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BIOLOGICALLY ACTIVE COMPOUNDS TRANSFORM DURING THE RIPENING STAGES IN GREENHOUSE TOMATOES

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SUMMARY

Currently, the selection of tomatoes with a high content of biologically active substances and antioxidant properties at the large green and breaker stage is relevant since mature tomatoes cannot tolerate storage and transportation. For this purpose, 11 tomato genotypes, chosen in a preliminary study in 2018–2020 from the Genetic Collection of Plant Resources of Federal State Budgetary Scientific Institution Federal Scientific Vegetable Center (FSBSI FSVC), Moscow, Russia, and Tomato Genetics Resource Center (TGRC), the University of California, Davis, USA, for further studies during 2020–2022 for antioxidant pool changes: measuring the contents of chlorophyll, lycopene, β-carotene, ascorbic acid, and lutein. The experiment arranged in a randomized complete block design proceeded in the film unheated greenhouses. The results showed five promising tomato genotypes, i.e., VFN Hi Sugar, VS-420, Paul Robeson, Black Cherry, and VS-410. The genotype Black Cherry fruits with breaker ripeness contained 42% lycopene and 93% β-carotene, while the genotype Paul Robeson at the same stage contained 80% β-carotene. The three other tomato genotypes, viz., VFN Hi Sugar, VS-420, and Paul Robeson, also gave a higher content of ascorbic acid in the fruits at the breaker ripening stage.

Key words: Tomato, ascorbic acid, β-carotene, chlorophyll, lutein, lycopene, total phenols

Key findings: The studied dynamics of various pigments with biological activity according to the tomato ripening stages revealed promising genotypes identified and selected for further studies.

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INTRODUCTION

In vegetable crops, tomato ranks first in popularity, with its extensive number of uses. On a global scale, tomato consumption is growing annually due to taste qualities and biological values of the most valued antioxidants, i.e., ascorbic acid, polyphenols, and vitamins B and E (Shende *et al*., 2012; Al-Kurtany *et al*., 2023). The most important biologically active substances in tomato fruits are carotenoids, represented by orange, red, and yellow pigments. These essential biological compounds are a vital part of the human diet, reducing the risk of oncological and cardiovascular diseases (Ford and Erdman, 2012; Mamedov *et al*., 2017). Carotenoids exist in small globules, especially at the outer pericarp of the fruit, having the highest total carotenoids, followed by industrial waste (i.e., tomato pomace, composed of tomato peels and seeds), whole tomato, and pulp (Amorim-Carrilho *et al*., 2014; Kumar *et al*., 2015).

The main factors affecting the carotenoid content and other biologically active substances are the genetic makeup of the genotypes, environmental conditions, production technology, and the maturity degree of fruits (Dumas *et al*., 2003). However, the vital role played by the genotypes and the process of fruit maturation manages the quantitative and qualitative variations (Cano *et al*., 2003; Binoy *et al*., 2004; Garcia and Barrett, 2006).

In red tomatoes, the most predominant carotenoid is trans-lycopene (83%), with the β-carotene as the minor component, accounting for 3%–7% of total carotenoids (Hayes *et al.,* 1998). During the ripening period, the lycopene content of tomatoes increases sharply from the pink stage onwards, still assessing the changes in other fruit antioxidants has had insufficient attempts so far (Dumas *et al*., 2003). Lutein, γ-carotene, cis-lycopene, prolycopene, and δ-carotene also exist in trace amounts in red tomatoes (García-Valverde *et al*., 2013). For tangerine tomatoes, their β-carotene, tetra-cis-lycopene, and δ-carotene content may be higher, with the all-trans-lycopene content reduced. Yellow tomatoes mostly contain lower lycopene and β-carotene and all other trace carotenoids (Yoo *et al*., 2017). Black tomatoes exhibited blackish-red skin because of retaining chlorophyll content and possessing higher lycopene content compared with red tomatoes, having β-carotene and lutein as minor carotenoids (Park *et al*., 2018).

In tomato genotypes, during the fruit ripening period, the variations in the biochemical composition may occur in different ways, and often, there is an increase in the biologically active substances from large green to full maturation of fruits. Studying such changes in these substances at different stages of fruit formation and ripening is crucial, especially when breeding, to improve the biochemical composition of the tomato fruits. Since, in greenhouse plants, along with biological ripeness, collections occur more often at the stage of large green and breaker fruit for better storage and transportation.

