THE INFLUENCE OF PARENTAL LINES ON LYCOPENE AND ß-CAROTENE CONTENT IN TOMATO F1 HYBRIDS (*Solanum lycopersicum* L)

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Abstract. *Tomatoes (Solanum lycopersicum L.) are the world's most consumed fruits, both fresh and processed, and a second important vegetable, after potatoes. The species belong to the Solanaceae family, the genus Solanum and the Lycopersicon section. Besides being consumed in many ways, both fresh and processed, tomatoes have an important role in health because they are an important source of vitamin C, potassium, folic acid and carotenoids such as lycopene and ß-carotene. Carotenoids are pigments synthesized during fruit maturation and responsible for the final colour of tomato fruits. Lycopene is a natural red pigment with significant antioxidant properties involved in reducing the incidence of various forms of cancer. Twelve tomato varieties, comprising four F1 hybrids (round breeding line tomatoes), and the parental forms from which they were created, were evaluated for their lycopene and ß-carotene content using high-performance liquid chromatography (HPLC) and spectrophotometry. Lycopene and ß-carotene content varied significantly among tomato hybrids, with USAB F_1 having the highest content of lycopene (33.34 mg/kg of fresh weight) and Banato F_1 in ß-carotene (1.59 mg/kg of fresh weight). Analyzing the behaviour of the hybrids versus their parents, it was observed that for β-carotene the recorded value was close to that of the patern genitor in all the studied hybrids, whereas in the case of lycopene there was no relationship between the values recorded in comparative hybrids with parental lines. The hybrids with high lycopene, ß-carotene has low values and vice versa. The lycopene content analysis shows the Crimson genes are not present in hybrids created by us. Results indicate that genetics may have a strong influence on tomato β-carotene content.*

Key words: *HPLC, lycopene, βcarotene, F1 tomato hybrids*

INTRODUCTION

Flavonoids, phenols, vitamin C, Vitamin E and carotenoids, mainly lycopene, are important bioactive molecules of ripe tomato fruits (LENUCCI, 2006; 2015).

Carotenoids are a large class of isoprenoid pigments synthesized by all photosynthetic organisms, as well as by some bacteria, fungi and aphids (CUTTRISS, 2011). They are synthesized during fruit ripening and are responsible for the final red colour of the tomatoes (PERVEEN, 2015)

Lycopene is the main carotenoid, representing about 90 % of total pigments, accumulated in ripe tomato fruits and has antioxidant properties that may reduce the incidence of certain cancers. Recent research has shown that lycopene is two to ten times more effective in quenching reactive oxygen species than β-carotene and α-tocopherol, respectively (DI MASCIO, 1995; and have highest trolox equivalent antioxidant capacity among all carotenoids (RICE-EVANS, 1997; STINCO, 2017)

This carotenoid with a linear structure is synthesized in red tomatoes fruit through a pathway starting from geranylgeranyl diphosphate which represent the precursor of most cyclic

carotenoids, including β-carotene. The conversion of lycopene to β-carotene is made by the action of lycopene beta-cyclase (β-Lcy), an enzyme introducing beta-ionone rings at both ends of the molecule (CUNNINGHAM, 1994; STOMMEL, 1994).

In this study we have to compare the levels of lycopene and β-carotene of ripe fruits from tomato F1 hybrids and their parental lines four main purposes: 1) to a better understanding of mechanisms for transmitting these characters from genitors to descendants and estimate the individual relative contribution of parents, 2) to compare the new hybrids between and with genitors. This information can be interesting for the farmers who will grow our hybrids and for the tomato processing industry that can optimize the use of lycopene and β-carotene as versatile ingredients in diet and human health.

MATERIAL AND METHODS

Tomato cultivation

The experiment was conducted in the year 2017 in the greenhouses of Plant Physiology Dept., Faculty of Horticulture and Forestry, in Banat University of Agricultural Sciences and Veterinary Medicine King Michael I st of Romania from Timisoara (45.7489° N, 21.2087° E). The tomato cultivars UASB, Sorada, Miruna și Banato are F_1 hybrids obtained by crossing of some local landraces from Banat region, cultivated in traditional peasant farms. It was grown in traditional technology în greenhouse conditions, under a controlled environment.

