 ± 26	23.9 ± 0.8	6.1 ± 0.4	37
BGV009518	1	Kyrgyzstan	Pink, 7	71 ± 3	74 ± 14	4.3 ± 0.8	43
BGV009529	1	Vietnam	Pink, 4	143 ± 41	35 ± 1	8.3 ± 0.1	26
BGV011359	1	Taiwan	Pink, 5	51 ± 7	24.5 ± 0.2	7.3 ± 0.2	44
BGV011512	1	Portugal	Yellow, 4	127 ± 13	1 ± 1	13.14 ± 0.01	17
BGV012344	1	The Philippines	Light pink, 4	160 ± 38	20.5 ± 0.3	3.89 ± 0.06	41
BGV012619	1	Taiwan	Red, 6	85.2 ± 0.6	51.7 ± 0.1	3.3 ± 0.1	45
BGV012620	1	Taiwan	Strawberry-reddish, 3	136 ± 33	52 ± 25	6.0 ± 0.1	32
BGV012630	1	Ethiopia	Yellow, 5	77 ± 58	0.5 ± 0.4	2.51 ± 0.06	48
BGV006753	2	Ecuador	Orange-reddish, 2	150 ± 77	91 ± 2	5.0 ± 0.6	28
BGV006777	2	Ecuador	Strawberry-reddish, 2	145 ± 5	102 ± 2	7 ± 2	18
BGV006824	2	Ecuador	Strawberry-reddish, 3	137 ± 70	28 ± 1	4.9 ± 0.3	38
BGV006825	2	Ecuador	Strawberry-reddish, 3	159 ± 35	47 ± 1	2.9 ± 0.3	39
BGV006857	2	Ecuador	Red, 2	131 ± 31	30.8 ± 0.5	3.9 ± 0.2	42
BGV006863	2	Ecuador	Orange, 2	162 ± 33	89 ± 2	12.1 ± 0.4	2
BGV006872	2	Ecuador	Orange-reddish, 1	57 ± 4	94.8 ± 0.3	7.1 ± 0.3	34
BGV006875	2	Ecuador	Pink, 2	132 ± 52	117 ± 4	8.0 ± 0.4	14
BGV006896	2	Ecuador	Orange-reddish, 1	169 ± 35	51 ± 7	7 ± 2	21
BGV006923	2	Ecuador	Orange-reddish, 3	113 ± 28	88 ± 7	11.8 ± 0.6	10
BGV008008	2	Bolivia	Orange, 2	52 ± 16	0.5 ± 0.3	0.94 ± 0.03	51
BGV008033	2	Peru	Strawberry-reddish, 2	142 ± 35	36 ± 4	8.8 ± 0.6	22
BGV008051	2	Mexico	Red, 1	155 ± 42	54 ± 0.7	11.39 ± 0.01	9
BGV008057	2	Malaysia	Strawberry, 2	231 ± 27	78.9 ± 0.2	13 ± 1	1
BGV008060	2	Peru	Orange, 2	166 ± 10	82 ± 1	11.7 ± 0.3	5
BGV008061	2	Mexico	Orange-reddish, 2	234 ± 41	60 ± 3	8 ± 1	11
BGV008065	2	Peru	Strawberry, 3	131 ± 30	31 ± 0.5	6.0 ± 0.1	35
BGV008070	2	Mexico	Yellow, 1	233 ± 76	1.4 ± 0.6	8.4 ± 0.1	23
BGV008109	2	Peru	Orange-reddish, 1	164 ± 18	88 ± 0.2	9.9 ± 0.3	7
BGV008148	2	Ecuador	Deep red, 1	85 ± 1	167 ± 2	8.6 ± 0.9	19
BGV008169	2	China	Yellow, 2	38 ± 3	0.7 ± 0.1	2.6 ± 0.1	50
BGV008224	2	Nicaragua	Pink, 2	196 ± 4	92 ± 0.4	7.8 ± 0.3	8
BGV008226	2	Panama	Pink, 2	141 ± 16	58 ± 3	7.5 ± 0.7	24
BGV008354	2	Costa Rica	Red, 3	299 ± 12	27 ± 1	12.3 ± 0.8	6
BGV009512	2	Guinea	Pink, 2	90 ± 35	30.5 ± 0.8	8.4 ± 0.4	33
BGV012627	2	Colombia	Red, 2	311 ± 118	58.0 ± 0.5	9.9 ± 0.3	4
BGV012639	2	Ecuador	Red, 2	200 ± 45	18.4 ± 0.1	10.1 ± 0.1	15
BGV012640	2	Peru	Red, 2	212 ± 45	11.7 ± 0.1	10.1 ± 0.2	16
BGV007825	3	Mexico	Orange, 1	44 ± 16	2 ± 2	7.5 ± 0.3	46
BGV007827	3	Mexico	Red, 2	38 ± 10	91.1 ± 0.5	7.6 ± 0.5	36
BGV008068	3	Peru	Red, 1	30 ± 1	120 ± 11	13 ± 1	20
BGV008166	3	Ghana	Red, 1	144 ± 23	271 ± 3	14.4 ± 0.1	3
BGV008230	3	Honduras	Red, 1	134 ± 37	7.7 ± 0.6	14.6 ± 0.1	12
BGV012625	3	Peru	Red, 2	105 ± 37	23 ± 2	8.8 ± 0.2	31
BGV012638	3	Ecuador	Deep red, 2	156 ± 3	82.2 ± 0.8	3.9 ± 0.2	30