In Russia, developing several tomato cultivars and hybrids has succeeded in protected soil cultivation with resistance to biotic and abiotic stresses and high yield. However, in recent years, much attention focused on the taste and antioxidant properties of cultivated genotypes. In this connection, selecting tomatoes for protected soil with increased content of biologically active substances in the fruits at the ripeness stage becomes relevant. Therefore, searching sources and creating source genetic material with high biochemical parameters at different stages of tomato crop maturation from green and breaker to red (biological ripeness) is imperative and could play a vital role in selecting protected ground tomato genotypes.

This study aimed to determine the trends related to the influence of the genotype and the stage of maturation on the main antioxidant compounds (chlorophyll, lycopene, β-carotene, and ascorbic acid) and at the selection of prime genetic material for use in breeding to improve the biochemical composition in the phases of large green and breaker fruit in tomatoes.

MATERIALS AND METHODS

The collection of genetic material comprised 11 tomato cultivars (VFN Hi Sugar, VS 420, VS 412, VS 410, Black Cherry, Paul Robeson, Big Yellow Red, Big Rainbow, Yellow Peach, White Beauty, and Samohval) from the Genetic Collection of Plant Resources of Federal State Budgetary Scientific Institution Federal Scientific Vegetable Center (FSBSI FSVC), Moscow, Russia, and Tomato Genetics Resource Center (TGRC), the University of California, Davis, USA to study the relationship between the signs of fruit coloring, biologically active substances, and antioxidant properties (chlorophyll, lycopene, and β-carotene). The

basis for selecting tomato genotypes was the diverse colors of the fruit in biological ripeness. The cultivation of tomato plants in unheated film greenhouses proceeded during 2020–2022 by the seedling method with recommended cultural practices. The experiment had a randomized complete block design with four replications each year corresponding to plots. The plot and subplot areas were 55 m^2 and 5 m^2, respectively. The total number of plants per subplot was 20.

The separation of fruits by degrees of maturity ensued through keen visual observations of the color of the fruit skin and the pulp shining through it. Selected three stages of fruit ripening for biochemical analysis comprised a) green fruit (green-ripe), b) breaker (around 10%–15% of the fruit surface turns to tarnish-pink or yellow color at the blossom end of tomatoes), and c) full biological ripeness.

Ascorbic acid (AsA) measurement used the methodology of Ranganna (2000). The 10 g sample maceration used 3% meta-phosphoric acid, with the volume adjusted to 100 mL. Taking an aliquot of 10 mL extract underwent titrating with Tillman's reagent (2,6-dichlorophenol indophenol) until a pale pink endpoint appeared, which persisted for 15–20 s. The expressed result was mg/g fresh weight (FW).

Determining the contents of chlorophylls a and b (Chl a and Chl b), lycopene (Lyc), and β-carotene (β-Car) was according to Nagata and Yamashita (1992). The sample weight (1 g) homogenization was in 10–20 ml of a mixture of acetone and hexane (4:6 v/v). The calculation was according to the following formulas:

$$Chl\ a\left[\frac{mg}{ml}\right]=\frac{0.999A_{663}-0.0989A_{645}}{100},$$

$$Chl\ b\left[\frac{mg}{ml}\right]=\frac{-0.328A_{663}+1.77A_{645}}{100},$$

$$Lyc\left[\frac{mg}{ml}\right]=\frac{-0.0458A_{663}+0.204A_{645}+0.372A_{505}-0.0806A_{453}}{100},$$

$$\beta-Car\left[\frac{mg}{ml}\right]=\frac{0.216A_{663}-1.22A_{645}-0.304A_{505}+0.452A_{453}}{100},$$

where:

A_{453}, A_{505}, A_{645}, and A_{663} were absorption at 453 nm, 505 nm, 645 nm, and 663 nm, respectively, and the thickness of the cuvette was 1 cm. The result, expressed as mg/g FW, used the following equation for calculation:

$$\text{Pigment}\left[\frac{mg}{g\ FW}\right]=\frac{c\cdot V}{m},$$

where c is the pigment concentration expressed as mg/ml, V is the total extract volume expressed in ml, and m is the extractable samples fresh weight, expressed as g FW.

Lutein determination in about 0.5 g of tomato employed extraction of the tomato samples with 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol (1:1, v/v), and analyzed by HPLC-DAD with a C18 column (250 mm × 4.6 mm, S-5 µm). The concentration of the standard solution calculation used the molar extinction coefficient 14.3 × 10^4 for lutein in hexane (Ilahy *et al*., 2011). Peaks detection was at 503 nm, with the results expressed in mg/g fresh weight (FW).

