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EVALUATION OF *SOLANUM LYCOPERSICUM* L. AS A SOURCE OF SECONDARY METABOLITES

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SUMMARY

Tomatoes are low in calories, however, they provide the precious content of vitamins, minerals, organic acids, carbohydrates, and, especially, carotenoids (β-carotene and lycopene), as essential requirements for the normal functioning of the human body. The latest study aimed to quantify the various compounds that exhibit antioxidant activities in different tomato cultivars. Tomato landraces with the supreme content of individual chemicals and complex of varied traits can serve as genetic sources in breeding for obtaining the tomato genotypes with the highest content of carotenoids, ascorbic acid, phenols, dry matter, and soluble sugars. Comparative analysis of tomato cultivars showed that to obtain genetic material with an enhanced content of secondary metabolites, the following tomato cultivars are recommendable for the desired hybridization to make possible cross combinations, i.e., Black Jack, Shirley, Bosare blue, and Christmas Blueberry and can suggest more precisely for high carotenoids content. Tomato cultivars VS169-19 and Paul Robeson can benefit as sources of soluble sugars and antioxidants, such as ascorbic acid and polyphenols.

Keywords: Tomato (*Solanum lycopersicum* L.), vitamins, minerals, carbohydrates, carotenoids, antioxidants, phenolic compounds, ascorbic acid, soluble sugars

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Key findings: The presented study determined the accumulation of secondary metabolites in the tomato fruits, with promising genotypes identified and selected for further probe through a hybridization program. A significant ($P \leq 0.05$) positive correlation between the β-carotene and lycopene content and between ascorbic acid and total phenolic content attained establishment.

INTRODUCTION

For a healthy lifestyle, the importance of diet is increasing in modern society (Ivanova *et al*., 2021). The lack of biologically active substances in our food, like vitamins, antioxidants, and minerals, is one of the vital factors. The biological values of food depend on its components that the human body cannot synthesize. The β-carotene and lycopene are among such components; their intake mainly depends upon their presence in the food (Gins *et al*., 2020). The carotenoid group also has antioxidant and anti-carcinogenic properties, playing a vital role in the human body (Salehi *et al*., 2019).

In the 21st century, there is a high demand for carotenoids. The global production of β-carotene supplements reaches approximately 5,000 to 10,000 tons yearly. Such complements have a broader use as a source of pro-vitamin-A for preventive purposes and as a food coloring in the industrial production of butter, margarine, and pasta. In addition, β-carotene can be a nutritional supplement in the livestock and poultry industries. Tomatoes and their processed byproducts, such as tomato juice, paste, sauce, and ketchup, are essential natural sources of carotenoids for body intake worldwide (Salehi *et al*., 2019).

In the present era, the tomato (*Solanum lycopersicum* L.) is one of the most popular crops due to its valuable nutritional and dietary properties. As a rich source of biologically active components, including those with antioxidant activity, i.e., vitamins, minerals, phenolic compounds (including flavonoids), and dietary fibers and proteins, the tomato also helps effectively fight various types of cancer. In addition, it reduces the risk of hypertension and other cardiovascular diseases (Sharma *et al*., 2021). Therefore, tomato production remains relevant, both domestically and internationally. The tomato crop area grows yearly, and its cultivation technology is improving. Additionally, new industrial technologies for tomato production progressed, further enhancing their profitability.

In 2021, the tomato global production estimates reached 189 million tons (FAOSTAT, 2021), with the major producers including China (67.6 million tons), India (21.2 million tons), Turkey (13.1 million tons), the USA (10.5 million tons), Egypt (6.2 million tons), Spain (4.8 million tons), Mexico (4.1 million tons), Nigeria (3.6 million tons), Iran (3.4 million tons), and Russia (3.1 million tons). In Russia, the tomato's main cultivation areas for industrial uses are Krasnodar, Stavropol, Rostov, and the Lower Volga regions.

Tomato is one of the rich sources of natural antioxidants. The chemical composition of the tomato fruit significantly depends on various factors, including genotype, cultivation methods, growth conditions, storage, and shelf life (Georgaki *et al*., 2023). In determining the level of antioxidant content in tomatoes, one of the most crucial factors is the fruit ripening stage at harvest (Dzhos *et al*., 2023). During maturity, the tomato fruit's exposure to various oxidative stresses consistently leads to considerable variations in the capacity of the antioxidant pool at harvest and during storage (Alenazi *et al*., 2020). Tomato coloration also depends upon the relative concentration of various pigments, i.e., carotenoids, chlorophylls, and flavonoids (Wang *et al*., 2019; Dzhos *et al*., 2023).

