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Genotypic variability of carotenoids in traditional tomato cultivars

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ABSTRACT

Traditional varieties constitute a wide source of genetic variation that can be used *per se* or to obtain new cultivars with high consumer appeal and nutritional value. This work focuses on the carotenoid and chlorophyll profiles and contents of 53 traditional tomato cultivars, paying particular attention to compounds with recognized health-promoting properties. The study includes fruits with different shapes (oblate, slightly flattened, rounded, heart-shaped, long oblong and pyriform), colors (yellow, pink and red) and sizes (very small to very large). In addition, black colored tomato fruits with yellow, pink or red background color were studied. The highest concentrations of lycopene, β-carotene, phytoene and phytofluene were found in pink and red tomatoes, while the highest concentrations of lutein, violaxanthin, neoxanthin and chlorophylls were found in fruits with a dark coloration, regardless of their background coloration. Finally, the highest concentrations of the studied compounds as a whole (except β-carotene) were found in red- and pink-black varieties. Findings will hopefully contribute to recovering many tomato traditional varieties for use, directly in the field or as donor parents for breeding programs, to increase the nutraceutical properties of commercial varieties.

1. Introduction

Tomato is one of the most widely consumed vegetables in the world all year round. In 2013, world total production was about 167 million tons fresh fruit, of which Asia contributed 60.5%, the Americas 15%, Europe 12.8%, Africa 11.4% and Oceania 0.3% (*Food and agriculture organization of the United Nations statistics division*, 2015). In addition to its economic relevance, tomato has been identified as a food of great interest because of its high content of health-related compounds. This protective effect has mainly been attributed to carotenoids, which are one of the major classes of bioactive compounds in this fruit. In particular, lycopene is a major carotenoid of tomatoes (Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005; Adalid, Rosello, & Nuez, 2010) that is responsible for the characteristic red color of tomatoes and other fruits, such as watermelons, pink grapefruits, pink guavas and papaya (Mangels, Holden, Beecher, Forman, & Lanza, 1993; Perkins-Veazie, Collins, Pair, & Roberts, 2001; Martins, Fabi, Mercadante, & de Rosso, 2016).

Lycopene and other carotenoids from tomato are reported to inhibit proliferation of many types or cancer cells, and possibly function as antioxidants against development of cardiovascular diseases, age-related macular degeneration and other eye diseases (Khachik et al., 2002; Friedman, 2013). The carotenoid concentration has been shown to vary widely among different tomato cultivars, traditional varieties, especially, constituting a wide source of genetic variation (Cortes-Olmos, Leiva-Brondo, Rosello, Raigon, & Cebolla-Cornejo, 2014; Kavitha et al., 2014). However, industrialized agriculture has favored the development of new cultivars with high yield potentials, uniform appearance, disease tolerance and long shelf life, to facilitate crop management and market distribution. Replacement of varied and locally adapted plant varieties by a limited number of commercial ones has led to a high degree of genetic uniformity with insufficient attention paid to the content of health-promoting compounds. Currently, the organoleptic and nutritional quality of fresh produces is becoming a crucial parameter for growers and tomato breeders 'fueled' by the increased interest of consumers in healthy and tasty food. Thus, both traditional and molecular methods are being used in several research studies and plant-breeding programs to enhance the levels of phytochemicals in vegetables, in particular lycopene and other carotenoids in tomatoes (Kinkade & Foolad, 2013; Rocha, Deliza, Correa, do Carmo, & Abboud, 2013). For that, the screening of large numbers of genotypes is important for crop improvement programs with respect to nutritional quality. The aim of this study was to evaluate the variations in the carotenoid content of selected traditional tomato varieties in an attempt to recover them for use directly in the field or as donor parents for breeding programs to increase the nutraceutical properties of commercial varieties.

2. Materials and methods

Fifty three traditional genotypes of tomato fruit (*Solanum lycopersicum* L.) were provided by the Murcia Institute of Agri-Food Research and Development (IMIDA) genebank (BAGERIM). Plants were cultivated under net house in the experimental farm 'Torreblanca' located in Torre-Pacheco (Murcia), SE Spain. Transplants were set in April, in single rows, 40 cm plant spacing within the row, and rows spaced

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Table 1
Fruit morphological characteristics and total soluble solids (TSS) in completely ripened fruits of the 53 studied tomato genotypes.