Tomatoes (each hybrid with parents) were grown on separate experimental plots, the surface of which was 60 m^2 . The care was taken to ensure similar agrotechnical and climatic conditions in each experimental design. During the vegetation season, the plants were regularly watered by the means of drip system with humidity sensors. Tomato fruits were collected in the same state of ripeness (red-ripe) from all experimental. The sampling procedure has been described below in the subsections.

Sample preparation for lycopene and β-carotene determination from tomato fruits (adapted from MOTIVA et al, 2014). Samples of tomato fruits (1 g) were ground in a mortar and pestle and 10 ml solvent mixture n-hexane/acetone/ethanol (50/25/25, v/v/v) were added. The mixture was homogenised with a vortex for 1 min and centrifuged at 5000 rpm for 20 min at 5 °C. The supernatant was collected and the extraction repeated until a colourless supernatant was obtained. The extract containing lycopene was kept in the dark!**.** Three extractions were necessary to complete extraction of the lycopene and the phenolic compounds from the tomato. The supernatants obtained from the extractions were put in an amber separation funnel and 5 ml of pure water were added. The funnel was shaken and two layers allowed to form; the upper layer (orange), the organic phase (hexane), containing the β-carotene, lycopene, and a lower layer (colourless) was the aqueous phase (acetone and ethanol) which contained the phenolic compounds. The two phases were collected in separate bottles, using an amber bottle for the organic phase.

The organic phase was again put in the separation funnel and 5 ml of pure-water was added to remove any residue of water-soluble compounds. After shaking, the two layers were collected in their respective bottles. After cleaning up the organic phase, the aqueous phase was put in a separation funnel and 5 ml of n-hexane was added in order to recover any residual lycopene. The separation funnel was shaken and again two layers allowed to form and collected in their respective bottles. This process was repeated one more time, and phases were

collected and placed in their respective bottles. Ultimately, two bottles were obtained, the first containing the organic phase (lycopene), which was orange in colour, and the second containing the aqueous phase (phenolic compounds), which was colourless. Before analysis samples were filtered using 25 mm Syringe filters (0,45µm PTFE membrane).

HPLC analysis

Analysis was performed using an ultra high-performance liquid chromatography (Nexera X2, Shimadzu, Tokyo, Japan) equipped with a diode array detector (M30A, Shimadzu, Tokyo, Japan) and a Nucleosil 100-3-C18 reversed-phase column (4.0 mm i.d. x 125 mm column length, 3 µm particle size, Macherey-Nagel GmbH, Duren, Germany).

Lycopene analysis

The column temperature was maintained at 30 ºC and the flow rate of 0.500 mL min^{-1}. The elution solvents used for the chromatographic analysis consisted of methanol (A) and acetone (B). The chromatographic elution program used was as follows: 50% A and 50% B, then the linear gradient grew to 75% B and maintained for 3 min, followed by a linear gradient of 50% B in 3 min. Total run time 12 minutes. The injected volume of samples and standards was 10 µL and it was done automatically using an auto-sampler. The spectra were acquired in the wavelength range: 200 - 600 nm.

β-carotene analysis

The column temperature was maintained at 20 ºC and the flow rate of 1 mL min^{-1}. The elution solvents used for the chromatographic analysis consisted of ultra-pure water with 0.1% TFA (A) and acetone (B). The chromatographic elution program used was as follows: 25% A and 75% B, then the linear gradient grew to 100% B and maintained for 3 min, followed by a linear gradient of 75% B, the gradient was kept in 75% B, 5 minutes. Total run time 25 minutes. The injected volume of samples and standards was 10 µL and it was done automatically using an auto-sampler. The spectra were acquired in the wavelength range: 350 - 700 nm.

Spectrophotometric analysis was made using a double beam spectrophotometer Specord 200 (Analytik Jena AG, Germany), with a 10 mm quartz cuvette. Absorbance was measured at a specific wavelength: for the organic phase: 450, 470, 503 nm, and for aqueous phase: 350, 470 nm.

