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APPLICATION OF THE MATHEMATICAL MODEL HJ-BIPLOT TO IDENTIFYING THE LINKS AMONG BIOACTIVE COMPOUNDS OF THE TOMATO VARIETY PANEКRA

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Abstract

Samples were collected from the Plovdiv, Bulgaria. The aim of this study was to is to compare the similarity and remoteness of different irrigation regimes: 1. Irrigation regime (50% of irrigation rate) and 50% fertilization; 2. Irrigation regime (75% of irrigation rate) and 50% fertilization; 3. Optimal irrigation regime (M-100%) with 50% fertilization; for the variety Panекra during the period 2016-2018 year and to identify the link among bioactive compounds of tomatoes through the mathematical model HJ-biplot. An important indicator of the quality of tomatoes is the content of total dyes. The highest values were found in tomato farmed irrigation regime (50% of irrigation rate) where total dyes attained up to 4.95 mg/100 g. The β-carotene content determines the orange color of the tomato fruit. The good combination of the two components (lycopene and β-carotene) with an antioxidant effect determines hybrids of tomatoes such as hybrids of high biological value. In the present study the content β-carotene ranges from 0.26 to 0.35 mg/100 g. The content of β-carotene is higher at a 50% of irrigation rate

Key words: *irrigation regimes, tomatoes, Panекra, HJ-biplot, Bulgaria.*

INTRODUCTION

The prevalence and widespread use of tomatoes is due primarily to the excellent food, taste and technological qualities of the fruit.

The chemical composition of fruits fluctuates quite widely, depending on the variety, the region, the growing conditions and the farming practices applied. However, the nutrient content of tomatoes depends mainly on genetic and ecological factors, and Javanmardi et al., 2008 consider maturation. Irrigation is also an agricultural practice that can influence the final content of these parts in the tomato fruit (Dumas et al., 2003). Water supply is limited globally and there is a growing need to reduce the amount of water used during irrigation practices (Zegbe-Domlnguez et al., 2003). The water deficit has a positive influence on the dry matter content, (Shao et al., 2014) and lycopene (Dumas et al., 2003).

The influence of irrigation practices on the processing tomato quality has not been sufficiently studied yet. Here are many studies related to the effects of quantity and frequency of irrigation on the quality of tomatoes (Machado et al., 2005; Zegbe-Dominguez et al., 2003) but still this question is relevant given the content of antioxidants in tomatoes. Examining the influence of water deficit there are established parameters of water deficit and productivity of irrigation water which result in stable yields and high quality (Lahoza et al., 2016; Nangare et al., 2016). Favati et al., 2009 evaluate the relationship between all quality parameters in tomatoes and seasonal water for irrigation. Tested and evaluated are the effects of different irrigation regimes, taking into account the physical and chemical characteristics of fruit and content of antioxidants. In recent years, as a result of expanding knowledge about the benefits of carotenoids for health, there is a significantly increasing attention of researchers to food taste and antioxidant properties of tomato fruits. A plurality of medical research has shown that dietary intake of lycopene-rich food limiting cases of some oncological and cardiovascular diseases, cataracts and the like. Color is principally associated with the lycopene content of tomato and is generally considered the most important attribute determining the product quality (Garcia et al., 2005).

The purpose of this paper is to compare the similarity and remoteness of different irrigation regimes the variety Panекra and to identify the link among bioactive compounds of tomatoes through the mathematical model HJ-biplot.

MATERIALS AND METHODS

Tomato Sampling

For the purpose of the study there were used test results of different irrigation regimes in tomato cultivar Panекra during the period 2016-2018 year (Figure 1).

Figure 1. Panекra

The Panекra variety is offered for early production in steel-glass and polyethylene greenhouses. The hybrid has many habitats, with houses between restoration and a good balance between vegetative and generative development. It has great potential for obtaining average yield height. The areas are large, up to very large (over 300 g.), solid, black in color, multicameral without parenchymal tissue inside the fetus. It is resistant to tobacco mosaic virus, Verticillium, Fusarium, mold list and nematode.
The experiment is based in the region of Plovdiv with geographical coordinates 42° and 09′ north latitude and 24° and 45′ East GMT (GPS). The experiment based on the block method on a flat surface in scheme 110 + 50 + 35 with the size of the parcel plot of 10 m^2 (Barov, 1982).