Sp: species 1 = *S. esculentum*; 2 = *S. esculentum* var. *cerasiforme*; 3 = *S. pimpinellifolium*.
[a] Size: 1, very small; 2, small; 3, small-medium; 4, medium; 5, medium-large; 6, large; 7, very large.
[b] Ideal index: accession ranking according to its proximity to the "ideal accession" (best mean and balanced content of antioxidants) used as reference.

modern tomato cultivars with normal levels of ascorbic acid and carotenoids were included as controls: a commercial hybrid (Cambria from Seminis Vegetable Seeds Iberica, Almería, Spain) and a tomato experimental line (BGV012406) from the COMAV genebank.

2.2. Experimental design and growing conditions

Twelve plants of each accession were grown in the spring–summer cycle in Valencia, Spain. Plants were grown hydroponically in pots filled with coconut fibre in a glass greenhouse with automated climate control. The temperature control system maintains optimal temperature ranges between 15–18 and 20–25 °C (night/day). Plants were staked and pruned. The composition of the nutrient solution used was (mequiv./L): 4.0 Mg^{2+}, 1.96 Na^{+}, 8.0 K^{+}, 8.5 Ca^{2+}, 1.0 NH_4^{+}, 2.25 Cl^{-}, 11.86 NO_3^{-}, 1.5 $H_2PO_4^{-}$, 7.50 SO_4^{2-}, and 0.5 HCO_3^{-}. Micronutrients were added using a commercial mixture (Nutrel C, Phosyn, Jaén, Spain) containing the following elements (mM): Cu, 0.76; Fe, 20.15; Mn, 9.01; Zn, 1.38; B, 9.71; and Mo, 0.31. The EC was 2.35 dS/m and the pH was 5.5. Fertirrigation was scheduled to obtain a daily mean drainage of 40%. Environmental variability in the greenhouse was reduced by means of a completely randomised plot design (three plots of four plants for each accession).

2.3. Sampling

Uniformly ripe, healthy fruits, at the red-ripe stage were harvested (Hanson et al., 2004; Kuti and Konuru, 2005; Lenucci et al., 2006). Accessions with fruits that are not red were harvested when fruits reached maximum colour intensity at ripe stage. A total of 5–20 representative fruits were collected from each plant (only from the first 3 trusses) to minimise intraplant variability (Borja et al., 1998). The homogenization of tomato sample was immediately carried out in volumes of 50 mL in cold bath at 4 °C and low light (to minimise antioxidant loss). After that, ten aliquots of 1.5 mL of each sample were frozen at −80 °C in cryovials (Daslab, Barcelona, Spain) until analysis. The laboratory homogenizer (Diax 900, Heidolph, Germany) was used with a generator 6G to disrupted seeds, skin and pulp which were blended to particle sizes <0.4 mm according to the manufacturer's technical specification.

