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THE BIOLOGICALLY ACTIVE (BIOACTIVE) COMPOUNDS IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) AS A FUNCTION OF GENOTYPE

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Abstract

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The 15 cherry tomato genotypes from the Institute for Vegetable Crops, Smederevska Palanka were grouped in order to define the start material for breeding. Genotypes were grouped according to: average content of carotenoids (lycopene, β-carotene), total sugars and total acidity in tomato fruits, through PCA (Principal Component Analysis). Correlation matrix showed low, both positive and negative correlation among the researched traits. The level of lycopene was negatively correlated to β-carotene, L-ascorbic acid and total acidity, while it was in positive correlation with total sugars. β-carotene was negatively correlated to l-ascorbic acid and total sugars, but in positive correlation with total acidity, while it was negatively correlated with total sugars. Total sugars were negatively correlated with total acids. The first three components explained 78.55%, while first two components explained 62.07% of total variability. Genotypes on the positive side of both main components (PC1 and PC2) had the highest mean values of L-ascorbic acid (GK67, GK64, GK33, GK19), while genotypes with highest mean values of total sugars were on the negative side of both components (GK153, GK71, GK2, GK75, GK91). Genotypes GK70 and GK1 had high level of β-carotene and total acidity, low level of L-ascorbic acid and minimal mean values of total sugars. GK10, GK20, GK88 and GK74 stood out in the quadrant of negative values of the first main component and positive values of the second main component.

Key words: cherry tomato, 15 genotypes, quality attributes, Principal Component Analysis

Introduction

In recent years, researchers are interested and focused on the identification of bioactive components in food that affects the health, and may also reduce the risk of some diseases. The research of bioactive components, particularly lycopene content includes very extensive studies both in conventional breeding and biotechnological researches, with special reference to the possibility to increase their content (Tedeschi et al., 2011). Many traditional groceries, including fruits and vegetables, contain components that are good for health. Within this group tomato was identified as functional and nutraceutical food (Canene-Adams et al., 2005). High consumption of tomato in the world throughout the year makes it one of the main sources of minerals, vitamins and antioxidants.

Due to carotenoids, lycopene and β-carotene, tomato has high nutritional value. Lycopene is the main carotenoid of tomato and is accumulated and highly concentrated in mature red fruits. Tomato decreases the risk from some types of cancer and heart diseases (Rao et al., 2000). β-carotene is provitamin of vitamin A and its deficiency can cause xerophthalmia, blindness and premature death (Mayne, 1996). Arnao et al. (2001) found that antioxidant capacity of lycopene is 1.16 times higher than β-carotene and 2.9 times higher than antioxidative capacity of vitamin C (L-ascorbic acid). It is believed that ascorbic acid is vital in preventing cardiovascular diseases, some cancers, cataracts, and also prevents mutations of DNA caused by oxidative stress (Byers and Guerrero, 1995; Lutsenko et al., 2002; Marchioli et al., 2001). The colour of tomato fruits, influenced by the level of lycopene, is

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significant for fresh consumption (just after the yield) or for storing and transportation. The moment of picking, stability of lycopene and other bioactive components in phases after picking are very important for choosing genotype for selection (Nikbakht et al., 2011, Brashlyanova and Ganeva, 2009). One aspect of the selection (for the higher concentration of biochemical parameters) is including other species of the tomato family in selection, in order to increase the content of bioactive components (Pavicharova et al., 2012)

Divergence in selection material from the morphological point of view, primary determinates type and direction of selection, Glogovac et al. (2010). Divergence of taste components (such as total sugars and total acidity) and related sensory traits are caused by different content and relation of potentially favourable and unfavourable traits of tomato fruit (Krumbein et al., 2004). Selection of the appropriate genotypes for the start of the selection depends largely on the primary identification and grouping of genotypes according to the characteristics relevant to the general and specific objectives of selection of this vegetable (Foolad, 2007). Selection of cherry tomato requires consideration of a number of basic morphological characteristics and chemical composition (Ganeva et al., 2006) on material as divergent as possible, since this type of tomato belongs to delicious one. The main purpose of these researches was to determinate the level of bioactive components (L-ascorbic acid, β-carotene and lycopene) total acidity and total sugars, depending on genotypes of cherry tomato.