Determining total soluble phenols in ethanol extracts used the Folin-Ciocalteau reagent (Golubkina *et al*., 2017), with one gram sample homogenized in 10 ml of 80% ethanol. The homogenate, placed in capped test tubes, received heat at 60 ºC in a water bath for one hour. The extract centrifugation was at 5000 rpm for 15 min, with the collected resulting supernatant used for the estimation of total phenolics. The results were as mg gallic acid equivalents (GAE) / g FW.

Statistical analysis

All the recorded data underwent statistical analysis using OriginPro 9.0 and R Statistical software.

RESULTS AND DISCUSSION

It is a fact that combining colored and unpainted skin and varying degrees of colored fruit flesh provides a wide range of colors in mature tomato fruits, i.e., yellow, slightly pink to purple-red. The analysis of consumer demand showed broad differences in coloring priorities in different regions of the Russian Federation, authenticating the need for the original shape of fruits with different colors and higher contents of antioxidants. In this regard, a study started on the genetic material taken from the Tomato Genetics Resource Center (TGRC, USA) and the breeding samples of the

Table 1. Morphological characteristics of the studied tomato cultivars.

Tomato cultivars	Origin / Catalog number	Type of plant	Leaf color	Fruit (shape)	Weight (g)
VFN Hi Sugar	TGRC/ LA 2086	Determinate	Dark green	Rounded	150
VS 420	FSVC	Determinate	Dark green	Plum-shaped	35-40
VS 412	FSVC	Indeterminate	Dark green	Rounded	20-25
VS 410	FSVC	Determinate	Dark green	Rounded	50-60
Black Cherry	TGRC/ LA 4451	Indeterminate	Green	Rounded	25-30
Paul Robeson	TGRC/ LA 4450	Semi-determinate	Green	Flat-rounded	150-200
Big Yellow Red	TGRC/ LA 2972	Semi-determinate	Green	Flat-rounded	200-250
Big Rainbow	TGRC/ LA 2973	Semi-determinate	Green	Flat ribbed	250-300
Yellow Peach	TGRC/ LA 2357	Indeterminate	Light green	Founded	40-50
White Beauty	TGRC/ LA 2464	Semi-determinate	Light green	Flat ribbed	100-150
Samohval	FSVC	Determinate	Light green	Rounded	90-100

FSBSI FSVC with different levels of biologically active substances differing in a variety of shapes and colors.

Focusing breeding work to create cultivars and hybrids with a high content of biologically active substances leads to substantial interest in evaluating numerous tomato genotypes with different fruit colors and shapes and plant habitus for their antioxidant status. The studied samples, represented by the healthy plants of the different tomato genotypes, had certain morphological features inherent in these forms. The studied tomato lines had characteristics of different habitus (determinate, semi-determinate, and indeterminate), the shape and weight of the fruit, and leaf color from light green to dark green (Table 1).

The ripening of tomato fruits includes various morphological, physiological, and biochemical changes, including a change in chlorophyll content, the synthesis of carotenoids, particularly lycopene, and β-carotene. In the large green fruit stage, most samples of tomato genotypes had a fairly high content of chlorophyll and approximately the same values of β-carotene, ranging from 0.006–0.019 mg/g FW. In fruits with a green color and a spot, the recorded amount of chlorophyll has a higher range (0.027–0.040 mg/g FW) compared with green and light green colors (Table 2). As the fruits ripen and enter the blanching ripeness phase, a sharp decrease occurs in the chlorophyll content, except for the samples of the tomato genotypes, viz., Black Cherry and Paul Robeson, which have a brown color at the biological ripeness stage. In said group, there was a slight decrease or even an increase in their content.

In the studied tomato genotypes, except for yellow-colored ones, the amount of beta-carotene and lycopene tends to increase at the biological ripening stage of the fruits. In samples with red fruits, a sizable increase in lycopene and β-carotene influenced the ripening process, and the genotype VS-420 with a pink-red hue accumulates lycopene twice as much as in other red fruits. The stages of maturation of large green and blanched fruit in red-fruited genotypes resulted with a low content of lycopene and β-carotene, except for genotype VS-410. In the said genotype at the breaker ripeness stage, the lycopene content and beta-carotene emerged within 23% and 75% of the biological maturation stage, which makes it promising in breeding for high content of biologically active substances at the breaker fruit stage.

In tomato genotypes with brown fruits, the content of lycopene and β-carotene by the time of biological ripeness were approximately equal. Moreover, in the genotype Black Cherry, there was a residual amount of chlorophyll up to 0.011 mg/g FW, affecting the fruit color. Furthermore, the cultivar Black Cherry, which has a greenish-red color at the stage of biological ripeness, contains both lycopene and beta-carotene. The said tomato genotype's fruits with blanched ripeness contain 42% of the final content of lycopene and 93% of β-carotene, which determines its prospects for use as a base genetic material in future breeding for a rich biochemical composition at the technical ripeness stage. The genotype, Paul Robeson, revealed a greenish-yellow color at the breaker fruit stage and contained 80% of the β-carotene in fruits.