No.	code	Color	Shape	Size	Weight (g)	TSS (°Brix)
1	Ly-83	Yellow	Pyriform	Very small	11.4 ± 0.4	6.5 ± 0.1
2	Ly-80	Yellow	Rounded	Very small	14.2 ± 0.8	8.8 ± 0.5
3	Ly-94	Yellow	Oblate	Large	166.0 ± 29.1	6.1 ± 0.3
4	Ly-101	Yellow	Oblate	Very large	283.9 ± 50.5	5.1 ± 0.4
5	Ly-85	Yellow	Oblate	Very large	334.8 ± 16.0	5.4 ± 0.1
6	Ly-110	Yellow	Oblate	Very large	483.2 ± 12.7	4.7 ± 0.1
7	Ly-72	Yellow	Rounded	Small	67.2 ± 5.7	4.7 ± 0.2
8	Ly-73	Yellow	Rounded	Small	56.3 ± 3.0	5.1 ± 0.3
9	Ly-121	Yellow-black	Oblate	Large	163.9 ± 19.3	4.2 ± 0.1
10	Ly-104	Yellow-black	Oblate	Very large	272.6 ± 21.5	4.5 ± 0.1
11	Ly-99	Yellow-black	Oblate	Large	198.4 ± 20.6	6.2 ± 0.1
12	Ly-91	Pink	Rounded	Medium	121.3 ± 18.2	5.7 ± 0.1
13	Ly-89	Pink	Flattened	Large	206.0 ± 14.0	4.6 ± 0.3
14	Ly-90	Pink	Oblate	Very large	281.7 ± 29.4	5.0 ± 0.4
15	Ly-79	Pink	Heart-shaped	Large	254.9 ± 28.0	5.6 ± 0.3
16	Ly-131	Pink	Oblate	Very large	417.3 ± 91.3	4.3 ± 0.3
17	Ly-132	Pink	Oblate	Very large	337.7 ± 48.6	5.3 ± 0.3
18	Ly-130	Pink	Oblate	Very large	437.8 ± 46.4	5.3 ± 0.2
19	Ly-120	Pink	Oblate	Very large	298.9 ± 31.8	5.7 ± 0.3
20	Ly-141	Pink	Oblate	Very large	282.7 ± 20.6	4.6 ± 0.2
21	Ly-155	Pink	Oblate	Large	246.0 ± 29.9	5.8 ± 0.2
22	Ly-137	Pink-black	Oblate	Large	203.9 ± 11.7	5.3 ± 0.6
23	Ly-135	Pink-black	Oblate	Large	164.3 ± 23.6	6.4 ± 0.5
24	Ly-92	Red	Flattened	Large	179.7 ± 11.2	5.7 ± 0.2
25	Ly-93	Red	Oblate	Large	179.2 ± 14.3	6.5 ± 0.2
26	Ly-71	Red	Rounded	Very small	13.8 ± 1.1	7.2 ± 0.3
27	Ly-86	Red	Pyriform	Small	68.7 ± 4.5	7.4 ± 0.1
28	Ly-87	Red	Cylindrical	Medium	73.1 ± 6.3	6.2 ± 0.1
29	Ly-81	Red	Cylindrical	Large	163.2 ± 21.5	5.6 ± 0.1
30	Ly-103	Red	Oblate	Large	249.1 ± 20.5	4.6 ± 0.2
31	Ly-140	Red	Flattened	Large	162.2 ± 15.5	4.8 ± 0.2
32	Ly-134	Red	Pyriform	Large	160.3 ± 16.1	4.0 ± 0.2
33	Ly-123	Red	Oblate	Large	253.5 ± 29.5	5.3 ± 0.2
34	Ly-98	Red	Flattened	Very small	9.2 ± 1.3	8.8 ± 0.2
35	Ly-145	Red	Oblate	Large	160.0 ± 10.3	4.2 ± 0.2
36	Ly-146	Red	Oblate	Large	214.5 ± 17.3	3.9 ± 0.3
37	Ly-111	Red	Heart-shaped	Large	185.8 ± 33.1	5.8 ± 0.2
38	Ly-118	Red	Rounded	Large	225.6 ± 10.0	4.8 ± 0.2
39	Ly-147	Red	Cylindrical	Large	195.1 ± 5.7	4.6 ± 0.1
40	Ly-112	Red	Cylindrical	Large	170.7 ± 11.5	5.9 ± 0.3
41	Ly-153	Red	Cylindrical	Medium	103.4 ± 3.9	5.8 ± 0.1
42	Ly-126	Red	Flattened	Large	246.4 ± 26.7	3.1 ± 0.1
43	Ly-148	Red	Rounded	Large	226.2 ± 16.6	4.5 ± 0.2
44	Ly-142	Red	Cylindrical	Medium	94.6 ± 6.9	5.8 ± 0.1
45	Ly-139	Red	Pyriform	Medium	122.1 ± 3.3	4.9 ± 0.2
46	Ly-88	Red-black	Rounded	Small	69.0 ± 5.0	7.2 ± 0.4
47	Ly-75	Red-black	Rounded	Small	67.1 ± 1.9	6.2 ± 0.1
48	Ly-96	Red-black	Oblate	Very large	298.2 ± 41.9	5.0 ± 0.2
49	Ly-122	Red-black	Cylindrical	Small	36.9 ± 1.7	6.2 ± 0.1
50	Ly-113	Red-black	Oblate	Very large	428.2 ± 50.1	5.3 ± 0.2
51	Ly-74	Red-black	Rounded	Small	27.6 ± 1.3	8.8 ± 0.6
52	Ly-102	Red-black	Oblate	Large	204.1 ± 17.8	5.5 ± 0.2
53	Ly-136	Red-black	Oblate	Very large	377.5 ± 29.9	4.6 ± 0.2