Material and Method

Plant material

The experiment was conducted in the spring cycle of tomato growing in the open field. The investigations included 15 tomato genotypes originating from the collection of cherry tomatoes from the Institute for Vegetable Crops, Smederevska Palanka. During its growing season all standard growing measures have been applied to researched tomato genotypes. For the purposes of this research, fruits were harvested at full maturity, six days from the change of the fruit colour. After the harvest, the samples were analyzed for the content of lycopene, β-carotene, L-ascorbic acid, total acidity and total sugars.

Carotenoid determination

Tomatoes (the samples) for carotenoid determination were being extracted by 96% ethanol for 24 hours in the process of cold maceration. The solutions have been filtrated after that time and ethanol was removed by a rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenija) under a vacuum and was dried at 40°C. The dried extracts were stored in glass bottles at 4^0C to prevent oxidative damage until analysis.

B-carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried ethanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 minute and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453. 505. 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations:

$$\text{Lycopene (mg/100 ml)} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453};$$

$$\text{B-carotene (mg/100 ml)} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}.$$

The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as milligrams of carotenoid/100 g of fresh tomato.

Ascorbic acid determination

The ascorbic acid in fresh fruits was measured by titration against 0.21% 2.6-dichlorophenolindophenol dye according to Albrecht (1993), after extraction of 5 g fresh sample in 5% metaphosphoric acid. Three parallel titrations were performed for each sample. For the calculation of L-ascorbic acid content in the tomato, the average values of the volumes of three titrations were taken.

Determination of total sugar and titrable acidity (TA) (acidity total)

Total sugar was measured using an Abbe refractometer (Carl Zeiss, Jena Germany). TA was measured according to AOAC Method 942.15 (AOAC, 1995) and expressed as % citric acid.

Statistical analysis (data analysis)

Correlative ratio among the traits according to researched genotypes has been determined by applying Pearson matrix at the significance level $P \leq 0.05$. The connection of genotypes and traits was done by multi-variation technique of PCA-Principal Component analysis using Statistical software: XLSTAT Version 2012.4.02 Copyright Addinsoft 1995-2012. The analysis was performed according to average values of the researched parameters.

Results and Discussion

Analysis of results of the level of lycopene, β-carotene and L-ascorbic acid (Table 1) in the researched cherry tomato genotypes proved significant differences among genotypes,

which opens the possibilities towards selection of desired level of bioactive components.

Lycopene is a pigment, responsible for the red colour of the mature tomato and its products (Shi et al., 2000). The lowest concentration of lycopene were found in yellow genotypes GK 33 (0.031 ± 0.01 mg/100 g), GK 19 (0.089 ± 0.03 mg/100 g) and GK 1 (0.093 ± 0.02 mg/100 g). A little higher level of lycopene was found in the orange genotypes GK 70 (0.190 ± 0.05 mg/100 g) and GK 71 (0.298 ± 0.06 mg/100 g). The highest concentration of lycopene, expectedly, was found in red genotypes GK 10 (4.330 ± 0.02 mg/100 g) and GK 20 (3.067 ± 0.02 mg/100 g). The obtained data are in accordance with values obtained by Lenucci et al. 2006. Tomato and its products are important sources of lycopene. The recommended daily doses of lycopene are 25.2 mg, according to Rao et al. (1998). According to this estimation, the usage of 100 g of fresh tomato (for the tested genotypes) gives about 20% of recommended daily lycopene intake.