2.4. Ascorbic acid determination

Ascorbic acid was quantified by Capillary Zone Electrophoresis (Galiana-Balaguer et al., 2001) using a P/ACE System MDQ (Beckman Instruments, Fullerton, USA), controlled by the Beckman 32 Karat V5 software. A 2 g sample was thawed in the dark in a refrigerator (K4270, Liebherr-International, Bulle, Switzerland) at 4 °C and centrifuged at 12,500 rpm in a refrigerated centrifuge (Centrifuge 5415R, Eppendorf, Hamburg, Germany). The supernatant was diluted in 2% metaphosphoric acid (Sigma Chemical, St. Louis, USA) to avoid ascorbic acid oxidation (Galiana-Balaguer et al., 2001). A known concentration (100 mg L^{-1}) of potassium hydrogen phthalate (Sigma Chemical, St. Louis, USA) was added in each sample as an internal standard to correct analysis variability. The stock solutions of metaphosphoric acid and internal standard were refrigerated at 4 °C when they were added to the samples to avoid loss of ascorbic acid due to room temperature. 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 25 °C. Three analytical replicates per sample were made. The intra-day and inter-day precision was calculated as a CV of 7.8% and 8.5%, respectively.

2.5. Carotenoid determination

Determination was based on a spectrophotometric analysis following the method originally developed by Zscheille and Porter (1947) and improved by Rousseaux et al. (2005) using a spectrophotometer with double-beam operation (model Lambda-25, PerkinElmer, Waltham, USA) that allows control to be measured and corrected samples in real-time. The samples were thawed in the dark in a refrigerator (K4270, Liebherr-International, Bulle, Switzerland) at 4 °C to avoid carotenoid oxidation. Carotenoid extractions were performed once in 0.1 g of thawed samples, which were shaken (Platform rocker STR6, Stuart, Staffordshire, UK) for 1 h using 7 mL of organic solvents (ethanol:hexane, 4:3) (Sigma Chemical, St. Louis, USA). 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 4 °C to avoid carotenoids loss. To obtain lycopene concentrations, a calibration line (r^2 = 0.998) which relates lycopene concentrations from standards (Sigma Chemical, St. Louis, USA) and absorbance at 510 nm was calculated. For β-carotene concentrations we calculated a calibration plane (r^2 = 0.987) which relates the concentrations from standards (Sigma Chemical, St. Louis, USA) and absorbances at 452 nm (positive correlation) and 510 nm (negative correlation). The lycopene interference in β-carotene concentrations calculation was thereby minimized. For calibration, seven standards with joint concentrations (randomly paired up) of lycopene and β-carotene were used. Three analytical replicates per sample were made. This method was validated with standard reference material BCR485 (lyophilized mixed vegetables) from the Institute of Reference Materials and Methods (Geel, Belgium). In order to carry out this validation, 2 g of the BCR485 were weighted and rehydrated with 5 mL distilled water at room temperature for 5 min. The carotenoid extraction and analysis were done using the methodology explained above.

2.6. Statistical methods

In addition to observing averages, a graphical multivariate statistical analysis, using the biplot method (Gabriel, 1971; Yan and Kang, 2003), was done to more easily study the relationships between accessions assayed and the antioxidant content and to better evaluate and select accessions of interest as packages of functional traits (Yan and Kang, 2003; Yan and Fregeau-Reid, 2008). In GGE biplot analysis, singular value decomposition (SVD) of the two-way data table of accessions (rows) and traits (columns) was used. In this SVD the singular values are entirely partitioned into row eigenvectors to preserve the row metric in order to graphically compare genotypes (Yan and Kang, 2003). However, prior to SVD, the original two-way data table must be adequately preprocessed (centered and scaled). The data centering was done in order to use the best model to show differences between accessions:

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

where y_{ij} is the phenotypic value of each cell of the two-way trait table, μ the grand mean, α_i the accession (row) main effect, β_j the trait (column) main effect, and ϕ_{ij} the specific interaction between the last two factors.

On the other hand, data standardization is essential when the traits have different units or scales. We use the standard deviation for column (trait) as scaling factor in order to have the same weight (importance) in all the traits.

Additionally, for a better global accession evaluation, an "ideal index" which relates the mean performance (average values of traits) and balance (deviation from the average composition for all traits) of the accessions tested, was used. This ideal index, constructed from GGE stability computations (Yan and Kang, 2003), was used to graphically rank the tested accessions in a rotated GGE biplot axes for an easy accession comparison and selection. All GGE biplot analyses and graphics were carried out with the GGE biplot software (licensed by Dr. Weikai Yan, Canada).