Beta-carotene predominates in the tomato genotypes with orange-colored fruits, while lycopene and beta-carotene in equal amounts prevail in the orange-red ones. Another dynamic appeared in yellow–fruited tomato species - lycopene and β-carotene did not practically show at the time of biological maturation. In these samples, the noted lutein content was also in a range of 0.011–0.013 mg/g FW (Table 3).

Table 2. The chlorophyll and carotenoid content (mg/g FW) in tomato cultivars with different fruit colors collected at three different stages of maturation.

Tomato cultivars & stage of fruit maturation	Chl a	Chl b	Chl a + b	Lycopene	β-carotene	Fruit color
VFN Hi Sugar						
Green	0.014±0.001	<0.003	0.017±0.001	<0.001	0.012±0.001	Green
Breaker	0.006±0.001	<0.001	0.007±0.001	0.011±0.001	0.016±0.001	Green orange
Biological ripeness	<0.001	<0.001	<0.002	0.100±0.005	0.050±0.003	Intensely red
VS 420						
Green	0.022±0.001	<0.005	0.027±0.001	<0.001	0.014±0.001	Green with a spot
Breaker	<0.004	<0.001	<0.005	0.018±0.001	0.018±0.001	Orange
Biological ripeness	<0.001	<0.002	<0.003	0.205±0.010	0.106±0.005	Rose-red
VS 412						
Green	0.032±0.002	0.009±0.001	0.041±0.002	<0.001	0.015±0.001	Intensely green
Breaker	0.012±0.001	<0.005	0.017±0.001	0.013±0.001	0.019±0.001	Orange yellowish
Biological ripeness	0.006±0.0010	0.002±0.001	0.008±0.001	0.129±0.006	0.060±0.003	Intensely red
VS 410						
Green	0.052±0.003	0.017±0.001	0.069±0.003	<0.001	0.019±0.001	Green with a dark spot
Breaker	0.012±0.001	0.007±0.001	0.019±0.001	0.027±0.001	0.033±0.002	Orange reddish
Biological ripeness	<0.005	<0.002	0.007±0.001	0.116±0.006	0.044±0.002	Red
Black Cherry						
Green	0.029±0.001	0.009±0.001	0.038±0.002	<0.001	0.018±0.001	Green with a spot
Breaker	0.018±0.001	<0.004	0.022±0.001	0.013±0.001	0.025±0.001	Greenish red
Biological ripeness	0.009±0.001	<0.002	0.011±0.001	0.031±0.002	0.027±0.001	Brown
Paul Robeson						
Green	0.032±0.002	0.008±0.001	0.040±0.002	<0.001	0.018±0.001	Green with a spot
Breaker	0.037±0.002	0.009±0.001	0.046±0.002	<0.001	0.020±0.001	Yellowish green
Biological ripeness	<0.003	<0.002	<0.005	0.037±0.002	0.025±0.001	Brown
Big Yellow Red						
Green	0.009±0.001	<0.002	0.011±0.001	<0.001	0.006±0.001	Light green
Breaker	<0.002	<0.001	<0.002	<0.002	0.008±0.001	Yellow
Biological ripeness	<0.001	<0.001	<0.001	0.035±0.002	0.028±0.001	Orange-red
Big Rainbow						
Green	0.020±0.001	<0.005	0.023±0.001	<0.001	0.013±0.001	Green
Breaker	<0.004	<0.001	<0.005	<0.001	0.007±0.001	Greenish yellow
Biological ripeness	<0.001	<0.001	<0.001	0.008±0.001	0.029±0.001	Orange
Yellow Peach						
Green	0.017±0.001	<0.004	0.021±0.001	<0.001	0.010±0.001	Green
Breaker	0.008±0.001	<0.001	0.009±0.001	<0.002	0.014±0.001	Greenish yellow
Biological ripeness	<0.001	<0.001	<0.001	<0.003	0.007±0.001	Pale yellow
White Beauty						
Green	0.030±0.002	0.007±0.001	0.037±0.002	<0.001	0.019±0.001	Light green with a spot
Breaker	<0.001	<0.001	<0.001	<0.001	<0.003	Pale yellow
Biological ripeness	<0.001	<0.001	<0.001	<0.001	<0.002	Pale yellow
Samohval						
Green	0.025±0.001	<0.005	0.030±0.002	<0.001	0.014±0.001	Intensely green
Breaker	<0.001	<0.001	<0.001	<0.001	0.008±0.001	Yellow
Biological ripeness	<0.001	<0.001	<0.001	<0.001	0.007±0.001	Yellow

The values shown represent the mean ± standard error from at least four biological replications.