Concentration of β-carotene is higher than the lycopene concentration in yellow and orange genotypes (decreases in the series GK1, GK19, GK33, GK71 and GK70). Red genotypes vary in β-carotene content from 0.111 mg/100 g in GK10 to 0.845 mg/100 g in GK 20. Holden et al. (1999) found that the average content of β-carotene in fresh tomato was 3.9 mg/kg, while Abushita et al. (2000) found that the β-carotene content was between 2.9 mg kg^{-1} to 6.2 mg kg^{-1}. It is believed that the differences among the contents depend upon the growing methods and climate conditions (Raffo et al., 2006), but from the traits of the researched tomato genotypes, too.

Large differences in L-ascorbic acid content among genotypes (Table 1) were found. The highest level was found in tomato GK 67A (37.05 mg /100 g), followed by genotypes GK 19, GK 33, GK 20. The lowest level of L-ascorbic acid had tomato GK 91 (13.32 mg /100 g) (Table 1). High divergence of this parameter was found by Saha et al. (2010) among 53 researched genotypes (CV 12-86 mg 100 g). The average value of L-ascorbic acid (26.5 mg /100 g) in the researched cherry tomato genotypes was higher than the average values (20 mg /100 g) obtained by Gould W.A. (1992). This suggests that besides the genotype, the content of L-ascorbic acid and its accumulation is influenced by terms of growing and environment (Dumas et al., 2003).

It was found that the total acidity of the genotypes is in the range of 0.193 g /100 g do 0.493 g /100 g. Gould (1992) found that tomato with 0.35% to 0.55% total acidity content, together with balanced sugar content, has a good potential quality that can make it suitable for proceeding.

Total sugar content in the researched genotypes was from 2.66% (GK1) to 6.38% (GK2) (Table 1). Most of the sugar content, which represents the component of fruit taste, enables the selection of genotypes with higher level of this trait, which is especially interesting in selecting cherry tomato genotypes.

Correlation matrix showed low both positive and negative correlations among the researched traits for genotypes includ-

Table 1
Variations of the measured compounds content in cherry tomato as a function of genotype

Genotype	Lycopene, mg /100 g	β-Carotene, mg /100 g	L-ascorbic acid, mg /100 g	Total acidity, g /100 g	Total sugars, %
GK1 ž	0.093 ± 0.02	4.536 ± 0.06	25.16 ± 0.15	0.490 ± 0.003	2.66 ± 0.31
GK19 ž	0.089 ± 0.03	1.572 ± 0.04	34.04 ± 0.27	0.276 ± 0.001	3.70 ± 0.19
GK20 c	3.067 ± 0.02	0.845 ± 0.05	31.08 ± 0.12	0.368 ± 0.002	3.99 ± 0.15
GK33 ž	0.031 ± 0.01	0.673 ± 0.03	34.04 ± 0.18	0.456 ± 0.003	4.00 ± 0.25
GK 67Ac	2.375 ± 0.01	0.333 ± 0.06	37.05 ± 0.09	0.394 ± 0.003	4.13 ± 0.54
GK74 c	2.072 ± 0.05	0.392 ± 0.04	21.83 ± 0.31	0.292 ± 0.002	3.49 ± 0.17
GK75 c	0.513 ± 0.07	0.154 ± 0.07	24.15 ± 0.20	0.193 ± 0.007	6.31 ± 0.33
GK10 c	4.330 ± 0.02	0.111 ± 0.05	26.37 ± 0.14	0.223 ± 0.002	3.28 ± 0.17
GK64 c	2.451 ± 0.02	0.177 ± 0.02	30.11 ± 0.16	0.493 ± 0.003	4.18 ± 0.42
GK88 c	2.015 ± 0.03	0.051 ± 0.05	28.43 ± 0.42	0.309 ± 0.002	3.60 ± 0.68
GK153 c	1.782 ± 0.05	0.346 ± 0.03	26.76 ± 0.10	0.448 ± 0.001	6.04 ± 0.15
GK2 c	2.911 ± 0.01	0.803 ± 0.02	19.24 ± 0.36	0.263 ± 0.004	6.38 ± 0.26
GK71 n	0.298 ± 0.06	0.937 ± 0.04	26.64 ± 0.07	0.267 ± 0.001	5.85 ± 0.19
GK91 c	1.962 ± 0.06	0.516 ± 0.04	13.32 ± 0.55	0.389 ± 0.001	5.59 ± 0.21
GK70 n	0.190 ± 0.05	0.893 ± 0.02	20.25 ± 0.28	0.403 ± 0.002	2.73 ± 0.36