3. Results and discussion

In the validation test of carotenoid quantification, the lycopene concentration (mean ± SD) obtained with the spectrophotometric method was 14.27 ± 0.99 and 14.14 ± 2.56 mg kg^{-1} dry weight intra-day and inter-day, respectively. These results were very similar to the value given in certificate of analysis of BCR485 (14.2 mg kg^{-1} dry weight). On the other hand, the spectrophotometric determination of the β-carotene content in the BCR485 was 44.74 ± 1.39 and 45.63 ± 2.07 mg kg^{-1} dry weight, intra-day and inter-day, respectively, which were values higher than the certified one (25.06 mg kg^{-1} dry weight).

Nevertheless, this fact has a simple explanation. The BCR485 is a mix of three vegetables (tomato, carrot and maize) with the presence of some carotenoids, as α-carotene, not present in tomato (Hart and Scott, 1995). This carotenoid has similar peaks of absorbance in hexane to that of β-carotene so its absorbance distorts our calculations of β-carotene. Obviously, the total β-carotene content that we obtain is not the sum of real α-carotene (9.80 mg kg^{-1} dry weight) and β-carotene content because, according to the Lambert–Beer law, as α-carotene has a higher extinction coefficient in hexane (145.5 mol^{-1} L^{-1}, at 446 nm) than β-carotene (136.91 mol^{-1} L^{-1}, at 452 nm) (Thumhan et al., 1988) use of functions based on β-carotene extinction coefficient overestimate the content of α-carotene. Despite it all, we have not found references reporting the presence of α-carotene in tomato (obviously excluding transgenic tomato which is not used in this study). So, if there is no α-carotene in tomato samples there is no problem with using the spectrophotometrical method.

On the other hand, in spite of the fact that other substances can absorb light in the same spectral region as β-carotene, normally in tomato samples none of these other carotenoids are detected at a completely mature stage (or they are present only as traces) and only small quantities of xanthophylls (mainly lutein) are present (Hart and Scott, 1995). Nevertheless, with the extraction procedure used, xanthopylls are retained in the lower organic phase (ethanol phase) which was discarded (Clausen and McCoord, 1936), so their possible interference could be considered as negligible. Moreover, the intra-day and inter-day precision of the lycopene (CV of 7.0% and 7.6%, respectively) and β-carotene (CV of 3.1% and 4.5%, respectively) spectrophotometric method used in this work was slightly better than those reported for the BCR485 certified product (CV of 10.0% for lycopene and 6.4% for β-carotene; Finglas et al., 1998).

So, despite the limitations of the spectrophotometric method for carotenoid quantifications, we consider that this technique is valid for the objective of the study that is to obtain a first tomato accession comparison of carotenoid content. In this sense, its simplicity, fast sample preparation and lower apparatus requirements will represent an important advantage for large field screening assays where high number of tomato samples will be evaluated.

Regarding the accessions evaluation results, the analytical results for ascorbic acid, lycopene and β-carotene content showed a high variability between accessions (Table 1), indicating that the selection of accessions with a desired content of bioactive components is possible.

For ascorbic acid, the controls used in this study (Cambria and BGV012406) showed ascorbic acid contents similar to the commonly accepted average level in the commercial tomato (200 mg kg^{-1}) (Gould, 1992), as in previous works (Roselló et al., 2006). Nevertheless, in the present study, their content was nearly half of this value (Table 1). So, we may consider that the environmental factors during this trial influenced and diminished ascorbic acid accumulation, as Dumas et al. (2003) and Toor et al. (2006) have suggested. The accession of the common tomato type with the highest content was BGV007022 (more than twice the content of controls). In other works (Abushita et al., 2000), some known cultivars have shown contents around the commonly accepted average level in the commercial tomato (Gould, 1992). However, the accessions of cherry type tomato presented the highest ascorbic acid content, particularly BGV012627 and BGV008354. These accessions presented more than 3 times the ascorbic acid content than controls and were also higher than the best values obtained by other researchers in cherry tomato cultivars (Lenucci et al., 2006).