Carotenoids are one of the prime groups of biologically active compounds found in tomatoes. Lycopene (Lyc) is a natural carotenoids that give a red color to various fruits and vegetables, such as tomatoes, rosehips, watermelon, and pink grapes. Lycopene efficiently ameliorates cancer insurgences, diabetes mellitus, cardiac complications, oxidative stress-mediated malfunctions, inflammatory events, skin and bone diseases, and hepatic, neural, and reproductive disorders (Imran *et al*., 2020). In fresh tomatoes, the lycopene accumulation has high influences from fertilizers. The increased potassium fertilizer amount also improves lycopene in fresh tomatoes (Serio *et al*., 2007). The second main carotenoids found in tomatoes are β-carotene (bCar), which is also responsible for the orange color. In addition to these two chief carotenoids, the least amount of γ-carotene, δ-carotene, lutein, and other carotenoids can also occur in fresh tomatoes. However, the ratio of carotenoids is not always constant in tomato fruits, and the lycopene and β-carotene concentrations vary during fruit ripening, increasing as the fruit approaches biological ripeness (Dzhos *et al*., 2023).

Ascorbic acid (AsA) has a significant role in maintaining a healthy human body (Salehi *et al*., 2019). A powerful antioxidant, ascorbic acid is vital in redox processes and critical for synthesizing adrenaline and hormones. The AsA inhibits the oxidation of LDL-protein, thereby reducing atherosclerosis. Vitamin C protects the immune system, reduces the severity of allergic reactions, and helps fight against various infections (Chambial *et al*., 2013). Fresh fruits and vegetables became the prime sources of vitamin C for humans since they have lost the ability to synthesize vitamin C due to a deficiency of the enzyme gulonolactone oxidase (Chambial *et al*., 2013). Tomato is also the second prime source of vitamin C after citrus fruits in the human diet due to their frequent consumption in raw form and larger quantities compared with other vegetables, fruits, and spicy herbs (Fenech *et al*., 2019). The vitamin C content in tomato fruits gradually increases as the fruit matures (Dzhos *et al*., 2023).

Polyphenols have considerably helped reduce oxidative stress and, thus, protect the human body from various diseases, including cardiovascular ailments and cancer (Briguglio *et al*., 2020; Bianchi *et al*., 2022). Tomato fruits also contain quercetin, naringenin, rutin, and chlorogenic acids as the chief phenolic compounds. Regarding flavonoids, they are around 10% of the total polyphenols (Cruz-Carrión *et al*., 2022). Past studies also reported that flavonoid content increases during ripening, and flavonoids are more abundant in peel tissues than in the flesh (Adato *et al*., 2009). Sugar content is an essential biochemical indicator in evaluating tomato cultivars because the sugar-acid index determines the taste qualities of the fruits. In tomato fruits, the sugar content always depends upon growing conditions and varietal characteristics. According to past studies, the total sugar content in tomato fruits rarely exceeds 5%–6% (per fresh weight of the fruit) and sometimes drops to 1.5% (Kurina *et al*., 2021).

The dry matter content in tomato fruits also varies to a broader extent (3.7% to 11.3%); however, various studies cited the different ratios (Nour *et al*., 2013; Kurina *et al*., 2021). This data generally refers to tomato culture and does not reflect the variability degree of varietal composition based on the locations and the cultivation techniques affecting the dry matter formation. Therefore, assessing tomatoes by this characteristic is highly beneficial for breeding work.

Given the invaluable role of helpful metabolites for normal physiological processes in the human body, the main problem of modern breeders is the development of new cultivars into production with an increased content of health-promoting compounds in tomato fruits. The breeder's strategy combines the maximum genetic productivity potential, biotic and abiotic stress tolerance, and improved nutritional properties in the cultivar. The promising research aims to study the genetic collection of tomatoes according to the content of secondary metabolites in their fruits.