ed in the analysis. The level of lycopene is negatively correlated to β-carotene, L-ascorbic acid and total acidity content but in positive correlation with total sugars. B-carotene is negatively correlated to L-ascorbic acid and total sugars, but it is positively correlated with total acidity. L-ascorbic acid correlates positively with total acidity but is in a negative correlation with total sugars. Total sugars correlate negatively with total acidity (Table 2).

Saha et al. (2010) found significant correlations ($p<0.05$. 0.01) among pericarp thickness and lycopene - 0.52 (medium strong and negative correlation), while in our research medium strong and negative correlation of lycopene and β carotene (-0.46) was found. Selection programmes that rely on colour and taste are very useful in selecting cherry tomato and tomato for industrial proceeding (Pevicharova and Ganeva, 2004). Correlative relations among these traits are very significant for directions of the breeding process.

Results in Table 3 proved that first three components explain 78.55%, while first three components explain 62.07% of total variability. If the data was to be presented on only one axis (PC1) we would be able to see even 38.86% of total variability of the obtained results.

Figure 1 shows correlation circle of first two components. L-ascorbic acid and total acidity were at the same quadrant of the circle, so it can be concluded that they were positively correlated. Traits: L-ascorbic acid and lycopene, lycopene and total sugars, as well as L-ascorbic acid and β-carotene were placed opposite each other, so it can be concluded that among these traits there was no significant correlation.

Total acidity content is near PC2 axis and it is the closest to the centre of the correlation circle. Two main components (PC1 and PC3) carry information regarding this trait (Table 4) and according to this correlation circle it is impossible to explain this trait.

Bold-marked values represent the highest value of cosine squared for certain trait and their connection with certain factors. The first main component was influenced by four of five observed traits. Our results cannot be directly compared to Saha et al. (2010), since the researched traits and their share in variation were different, while 66% of variations were for five main components. The second main component was influenced by L-ascorbic acid.

These results were helpful in interpreting the next graph. Genotypes on the positive side of both components (PC1 and PC2) had the highest middle values of L-ascorbic acid (GK67, GK64, GK33, GK19), while genotypes with higher middle values of total sugars were on the negative side of both main components (GK153, GK71, GK2, GK75, GK91). Genotypes GK70 and GK1 had high content of β-carotene and total acidity, low L-ascorbic acid and minimal middle values of total sugars. GK10, GK20, GK88 and GK74 stood out in the quadrant of negative values of the first principal component and the positive values of the other major components. These genotypes had high values of lycopene and low values of total sugars (Figure 2). De Nardo et al. (2009) compared two methods for determination of the level of lycopene by applying PCA multivariation technique and came up with the similar genotype grouping as presented in our research. Results of the analysis for this multivariation model depend on the number of traits and the way the assays were carried out. Lavelli et al. (2001) found that the first 3 components (78%) were responsible for total variance of the samples, where 60% were within the first component while 18% were in the second.

Since PC1 component is characterised with four traits (lycopene, β-carotene, total sugars and total acids), the selection process begun with genotypes grouped around these traits. The research of 15 genotypes of cherry tomato proved that the progeny will have the desired traits.

If the selection process goes toward increase of l-ascorbic acid, genotypes GK64, GK67 and maybe GK20 will be chosen. We must have in mind that *total sugar* trait correlates positively and low with the level of lycopene. On the other hand, it correlates medium low and negatively with level of: β-carotene, l-ascorbic acid and total acids (Table 2).