100 cm apart. The plots were maintained according to a standard processing tomato production management system. Four sub-samples of at least 10 ripe fruits were randomly picked in July. The tomato accessions were morphologically characterized following FAO/IPGRI descriptors (IPGRI, 1996). In addition, fruits were classified according to their weight as: very small (<15 g), small (≥15 and <70 g), medium (≥70 and <130 g), large (≥130 and <260 g) and very large (≥270 g) using a Mettler Toledo PG 6002-S (Uznach, Switzerland) balance. Afterwards, the fruits were cut into pieces, homogenized with liquid N_2 and frozen at −80 °C until subsequent analysis. Total soluble solids (TSS) were measured using a Digital Hand-Held "Pocket" Refractometer PAL 1 (Atago, USA).

Carotenoids were extracted by homogenizing 1 g of tomato sample in a Polytron (PT-MR 3100, Switzerland) with 20 mL methanol/tetrahydrofuran (1:1, v/v) containing 200 mg MgO (Merck, Darmstadt, Germany) and 0.1% (w/v) BHT (Sigma-Aldrich, Saint Louis, USA) in methanol/tetrahydrofuran (1:1 v/v). β-apo-8′-carotenal (96.0%) (Sigma-Aldrich, Saint Louis, USA) was added as internal standard before solvent extraction to assess losses during extraction procedure. A working solution of β-apo-8′-carotenal was prepared in methanol/tetrahydrofuran (1:1 v/v) and used as stock solution for further dilution to obtain the desired concentration. The homogenate was centrifuged at 10,000*g* for 10 min, at 4 °C. This procedure was repeated twice. The combined filtrates were evaporated to dryness in a vacuum vortex evaporator Syncore (Flawil, Switzerland). The residue was dissolved in 2 mL methanol/methyl tert-butyl ether (1:1, v/v) and the final solution was filtered through PTFE membrane filters 0.45 μm. Each sample was extracted in duplicate. Carotenoids were determined following the methodology validated by Motilva et al. (2014) using a Hewlett-Packard 1100 HPLC system (Waldbronn, Germany) equipped with a photodiode array UV/Vis detector operating in the spectral range from 250 to 800 nm. Separation was achieved in a 250 mm × 4.6 mm i.d., 3 μm Prontosil C_{30} column (Bischoff, Leonberg, Germany) with methanol (solvent A) and methyl tert-butyl ether (solvent B) as mobile phase.