The average lycopene content of raw tomatoes has been reported at 30 mg kg^{-1} (Holden et al., 1999; Kuti and Konuru, 2005). Cultivars broadly grown and consumed in Spain, such as 'Rambo', 'Daniella' and 'Durina', have shown contents of 32, 36 and 65 mg kg^{-1}, respectively (Martinez-Valverde et al., 2002). So, we may consider the range between 30 and 60 mg kg^{-1} to be the most common lycopene content under our conditions. Despite the reported influence of environmental factors (temperature, light, growing season and location), and the agricultural techniques used in lycopene accumulation (Dumas et al., 2003; Rosenfeld, 1999; Toor et al., 2006), in this trial, our controls, Cambria and BGV012406, showed a normal lycopene content (29 and 49 mg kg^{-1}, respectively). So we believe that our trial conditions did not diminish the potential lycopene accumulation of the accessions tested. Regarding common tomato type accessions, BGV007022 and BGV009518 accumulated more than 1.5 times the lycopene content of the best control (BGV012406). Cherry tomato types usually showed a higher lycopene content than common tomatoes with values that were generally close to the upper level of the normal considered range (60 mg kg^{-1}). For example, 'Rubino Top', 'Gardener's Delight', 'Naomi' and 'Sugar Lump' showed contents of 43, 48.9, 60 and 63.6 mg kg^{-1}, respectively (Kuti and Konuru, 2005; Lenucci et al., 2006). These lycopene contents are higher than the accepted average content, but in the same order of magnitude. In our trial, the cherry tomato type accessions BGV008148 and BGV006875 were 3.4 and 2 times higher, respectively, than the best control content. However, the most outstanding accession for lycopene content was BGV008166 (*S. pimpinellifolium*), with a content 9.3 times higher than the hybrid control and 5.5 times higher than the BGV012406 local cultivar. This lycopene level means that this accession could be an interesting donor parent in breeding programmes for developing new cultivars.

The average β-carotene content of raw tomatoes has been reported at 3.9 mg kg^{-1} (Holden et al., 1999). Abushita et al. (2000) found a range of between 2.9 mg kg^{-1} (cv 'Fanny') to 6.2 mg kg^{-1} (cv 'Monika'). The β-carotene contents of our controls were 6.7 and 10 mg kg^{-1} for Cambria and BGV012406, respectively. As in previous trials, these controls have shown lower β-carotene contents (Adalid et al., 2008), and we may consider that the environmental and/or agricultural practices (Abushita et al., 2000; Dumas et al., 2003; Raffo et al., 2006) of our trial have enhanced the potential accumulation of β-carotene. Regarding controls, this increased β-carotene accumulation was approximately 1.5 times higher than the normal average content. Of all the accessions tested, BGV011512 and BGV003095 among the common tomato types presented remarkable values, and included yellow and

orange tomatoes, respectively, with very low lycopene content. They presented twice the Cambria content and more than 20% of the BGV012406 control. In the cherry tomato type, BGV008057 showed the highest content (13 mg kg^{-1}). In previous works, cultivars 'LS203′ and 'Corbus' showed the highest content, this being around 10 mg kg^{-1} (Lenucci et al., 2006). The *S. pimpinellifolium* accessions, BGV008230 and BGV008166, were those with the highest contents. They presented more than twice the Cambria content, and 45% more than the BGV012406 content. However, the fact that their β-carotene content was only slightly above the best common tomato type accessions (BGV011512 and BGV003095) does not justify their use as donor parents in breeding programmes because of the greater laboriousness in the recovery of fruit weight.

Nevertheless, the average chemical analysis values do not completely exploit all the subjacent information to select cultivars and accessions with desirable contents of several antioxidants. In order to make easier this simultaneous evaluation of several components of nutraceutical quality and to make a better selection of the most desirable cultivars and accessions, we adopted multivariate statistical analyses using the GGE biplot methodology (Gabriel, 1971; Yan and Fregeau-Reid, 2008; Yan and Kang, 2003).

The GGE biplot analysis performed shows that the data fit well and account for 81.9% of the variability and, with appropriate views, could achieve direct and wide accession evaluations and comparisons (Yan and Rajcan, 2002). For this purpose, we used an average-antioxidant evaluation (AAE) view of the GGE biplot in which a singular value partitioning with accession-metric preserving (SVP = 1) was used (Yan and Tinker, 2005). In this plot (Fig. 1), the tomato accessions tested should be evaluated for both the mean antioxidant accumulation and equilibrated content of all the antioxidants studied.