1. Irrigation regime (50% of irrigation rate) and 50% fertilization; 2. Irrigation regime (75% of irrigation rate) and 50% fertilization; 3. Optimal irrigation regime (M-100%) with 50% fertilization.
Greenhouse production is intense and it requires the application of high doses of fertilizers. For the purposes of the experiment, different doses of basic fertilization and feeding during the growing season were tested. The main fertilization was carried out with P 230 kg/ha in the form of P_2O_5) and K 250 kg/ha (as K_2SO_4). Feeding through vegetation was performed with N 500 kg/ha as NH_4NO_3) and K 660 kg/ha as KNO_3) according to the experimental methodology. Submission of irrigation water was realized with the drip irrigation system. During the three years of the study various watering rates have been implemented depending on the requirements of culture and the length of the growing season.

Sample Preparation

Each sample was weighed, and the weight was divided by the number of tomatoes to give the weight per tomato. Then, three tomatoes were selected at random from each sample for analysis. They were hand-rinsed with ultra-pure water, shaken to remove any excess water, and gently blotted with a paper towel. A small portion (approximately 3 g weight) of each tomato was cut and put into 10 mL of 3% H_3PO_4 as subsamples for the ascorbic acid assay. The tomatoes were then mixed and homogenized to homogeneous puree. A fraction of this puree was desiccated at 105°C, homogenized again, and stored in a polyethylene tube at room temperature until assay for metals and protein. The rest was immediately stored in a polyethylene tube at -80°C in order to avoid enzymatic changes and for the measurement of the other chemical variables.

Analytical Methods

All assays were performed in triplicate, and the results expressed per fresh weight (FW). Moisture was determined using the oven-drying method (AOAC, 2006). The residue was heated at 550°C for 24 h for the ash determination (AOAC, 2006). Organic nitrogen was determined by the Kjeldahl method (AOAC, 2006), and the protein concentration estimated using 6.25 as the conversion factor. All assays were performed in triplicate, and the results expressed per fresh weight (FW). Sugars were determined as described by Galdon et al., 2009. In the determination of mineral elements were determined by Atomno absorption spectrometer "AAnalyst 800 with graphite furnace HGA" Company "Perkin Elmer". The tomato samples were previously acid-digested in nitric acid, in accordance with the procedure described by Hernandez Suarez et al., 2008. Ascorbic acid was determined in the individual tomatoes using the 2,6-dichlorophenolindophenol titration procedure (AOAC, 2006). The lycopene concentration was determined spectrophotometrically at 503 nm following extraction in 20 mL of acetone: ethanol: hexane (5: 5: 10 v/v) in the dark, in accordance with the method described by Fish et al., 2002. Organic acids were determined by HPLC as described by Hernandez Suarez et al., 2008. β-carotene was determined by HPLC as described by Hernandez Suarez et al., 2007.

Statistical Analysis

Statistical Software

All statistical computing, analysis and all charts were performed with the statistical software R program Version 3.5.2.

The log-ratio transformation

In order to apply standard statistical multivariate analysis, the raw compositional data were subjected to a clr-transformation. This transformation is symmetric with respect to the compositional parts, and maintains the same number of components as the number of parts in the composition. Its advantage is that it is an isometric transformation of the simplex with Aitchison metric onto a subspace of real space with the ordinary Euclidean metric (Egozcue et al., 2003; Aitchison et al., 2002; Aitchison, 1986). The clr-transformation is given by the expression:

$$clr(x) = \left[\ln \frac{x_1}{g_m(x)}, \ln \frac{x_2}{g_m(x)}, \ldots, \ln \frac{x_D}{g_m(x)} \right],$$

where: $x = (x_1, x_2, \ldots, x_D)$ is a compositional data vector; x_i are the chemical variables all of which must be expressed in the same units; $g_m(x)$ is the geometric mean of that compositional data vector.