Calculation of lycopene levels. Lycopene levels in the organic phase were calculated according to:

Lycopene (mg/kg fresh wt.) = (A_{503}x537x10x0.55)/(1x172)

Where:

- 537 g/mole is the molecular weight of lycopene,
- 10 mL is the volume of mixed solvent,
- 0.55 is the volume ratio of the upper layer to the mixed solvents,
- 1 g is the weight of tomato added,
- 172 mM^{-1} is the extinction coefficient for lycopene in hexane.

Data were statistically processed using analysis of variance and t-test. The significance of differences was expressed based on letters, being considered as significant the differences between variants marked with different letters.

RESULTS AND DISCUSSIONS

Tomato fruits contain a mixture of bioactive components that serve as a source of nutrients with antioxidant effects such as carotenoids, including lycopene, β-carotene and lutein. The mechanisms that control the metabolism of carotenoids synthesis during fruit maturation are extremely complex (LIU, 2015).

Tomato fruits are the most important accumulator of the carotenoid pigment lycopene, which can be readily cyclised to β-carotene by the plant enzyme *lycopene β-cyclase* (*lcy-b*). Beta-carotene is the major dietary precursor of vitamin A, together with other carotenoids containing unsubstituted beta-ionone rings (LAKSHMAN, 1993).

The amounts of lycopene, in the F 1 hybrids are reported in figure 1. Lycopene content in tomato fruit harvested on red-ripe stage ranged from 25,71 mg/kg fw in Sorada F1 to 33,34 mg/kg fw in USAB F1. Therefore, compared to USAB F1, the hybrid Sorada F 1 accumulates less 33% lycopene in fruits. The conversion of lycopene to β-carotene is made by the action of lycopene beta-cyclase (β-Lcy) enzyme, as demonstrated by the results shown in figure 2.

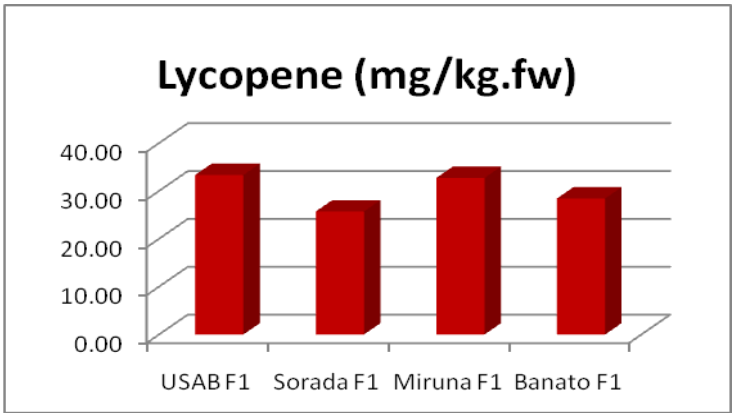

Fig.1. Lycopene content in whole fruits of tomato hybrids

The analysis of the experimental data presented in figure 2 shows that the highest beta carotene content of 1.59 mg/g fw was recorded at Banato F_1 and the lowest in the hybrid Miruna of 0.96 mg/g fw.

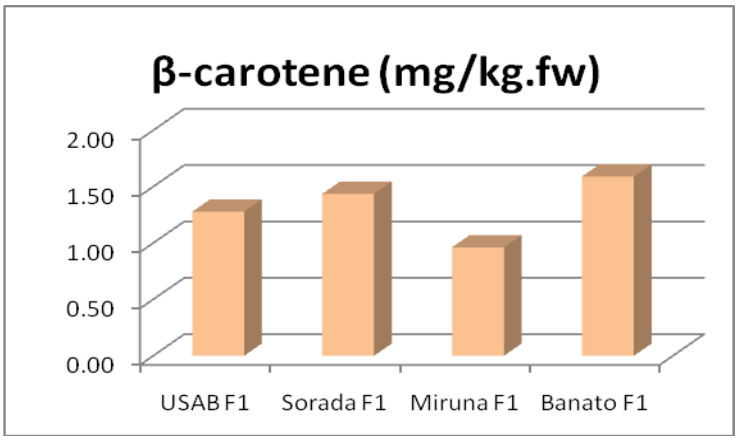

Fig.2. β-carotene content in whole fruits of tomato hybrids

Our results are in line with other reported by BRANDT, 2006; CHOI, 2014; DEAK, 2015; ILAHY, 2015, 2016; LENUCCI, 2006, ranging from 28 to 893 mg/kg fw measured in different tomato cultivars grown under controlled or field conditions from different geographical areas.