MATERIALS AND METHODS

The latest research on tomatoes commenced during the summer of 2019–2021 at the Federal Scientific Vegetable Center, Moscow region, Russia (55°39.51′ N, 37°12.23′ E). The area lies at a height of about 150 masl. Soils of the experimental site belong to the soddy-podzolic type, with medium-loamy texture, humus content (2.5%–3.2%), and pH (5.6–5.8). In the complete dry soil, the macro-elements contained were P_2O_5 (20.1–25.0 mg per 100 g) and K_2O (8.1–17.0 mg per 100 g). The research progressed on the different tomato cultivars, with the seeds procured from the a) Genetic Collection of Plant Resources of Federal State Budgetary Scientific Institution Federal Scientific Vegetable Center (FSBSI FSVC), Moscow, Russia, b) Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG SB RAS), Novosibirsk, Russia, and c) Tomato Genetics Resource Center (TGRC), University of California, Davis, USA (Table 1). Agronomic cultivation techniques included generally accepted methods for seedling tomato culture with a planting density of three plants per m^2. All the cultivated tomato cultivars were under the conditions of a film greenhouse with temperatures (22 °C–27 °C), humidity (65%–90%), illumination (15–20 klx), and photosynthetic photon flux density (260–350 $\mu mol/m^2/s$).

The selection of tomatoes occurred at full biological ripeness for biochemical analysis. For lycopene and β-carotene contents estimation of the tomato fruits, adding a 4:6 mixture of acetone: hexane (10 mL) to a gram of tomato sample proceeded in a 15 ml test tube, with the mixture homogenized. Determining the optical density of the polar extract supernatant was at 663, 645, 505, and 453 nm in a glass cuvette (Verdoliva *et al*., 2021; Dzhos *et al*., 2023). The total phenolic content (TPC) determination used the Folin-Ciocalteu assay with some modifications (Dzhos *et al*., 2023). Gallic acid served as the standard, with the TPC expressed as mg gallic acid equivalents (GAE)/g. Fresh samples (one g) bore thorough crushing and homogenization in 10 ml of 80% ethanol. The homogenate, placed in capped test tubes, received heat at 60 °C in a water bath for 1 h. The extract centrifugation was at 6000 g for 15 min, with the collected resulting supernatant used for the estimation of total phenolics. Each sample (20 µl) received 480 µl bi-distilled water and Folin-Ciocalteu reagent (one mL) and incubated at room temperature for three minutes. Then, adding one mL of 7% Na_2CO_3 continued to each mixture. The absorbance measurement was at 765 nm against a blank after 30 min of incubation at room temperature. The dry matter content (DMC) identification had the sample dried to a constant mass (Shafigullin *et al*., 2021).

In measuring the ascorbic acid content, macerating 10 g of the sample used 3% meta-phosphoric acid, with the volume adjusted to 100 mL with meta-phosphoric acid. An aliquot of 10 mL of the extract was taken and titrated with Tillman's reagent (2,6-dichlorophenol indophenol) until a pale pink endpoint was visible, which persisted for 15–20 s (Dzhos *et al*., 2023). The soluble sugar content (%) determination utilized the anthrone colorimetric method (Liu *et al*., 2018; Shafigullin *et al*., 2020). Sugar extraction with

Table 1. Morphological characteristics and yield of the studied tomato cultivars.

Tomato cultivars	Origin	ID	Type of plant	Fruit color	Fruit shape	Fruit weight (g)	Plant yield (kg $plant^{-1}$)
Black Jack	TGRC	BJ	semi-determinate	red-purple	rounded	20-25	1.5-1.9
Bosare blue	CSBG SB RAS	BB	Indeterminate	red-purple	rounded	35-45	2.0-2.7
Christmas Blueberry	TGRC	CB	Indeterminate	red-purple	rounded	30-40	1.7-2.2
Garmonica	FSVC	GA	semi-determinate	red	flat ribbed	80-120	2.8-3.6
Paul Robeson	TGRC	PR	semi-determinate	red	flat-rounded	150-200	3.0-3.8
VS166-19	FSVC	VS166	Indeterminate	pink	rounded	150-200	3.3-4.2
Shirley	TGRC	SH	Indeterminate	red	flat-rounded	60-70	2.4-3.1
VS167-19	FSVC	VS167	Indeterminate	yellow	rounded	25-30	1.8-2.3
VS169-19	FSVC	VS169	Indeterminate	yellow-orange	plum-shaped	15-20	1.4-1.7

bi-distilled water ensued in a water bath at 80 °C for 30 min, followed by acid hydrolysis of sucrose. After adding sulfuric acid and anthrone, the absorbance was at 625 nm. The D-glucose served as a standard, with all the data converted on dry weight (DW).