HJ-Biplot Method

The statistical technique used was the HJ-Biplot multidimensional data classification technique (Galindo, 1986), which is a variant of the Biplot graphic display proposed by Gabriel (Gabriel, 1971). This technique makes it possible to plot the rows and columns of the data matrix as points on a low dimension vectorial space. It has been shown theoretically (Galindo, 1986) that the quality of plotting both for individuals and for ical technique has been demonstrated in other studies (Castela et al., 2010; Lodoño et al., 2007; Galante et al., 1991). Also, the discriminatory power of this statistical technique has been demonstrated in other studies (Castela et al., 2010; Lodoño et al., 2007; Galante et al., 1991).

The HJ-biplot is a joint representation in a low-dimensional vector space (usually a plane) of the rows and columns of a data matrix X, using markers (points/vectors) $j_1, j_2, \ldots, j_n$ for its rows and $h_1, h_2, \ldots, h_p$ for its columns. The markers are obtained from the usual singular value decomposition (SVD) of the data matrix $X = U\Sigma V^T$, where U is formed by the eigenvectors of the matrix XX^T, V by the eigenvectors of the matrix X^TX, and Σ is a diagonal matrix containing the singular values (i.e., the square roots of the non-zero eigenvalues of both XX^T and X^TX), taking as rows the marker rows of $J = U\Sigma$ and as columns the marker rows of $H = V\Sigma$, in the appropriate dimensions. Thus, the matrix X is formed by clr-transformed data, and then double-centered through its SVD to ensure that the components are analyzed on a ratio scale (Aitchison, 2002) in the appropriate dimensions.

A biplot of compositional data consists of the elements shown in Figure 2 (Pawlowsky-Glahn et al., 2015; Aitchison et al., 2002):

- ➢ The similarity (Sij) between two samples or individuals is taken to be an inverse function of their distance, in such a way that closer samples are more similar.

- The centroid represents the center-of-gravity formed by the geometric mean of the compositional parts used in the clr-transformation.
- The ray provides information on the variance of the corresponding log-ratio with respect to the geometric mean (g_m):

$$\mathrm{var}\left(\ln\frac{x_i}{g_m(x)}\right)$$

The square root of this expression is the standard deviation of the clr-transformed variable Xi, and is represented by the length of the ray. The cosine of the angle (α) between two rays represents the approximate correlation coefficient between the corresponding variables.

The length of a link between the vertices of two rays represents the standard deviation of the log-ratio between the associated compositional parts, with the corresponding variance being defined as:

$$\mathrm{var}\left(\ln\frac{x_i}{x_j}\right).$$

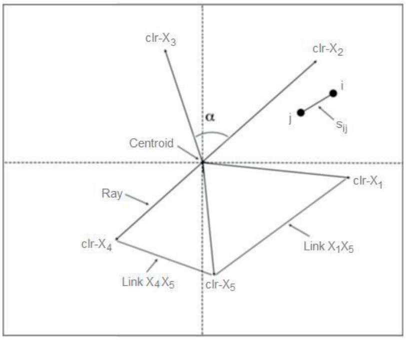

Figure 2. Elements of a compositional biploot (Pawlowsky-Glahn et al., 2015; Aitchison et al., 2002)

Angles between links provide information on the relationships between pairs of variables. If two links intersect at a right angle then this is an indication that the pairs of variables are possibly uncorrelated (in Figure 2, links X_1X_5 and X_4X_5), while if they are parallel (or the angle is obtuse) then the pairs of parameters may be strongly correlated (in Figure 2, links X_1X_5 and X_4X_3).

Coincident vertices or short links mean that the two variables are linearly proportional, so that the two parts involved can be assumed to be redundant. If a subset of links is collinear, this might indicate a possible one-dimensional variability.

RESULTS AND DISCUSSIONS

Tables 1 give the results of the influence of irrigation regime on the chemical composition of the tomato fruit (Ранекга) (proximate composition, mineral elements, organic acids, and antioxidant compounds).