Genotypes (GK2, GK75 GK, 71, GK91 and GK153), grouped around trait in the lower left quadrant of the coordinate system, point to the genotypes with high level of sugar, which is very important for cherry tomato. For β-carotene content, however, we will select GK1 and for lycopene GK10 (Figure 2).

Table 2
Pearson correlation matrix of tomato characteristics

Variables	Lycopene	β-Carotene	L-ascorbic acid	Total acidity	Total sugars
Lycopene	1				
β-Carotene	-0.46	**1**			
L-ascorbic acid	-0.07	-0.02	**1**		
Total acidity	-0.19	0.35	0.17	**1**	
Total sugars	0.05	-0.36	-0.28	-0.30	**1**

Table 3
Eigenvalues of 5 principle components

PC	Eigenvalues	Percent of variance	Cumulative, %
1	1.94	38.86	38.86
2	1.16	23.21	62.07
3	0.82	16.48	78.55
4	0.70	13.92	92.47
5	0.38	7.54	100.00

Table 4
Squared cosines of the observed traits:

Trait	Principal component				
	PC1	PC2	PC3	PC4	PC5
Lycopene	**0.34**	0.29	0.29	0.00	0.09
β-Carotene	**0.61**	0.15	0.02	0.04	0.18
L-ascorbic acid	0.12	**0.52**	0.33	0.00	0.04
Total acidity	**0.46**	0.01	0.05	**0.47**	0.00
Total sugars	**0.42**	0.19	0.13	0.18	0.08

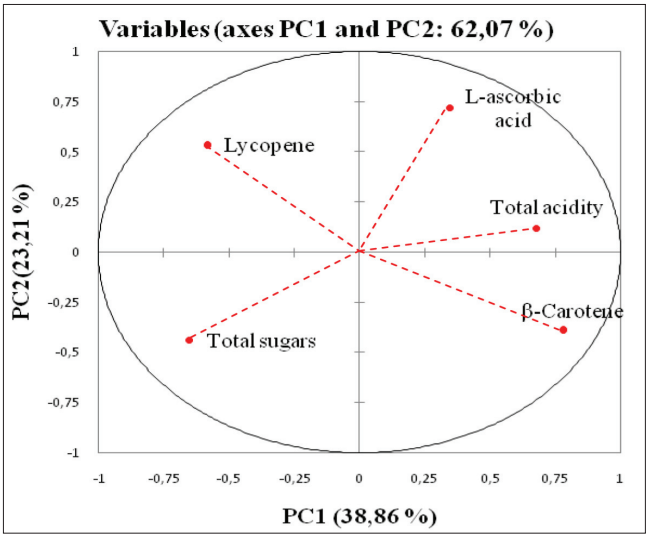

Fig. 1. Correlation circle (axes PC1 and PC2)

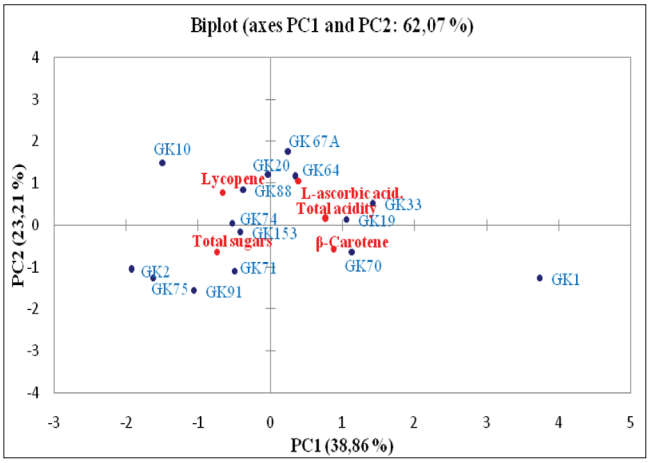

Fig. 2. PCA analysis biplot of first and second principle component

Results of this analysis can help making a new plan of selection of cherry tomato. Relationships of traits and their correlation as well as genotypes carrying desired traits could be a start material for further selection of cherry type tomato.

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