This figure is constructed to relate the accessions tested with an "ideal accession" (the centre of the concentric circles) which have both a high mean antioxidant accumulation and an equilibrated content of all the antioxidants studied (Yan, 2001). The single-arrowed line is the AAE abscissa which indicates a higher mean antioxidant content. Thus, BGV008166 presented the highest mean antioxidant content, followed by BGV008354, BGV008057, BGV012627, etc.; the BGV012406 control presented a mean antioxidant content similar to the overall mean, and is therefore a good reference control located near the origin of the axis; BGV008008 (51) showed the lowest mean antioxidant content. The double-arrowed line is the AAE ordinate; it indicates a greater disequilibrium in antioxidant content in either direction. Thus, BGV008166 (higher lycopene and lower ascorbic acid content than the mean) and BGV008354 (higher ascorbic acid and lower lycopene content than the mean) were highly disequilibrated whereas BGV008060 was highly equilibrated.

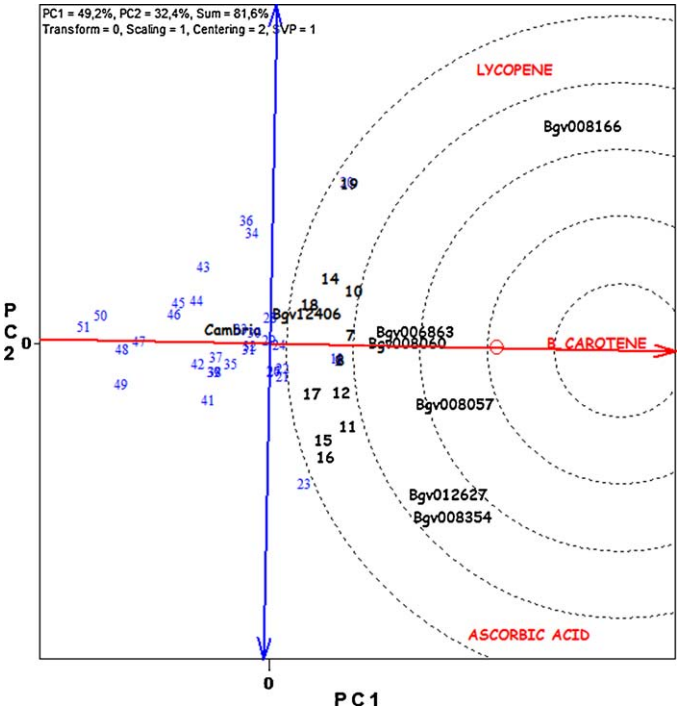

Fig. 1. Accession evaluation based on an ideal entry with a high and equilibrated antioxidant (lycopene, β-carotene and ascorbic acid) content. The position of the accession on the plot is on the left end of its label or number.

Ranking the tomato accessions related to the "ideal accession" could offer an integral evaluation of their nutraceutical capabilities. Moreover, the accessions located closer to the "ideal accession" are more ideal (the highest mean antioxidant content in all the substances evaluated) than others (Yan, 2001). In Fig. 1, this scenario is graphically shown by the concentric circles centring on the "ideal accession". Evaluating the tomato accessions revealed two interesting groups (located on the right hand side of the AAE axis and, consequently, with a higher mean antioxidant content than controls).

The first group of the "more ideal" accessions includes all the accessions inside the fourth circle (1–6 in the ideal index). In this group, there are five accessions of the cherry tomato type (*S. lycopersicum* var. *cerasiforme*) and one *S. pimpinellifolium* accession, thus confirming previous reports which indicated that these species are the best sources of antioxidants with nutraceutical properties (George et al., 2004; Hanson et al., 2004; Lincoln et al., 1943). The cherry type accessions included in this first ideal group (BGV008057, BGV006863, BGV012627, BGV008060 and BGV008354) are very desirable for direct consumption given their high and balanced nutraceutical properties. These tomatoes are usually consumed raw in salads with olive oil, which increases the bioavailability of such molecules, and, consequently, their salutary effects on human health (Bohm and Bitsch, 1999). The *S. pimpinellifolium* accession (BGV008166) included in this group would prove to be desirable as a donor parent in breeding programmes to increase the lycopene content of commercial varieties. Similarly, BGV012627 and BGV008354 cherry type accessions could also be used as donor parents in breeding programmes to increase the ascorbic acid content of new varieties.