The highest values were found in tomato farmed irrigation regime (75% of irrigation rate and 50% fertilization) where ascorbic acid attained up to 33.58 mg/100 g. Ascorbic acid contents in tomato farmed irrigation regime (50% of irrigation rate and 50% fertilization) declined to 27.84 mg/100 g. A similar trend was noted in Vasileva et al., 2016, in an analysis of the influence of potassium fertilization.

An important indicator of the quality of tomatoes is the content of total dyes. The two main groups of pigments in the fruit of tomato carotenoids and chlorophylls but the final color is determined by the total quantity and ratio of different carotenoids (Danailov, 2012). The highest values were found in tomato farmed irrigation regime (50% of irrigation rate and 50% fertilization) where total dyes attained up to 4.95 mg/100 g. Total dyes contents in tomato farmed irrigation regime (100% of irrigation rate and 50% fertilization) declined to 3.07 mg/100 g.

Lycopene is a phytochemical from the group of carotenoid pigments. Its antioxidant activity predetermines the interest in increasing its content in tomatoes. Researchers have found that lycopene accounts for between 75 and 83% of the total content of pigments in tomatoes (Gould, 1992; Abushita et al., 1997) making it an important biochemical quality indicator. According to some authors the content of lycopene is varietal characteristic and fruit of tomatoes respond to fertilization with potassium by increasing the content of antioxidants (Hartz et al., 2005; Henry et al., 2008).

Table 1. Proximate and mineral elements composition (mean ± standard deviation, data expressed in fresh weight, FW) of tomato samples

Irrigation regime (50% of irrigation rate) and 50% fertilization	Proximate Composition (% FW)			
	Compound	2016	2017	2018
	Moisture	92.84 ± 0.52	93.41 ± 0.46	93.11 ± 0.36
	Ash	0.72 ± 0.07	0.74 ± 0.11	0.73 ± 0.08
	Protein	0.69 ± 0.09	0.67 ± 0.06	0.67 ± 0.09
	Fructose	1.35 ± 0.11	1.36 ± 0.15	1.35 ± 0.18
	Glucose	1.44 ± 0.12	1.43 ± 0.22	1.43 ± 0.23
	Mineral Elements (mg/kg FW)			
	P	264 ± 28	283 ± 31	261 ± 27
	K	2820 ± 325	3025 ± 342	2870 ± 328
	Mg	135 ± 15	141 ± 18	137 ± 16
	Organic Acids and Antioxidant Compounds (mg/100 g FW)			
	Ascorbic acid	27.84 ± 0.48	32.61 ± 0.56	29.88 ± 0.68
	Titratable acidity	0.32 ± 0.09	0.39 ± 0.11	0.21 ± 0.06
	Total dyes	4.95 ± 0.32	3.89 ±0.28	4.95 ± 0.31
	Lycopene	4.63 ± 0.18	3.79 ± 0.15	5.00 ± 0.21
	β-carotene	0.32 ± 0.08	0.35 ± 0.07	0.34 ± 0.08
Irrigation regime (75% of irrigation rate) and 50% fertilization	Proximate Composition (% FW)			
	Moisture	93.21 ± 0.38	93.85 ± 0.43	93.54 ± 0.35
	Ash	0.69 ± 0.08	0.71 ± 0.13	0.70 ± 0.08
	Protein	0.71 ± 0.10	0.69 ± 0.08	0.71 ± 0.09
	Fructose	1.34 ± 0.12	1.36 ±0.11	1.34 ± 0.14
	Glucose	1.42 ± 0.11	1.44 ± 0.22	1.42 ± 0.18
	Mineral Elements (mg/kg FW)			
	P	248 ± 25	265 ± 27	245 ± 21
	K	2654 ± 307	2863 ± 328	2680 ± 305
	Mg	127 ± 12	132 ± 16	129 ± 14
	Organic Acids and Antioxidant Compounds (mg/100 g FW)			
	Ascorbic acid	30.57 ± 0.46	33.58 ± 0.52	29.88 ± 0.62
	Titratable acidity	0.3 ± 0.08	0.4 ± 0.12	0.25 ± 0.07
	Total dyes	4.58 ± 0.18	3.47 ± 0.25	4.57 ± 0.28
	Lycopene	3.92 ± 0.15	3.47 ± 0.14	4.64 ± 0.25
	β-carotene	0.30 ± 0.07	0.28 ± 0.08	0.29 ± 0.08
Optimal irrigation regime (M-100%) with 50% fertilization	Proximate Composition (% FW)			
	Moisture	93.53 ± 0.44	94.21 ± 0.48	93.92 ± 0.41
	Ash	0.68 ± 0.08	0.70 ± 0.12	0.69 ± 0.09
	Protein	0.70 ± 0.11	0.68 ± 0.09	0.68 ± 0.08
	Fructose	1.34 ± 0.11	1.36 ± 0.15	1.33 ± 0.16
	Glucose	1.43 ± 0.12	1.44 ± 0.21	1.39 ± 0.21
	Mineral Elements (mg/kg FW)			
	P	241 ± 27	256 ± 28	241 ± 23
	K	2574 ± 285	2728 ± 195	2570 ± 258
	Mg	119 ± 14	127 ± 17	123 ± 11
	Organic Acids and Antioxidant Compounds (mg/100 g FW)			
	Ascorbic acid	32.31 ± 0.41	30.17 ± 0.39	30.43 ± 0.38
	Titratable acidity	0.32 ± 0.08	0.32 ± 0.08	0.22 ± 0.08
	Total dyes	4.11 ± 0.15	3.07 ± 0.13	4.19 ± 0.11
	Lycopene	3.37 ± 0.12	3.05 ± 0.11	4.29 ± 0.11
	β-carotene	0.27 ± 0.08	0.26 ± 0.09	0.28 ± 0.08