Table 3. Lutein content in tomato genotypes with yellow-colored fruits (biological ripeness).

Tomato cultivars	Lutein (mg/g FW)
Yellow Peach	0.013±0.001
White Beauty	0.012±0.001
Samohval	0.011±0.001

The values shown are the mean ± standard error from at least four biological replications.

Table 4. Ascorbic acid content during fruit ripening (mg/g FW).

Tomato cultivars	Stage of fruit development	AsA
VFN Hi Sugar	Green	0.158±0.007
	Breaker	0.264±0.008
	Biological ripeness	0.334±0.008
VS 420	Green	0.193±0.009
	Breaker	0.228±0.008
	Biological ripeness	0.316±0.011
VS 412	Green	0.110±0.004
	Breaker	0.185±0.008
	Biological ripeness	0.246±0.008
VS 410	Green	0.105±0.004
	Breaker	0.135±0.005
	Biological ripeness	0.211±0.009
Black Cherry	Green	0.176±0.007
	Breaker	0.246±0.008
	Biological ripeness	0.299±0.009
Paul Robeson	Green	0.158±0.006
	Breaker	0.258±0.009
	Biological ripeness	0.404±0.011
Big Yellow Red	Green	0.140±0.007
	Breaker	0.193±0.008
	Biological ripeness	0.281±0.010
Big Rainbow	Green	0.105±0.005
	Breaker	0.140±0.007
	Biological ripeness	0.211±0.010
Yellow Peach	Green	0.176±0.008
	Breaker	0.193±0.009
	Biological ripeness	0.264±0.010
White Beauty	Green	0.211±0.010
	Breaker	0.228±0.009
	Biological ripeness	0.334±0.011
Samohval	Green	0.158±0.005
	Breaker	0.193±0.007
	Biological ripeness	0.246±0.010

The values shown are the mean ± standard error from at least four biological replications.

Along with carotenoids, vitamin C is also the most significant antioxidant in the human body, supporting the health of all cells. This compound protects against several types of reactive oxygen species, i.e., free radicals and peroxides (Sharifi-Rad *et al*., 2020). In the studied tomato genotypes, the vitamin C content significantly differed at the various stages of the maturation period (Table 4). During maturation, varying total ascorbic acid content depends upon the genotype; however, at the biological fruit maturation stage, its greatest accumulation emerged in all the tomato cultivars. In the context of genotypes, the noted higher content of ascorbic acid at the biological ripeness was in the genotype Paul Robeson (0.404 mg/g FW). Moreover, in the breaker stage of maturation, the said cultivar has sufficiently high ascorbic acid values (0.258 mg/g FW), compared with other genotypes, i.e., Big Yellow Red, Black Cherry, Yellow Peach, VS 412, and Samohval with biological maturation. As a base breeding material for selection for high ascorbic acid content, the tomato genotypes VFN Hi Sugar, VS-420, Paul Robeson, and White Beauty have a higher vitamin C in the fruits at the breaker stage of maturation. Also, two cultivars, VFN Hi Sugar and VS-420, have a higher lycopene content with vitamin C.

Phenolic compounds in the studied tomato cultivars showed their genotype specificity, with the highest content noted in the cultivars Paul Robeson and Black Cherry with brown fruit color (0.84–1.05 mg GAE / g FW). Past studies also reported that tomato genotypes with a high lycopene content in fruits caused a decrease in phenolic compounds contents during ripening. A study reported a gradual decline in the concentration of chlorogenic acid, one of the common phenols in tomatoes, during the ripening of 'Naomi' cherry tomatoes grown in the greenhouse (Raffo *et al*., 2006).

CONCLUSIONS

The augmented amount of lycopene, β-carotene, and vitamin C is crucial and desirable in tomato cultivars and their hybrids grown in protected soil and used in the immature fruit stage. Using lycopene-rich tomato cultivars and hybrids for fresh consumption and processing will enhance the consumption of antioxidants. Assessment of the accumulation level of various components with high antioxidant activity allows for identifying rich sources and including them in future breeding programs. As a base breeding material for developing new cultivars and hybrids with high antioxidant capacity at the large green and breaker fruit phases, the tomato cultivars, i.e., VFN Hi Sugar, VS-420, Paul Robeson, Black Cherry, and VS 410, come highly recommended.

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