The second "ideal group" includes all the accessions inside the fifth concentric circle (7–20 in the ideal index). This group includes two common tomato type accessions (13 and 17 in the ideal index), 11 cherry tomato accessions and two *S. pimpinellifolium* accessions (12 and 20 in the ideal index). All these accessions are beyond the controls of antioxidant content. These *S. pimpinellifolium* accessions are of no interest as potential donor parents because, despite their equilibrated antioxidant content, they are not outstanding in at least one antioxidant content which is an essential requirement to start a breeding programme. The remaining accessions can be selected and used directly for human consumption because of their equilibrated and nutraceutical content.

4. Conclusion

In conclusion, this work has shown the great variability in the bioactive component content of tomato fruit that can be found in genebanks in underutilized cultivars and related species. All the accessions selected in this evaluation trial were of interest given their nutraceutical properties and their bioactive components, in connection with the increasing interest of consumers in the relationship between diet and health. In the near future, these accessions may be used directly for human consumption or in

breeding programmes of new cultivars, increasing the agrobiodiversity of our fields.

Acknowledgement

This research was financed by The Spanish Ministry of Science and Innovation (MICINN) (project AGL2005-08083-C03-01).

References

Abushita, A.A., Daood, H.G., Biacs, P.A., 2000. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. Journal of Agricultural and Food Chemistry 48, 2075–2081.

Adalid, A.M., Roselló, S., Cebolla-Cornejo, J., Nuez, F., 2008. Evaluation and selection of Lycopersicon accessions for high carotenoid and vitamin C content. In: Proceedings of the 15th Meeting of Eucarpia Tomato Working Group, Vol. 789, Bari, Italy, pp. 221–228.

Bohm, V., Bitsch, R., 1999. Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma. European Journal of Nutrition 38, 118–125.

Borja, A., Angosto, T., Capel, J., Abad, J., Anatasio, G., Lozano, R., 1998. Variaciones intra e interindividuales en componentes del sabor de tomate de consumo fresco y líneas silvestres. In: Proceedings of XI Jornadas de Selección y Mejora de Plantas Hortícolas, Córdoba, Spain, pp. 49–56.

Byers, T., Guerrero, N., 1995. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. American Journal of Clinical Nutrition 62, 1385S–1392S.

Canene-Adams, K., Campbell, J.K., Zaripheh, S., Jeffery, E.H., Erdman, J.W., 2005. The tomato as a functional food. Journal of Nutrition 135, 1226–1230.

Clausen, S.W., McCoord, A.B., 1936. The determination of carotene and xanthophyll by a single distribution between liquid phases. The Journal of Biological Chemistry 113 (1), 89–104.

Dumas, Y., Dadomo, M., Lucca, G.D., Grolier, P., 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of Science of Food and Agriculture 83, 369–382.

Esquinas-Alcázar, J.T., Nuez, F., 1995. Situación taxonómica, domesticación y difusión del tomate. In: Nuez, F. (Ed.), El cultivo del tomate. Ed Mundi Prensa, Madrid, Spain, pp. 13–42.

Finglas, P.M., Scott, K.J., Witthöft, C.M., Van Den Berg, H., De Froidmont-Görtz, I., 1998. The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM121), milk powder (CRM421), lyophilized mixed vegetables (CRM485) and lyophilized pigs liver (CRM487). Report EUR18320 EN. Belgium.

Gabriel, K., 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika 58, 453–467.

Galiana-Balaguer, L., Roselló, S., Herrero-Martínez, J.M., Maqueira, A., Nuez, F., 2001. Determination of l-ascorbic acid in *Lycopersicon* fruits by capillary zone electrophoresis. Analytical Biochemistry 296, 218–224.

George, B., Kaur, C., Khurdiya, D.S., Kapoor, H.C., 2004. Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. Food Chemistry 84, 45–51.

Gould, W.A., 1992. Tomato Production, Processing and Technology. CTI Publications, Baltimore, USA.

Hanson, P.M., Yang, R.Y., Wu, J., Chen, J.T., Ledesma, D., Tsou, S., Lee, T.C., 2004. Variation for antioxidant activity and antioxidants in tomato. Journal of the American Society for Horticultural Science 129, 704–711.

Hart, D.J., Scott, K.J., 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chemistry 54, 101–111.