According to the FAO, the lycopene content ranges from 7 to 13 mg/100 g (Rath et al., 2009). In the present study the content ranges from 3.05 to 5.0 mg/100 g. Table 2 shows that the content of lycopene is higher at a 50% of irrigation rate. The water deficit has a positive influence on the content of lycopene as well as Dumas et al., 2003.

The β-carotene content determines the orange color of the tomato fruit. The good combination of the two components (lycopene and β-carotene) with an antioxidant effect determines hybrids of tomatoes such as hybrids of high biological value (Pevicharova et al., 2012). In the present study the content β-carotene ranges from 0.26 to 0.35 mg/100 g. The content of β-carotene is higher at a 50% of irrigation rate. Our results are in line with those of Mozafar, 1994. β-carotene content in fruit increases with increasing levels of K, Mg, Mn, B, Cu and Zn. Phosphorus may also increase the fruit concentration of phytochemicals such as ascorbic acid, flavonoids and lycopene (Dorais et al., 2008).

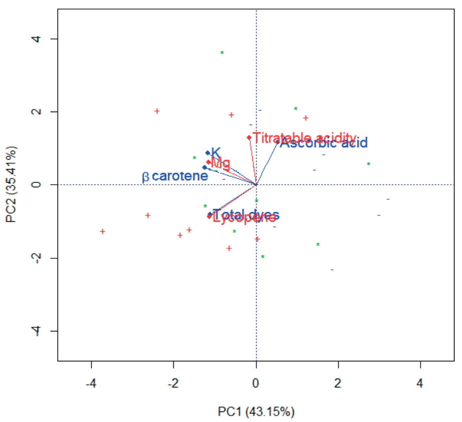

Figure 3. Standard HJ-biplot

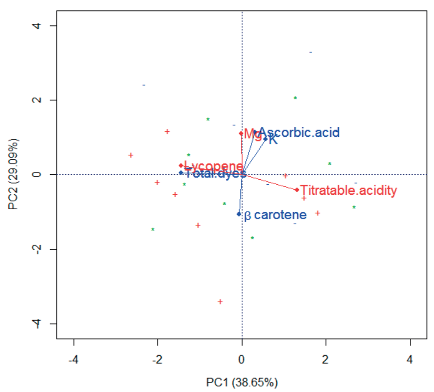

Figure 4. Compositional HJ-biplot of the chemical composition data

It is important to emphasize how the problem of spurious correlations is resolved with compositional statistics. By way of illustration, a standard HJ-biplot (Figure 3) and a compositional HJ-biplot (Figure 4) were constructed for K, Mg, Ascorbic acid, Titratable acidity, Total dyes, Lycopene and β-carotene. The HJ-biplot was coustructed with data expressed as percentages, and the compositional HJ-biplot with centred log-ratios, in both case with a double-centring transformation of the matrix. The angles between the vectors approximate the correlations between variables in such a way that small acute angles are associated with variables that are strongly positively correlated, obtuse angles close to 180° with variables that are strongly negatively correlated, and right angles with uncorrelated variables. One observes different correlations between the variables, and different percentages of the variance explained in each biplot, with an overall fit of 78.56% in the HJ-biplot and 67.74% in the compositional HJ-biplot.

Regarding compositional HJ-biplot (Figure 4), samples near the centroid have values similar to the geometric mean for each component, while those far from the centre can be diagnosed as having a large relative variation with respect to the geometric mean for the clr-transformed chemical variables that the points are near. In the case of the rays, Lycopene and Total dyes has the greatest variation with respect to theirs mean, while the smallest variation corresponds to K. The samples are mainly located near the rays of Lycopene, Total dyes, β carotene and Titratable acidity.