The carotenoid pigments deposition is responsible for the characteristic colour of ripe tomato fruits. The parents, in the present study, had a considerable variation for β-carotene content, from 1,08 mg/kg fw for Sanmartin♀ to 2,56 mg/kg fw in Gradinari♀ which has a positive significantly value compared with the average of the experiment. There is no heterosis in F1 hybrids, regarding the β-carotene content, but it can be observed that they have a closer amount of β-carotene to male than female genitors (tab.1).

Table 1

The assessment of β-carotene content for tomato hybrids and their parents

Genotypes	β-carotene (mg/kg fw)	Compared with exp. mean	
		Relative value (%)	Difference/Significance
Sanmartin♀	1.08 d	72.97	-0.40
USAB F1	1.28 bcd	86.49	-0.20
Pordeanu 19♂	1.18 cd	79.73	-0.30
Cruceni102 ♀	1.70 b	114.86	0.22
Sorada F_1	1.44 bcd	97.30	-0.04
Rudna 143 ♂	1.30 bcd	87.84	-0.18
Cruceni102 ♀	1.70 b	114.86	0.22
Miruna F1	0.96 d	64.86	-0.52^{0}
Livezile 498 ♂	1.23 bcd	83.11	-0.25
Gradinari♀	2.56 a	172.97	1.08***
Banato F1	1.59 bc	107.43	0.11
Dudestii V. 883b♂	1.69 b	114.19	0.21
Exp. mean	1.48	100	Control

$LSD_{5\%}$=0.49 $LSD_{1\%}$=0.69 $LSD_{0,1\%}$= 0.97

The differences between genotypes marked with different letters are significant at p= 0.05

The analysis of lycopene content in parental forms shows a variation between 30.08 mg/kg fw (Pordeanu 19♂) and 40.88 mg/kg fw (Rudna 143♂). With the exception of the hybrid USAB, all others recorded lower lycopene content compared to the parental forms that were used as the genitors (tab.2). The results are in accord with other studies, DAGADE, 2015; PANTHEE 2015.

Table 2

The assessment of lycopene content for different tomato hybrids and parents

Genotypes	Lycopene (mg/kg fw)	Compared with exp. mean	
		Relative value (%)	Difference/Significance
Sanmartin♀	38.27 abcd	109.75	3.40
USAB F1	33.34 cdef	95.61	-1.53
Pordeanu 19♂	30.08 efg	86.26	-4.79
Cruceni102 ♀	38.42 abc	110.18	3.55
Sorada F_1	25.71 g	73.73	-9.16^{00}
Rudna 143 ♂	40.88 a	117.24	6.01*
Cruceni102 ♀	38.42 abc	110.18	3.55
Miruna F1	32.73 def	93.86	-2.14
Livezile 498 ♂	37.79 abcd	108.37	2.92
Gradinari♀	39.22 ab	112.47	4.35
Banato F1	28.42 fg	81.50	-6.45^{0}
Dudestii V. 883b♂	35.16 bcde	100.83	0.29
Exp. mean	34.87	100	Control

$LSD_{5\%}$=5.58 $LSD_{1\%}$=7.81 $LSD_{0,1\%}$= 11.06

The differences between genotypes marked with different letters are significant at p= 0.05

CONCLUSIONS

Tomatoes fruit may show different colours from yellow light up the deep blue with the whole range of shades between. Among the multitude of pigments affecting the fruit colour, carotenoids play a decisive role.

Identification of genetic factors that affect the accumulation of carotenoids in tomatoes is time labour intensive and time-consuming. Were investigated two distinct carotenoids profiles (lycopene and β-carotene) in seven parental lines, to study the way of transmission of these characters in the first hybrid generation. Metabolite analysis using HPLC technique is an effective tool for identifying genes involved in the control of carotenoid biosynthesis in tomatoes.

Creating hybrids with high heterosis is a constant goal in the process of breeding tomatoes, but the best results are obtained when the parental forms are genetically removed, increasing the chance of complementarity and allelic heterozygosity in the cross (BODNARESCU et al., 2018).

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