Holden, J.M., Eldridge, A.L., Beecher, G.R., Buzzard, I.M., Bhagwat, S., Davis, C.S., Douglass, L.W., Gebhardt, S., Haytowitz, D., Schakel, S., 1999. Carotenoid content of US Foods: an update of the database. Journal of Food Composition and Analysis 12, 169–196.

Jack, D.B., 1995. Keep taking the tomatoes—the exciting world of nutraceuticals. Molecular Medicine Today 1, 118–121.

Kun, Y., Lule, U.S., Xiao-Lin, D., 2006. Lycopene: its properties and relationship to human health. Food Reviews International 22, 309–333.

Kuti, J.O., Konuru, H.B., 2005. Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes. Journal of the Science of Food and Agriculture 85, 2021–2026.

Lenucci, M.S., Cadinu, D., Taurino, M., Piro, G., Dalessandro, G., 2006. Antioxidant composition in cherry and high-pigment tomato cultivars. Journal of Agricultural and Food Chemistry 54, 2606–2613.

Lincoln, R.E., Zscheile, F.P., Porter, J.W., Kohler, G.W., Caldwell, R.M., 1943. Provitamin A and vitamin C in the genus *Lycopersicon*. Botanical Gazette 105, 113–115.

Lutsenko, E.A., Carcamo, J.M., Golde, D.W., 2002. Vitamin C prevents DNA mutation induced by oxidative stress. Journal of Biological Chemistry 277, 16895–16899.

Marchioli, R., Schweiger, C., Levantesi, G., Tavazzi, L., Valagussa, F., 2001. Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids 36, S53–S63.

Martinez-Valverde, I., Periago, M.J., Provan, G., Chesson, A., 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture 82, 323–330.

Mayne, S.T., 1996. Beta-carotene, carotenoids and disease prevention in humans. FASEB Journal 10, 690–701.

Omoni, A.O., Aluko, R.E., 2005. The anticarcinogenic and anti-atherogenic effects of lycopene: a review. Trends in Food Science and Technology 16, 344–350.

Raffo, A., La Malfa, G., Fogliano, V., Maiani, G., Quaqlia, G., 2006. Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1). Journal of Food Composition and Analysis 19, 11–19.

Roselló, S., Galiana-Balaguer, L., Adalid, A.M., Nuez, F., 2006. Influencia del ambiente en la evaluación del contenido de vitamin C en germoplasma de *Lycopersicon*. Actas de Horticultura 45, 73–74.

Rosenfeld, H.J., 1999. Quality improvement of vegetables by cultural practices. International Symposium on Quality of Fresh and Fermented Vegetables 483, 57–67.

Rousseaux, M.C., Jones, C.M., Adams, D., Chetelat, R., Bennet, A., Powell, A., 2005. QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. Theoretical and Applied Genetics 111, 1396–1408.

Thumhan, D.I., Smith, E., Flora, P.S., 1988. Concurrent liquid-chromatographic assay of retinol, α-tocopherol, β-carotene, α-carotene, lycopene and β-cryptoxanthin in plasma, with tocopherol acetate as internal standard. Clinical Chemistry 34 (2), 377–381.

Toledo, A., Burlingame, B., 2006. Biodiversity and nutrition: a common path toward global food security and sustainable development. Journal of Food Composition and Analysis 19, 477–483.

Toor, R.K., Savage, G.P., Lister, C.E., 2006. Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. Journal of Food Composition and Analysis 19, 1–10.

Yan, W., 2001. GGEbiplot—a Windows application for graphical analysis of multienvironment trial data and other types of two-way data. Agronomy Journal 93, 1111–1118.

Yan, W., Fregeau-Reid, J., 2008. Breeding line selection based on multiple traits. Crop Science 48, 417–423.

Yan, W., Kang, M.S., 2003. GGE Biplot Analysis. A Graphical Tool for Breeders, Geneticist and Agronomist. CRC Press, Boca Raton, USA.

Yan, W., Rajcan, I., 2002. Biplot evaluation of test sites and trait relations of soybean in Ontario. Crop Science 42, 11–20.

Yan, W., Tinker, N.A., 2005. An integrated system of biplot analysis for displaying, interpreting, and exploring genotype by environment interactions. Crop Science 45, 1004–1016.

Zscheille, F., Porter, J.W., 1947. Analytical methods for carotenes of *Lycopersicon* species and strains. Analytical Chemistry 19, 47–51.