The acute angles for Total dyes/Lycopene and Ascorbic acid/Mg indicate positive correlations, a correlation that is especially strong in the case of Total dyes. This is coherent with the shortness of the Total dyes/Lycopene link. In order to quantify the relationship, the values of the variance given in Table 2 must be considered. For the Total dyes/Lycopene log-ratio, the "variance corresponds to a standard deviation of 0.0067. Since this value is expressed on a logarithmic scale, it must be back-converted to a Euclidean scale, i.e., 0.0398. Thus, an almost linear increase in the lycopene content is due to the Total dyes content of the tomato fruit. However, Ascorbic acid is more strongly correlated with the ascorbic acid than the Mg content, with an estimated standard deviation of 0.01436.

In the case, of β-carotene/Ascorbic acid, the obtuse, close to 180°, angle between their rays indicates a possible strong negative correlation. Right angles mean uncorrelated variables, which here are Titratable acidity/Ascorbic acid and Total dyes/β-carotene.

Table 2. Variation array of functional compounds

	K	Mg	Ascorbic acid	Titratable acidity	Total dyes	Lycopene
Mg	0.0047					
Ascorbic acid	0.0128	0.01436				
Titratable acidity	0.0837	0.0996	0.0936			
Total dyes	0.0306	0.0288	0.0385	0.1619		
Lycopene	0.0303	0.0273	0.0402	0.1742	0.0067	
Beta carotene	0.0377	0.0426	0.0701	0.1256	0.0590	0.0610

CONCLUSIONS

An important indicator of the quality of tomatoes is the content of total dyes. The highest values were found in tomato farmed irrigation regime (50% of irrigation rate and 50% fertilization) where total dyes attained up to 4.95 mg/100 g. Total dyes contents in tomato farmed irrigation regime (100% of irrigation rate and 50% fertilization) declined to 3.07 mg/100 g.

The β-carotene content determines the orange colour of the tomato fruit. The good combination of the two components (lycopene and β-carotene) with an antioxidant effect determines hybrids of tomatoes such as hybrids of high biological value. In the present study the content β-carotene ranges from 0.26 to 0.35 mg/100 g. The content of β-carotene is higher at a 50% of irrigation rate.

Compositional Data Analysis (CoDA) refers to the analysis of data, which have been defined as random vectors with strictly positive components whose sum is constant.

Using this novel statistical technique, linear relationships or links between the variables were identified that are different from those provided by the usual correlation coefficient studies.

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