The experiment had a randomized complete block design (RCBD) with three replications each year, corresponding to plots. All the recorded data analysis used R and R-Studio. Pearson's correlation coefficient aided the estimation of correlation among various traits. Means comparison and separation employed the Least Significant Difference (LSD) test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Like other crop plants, the chemical composition of tomatoes is also considerably susceptible to the variability of the soil, agrotechnics, environmental conditions, and varietal characteristics. In the crop seasons of 2019–2021, a biochemical assessment of the nutritional values of the tomato fruits. i.e., the content of β-carotene, lycopene, ascorbic acid, dry matter, total phenolic, and soluble sugar content (SSC) proceeded at the Federal Scientific Vegetable Center, Russia. Data on the biochemical compounds is available in Table 2. Dry matter content ranged from 4.3% (tomato cultivar, Bosare blue) to 7.7% (cultivar VS169-19). The pertinent results were closely analogous to the values (3.72%–8.88%) observed in the fruits of cultivated tomatoes in past studies (Kurina *et al*., 2021; Chaerunnisa *et al*., 2024).

The dry matter of the tomato fruits mainly consisted of carbohydrates, specifically soluble sugars. The soluble sugar content in the fruits ranged around 54%–80% DW and 2.8%–6.0% FW. These results also agree with the findings of Li *et al*. (2017), who found that SSC in tomatoes measured via the anthrone colorimetric had a range of 4.44%–6.03% FW. The cultivar Paul Robenson presented the highest content of soluble sugars (80% DW), followed by the tomato cultivars VS167-19 and VS169-19 (78% DW).

According to the obtained data, the maximum content of lycopene emerged in the tomato cultivars Bosare blue (2.70 mg/g DW) and Christmas Blueberry (3.24 mg/g DW), and these genotypes also lead in the β-carotene content (1.56-1.57 mg/g DW). In studying 185 tomato accessions, Anjum *et al*. (2020) found that the maximum content of lycopene reached up to 0.232 mg/g FW and β-carotene up to 0.076 mg/g FW. Considering that the average

Table 2. Analysis of the biochemical compounds in tomato cultivar fruits at biological ripeness.

Parameters	Tomato cultivars												LSD_{05}
	Garmonica	VS166-19	VS167-19	Average of Gr1	Paul Robeson	VS169-19	Average of Gr2	Bosare blue	Christmas Blueberry	Black Jack	Shirley	Average of Gr3	
Lycopene (mg/g DW)	0.61	1.77	0.05	0.81	0.53	0.08	0.31	2.70	3.24	1.39	0.93	2.07	0.02
β-carotene (mg/g DW)	0.40	0.86	0.10	0.45	0.36	0.58	0.47	1.56	1.57	1.05	0.63	1.20	0.02
Ascorbic acid (mg/g DW)	2.8	1.6	2.8	2.4	5.8	4.3	5.1	5.3	4.6	6.2	6.5	5.7	0.3
Total phenolic content (mg GAE/g DW)	11.4	11.1	9.7	10.7	15.7	13.9	14.8	12.8	12.3	13.0	15.7	13.5	0.5
Soluble sugar content (% DW)	54	68	78	67	80	78	79	65	61	68	62	64	2
Dry matter content (%)	7.0	6.5	7.5	7.0	7.0	7.7	7.4	4.3	7.2	5.7	5.4	5.7	0.3

dry matter content was 6% then, one gets 3.86 mg/g DW and 1.26 mg/g DW, respectively, and these values align with the present results.

Among the studied tomato samples, the highest accumulation of AsA resulted in the three tomato cultivars Shirley (6.5 mg/g DW), Black Jack (6.2 mg/g DW), and Paul Robeson (5.8 mg/g DW). The current findings were higher than values obtained in tomato fruits from past studies (Anjum *et al*., 2020; Kurina *et al*., 2021). They also noted that in the tomato samples with a high ascorbic acid content, their content reached 0.3 mg/g FW and 5.0 mg/g DW. Therefore, it is noteworthy that the ascorbic acid content of tomatoes has regulations from culture practices and environmental conditions, such as light and higher levels of irradiation, resulting in higher accumulation of AsA (Zushi *et al*., 2020). Hence, one may assume that in the presented study, the environmental factors also influenced and increased the ascorbic acid accumulation.

The presented results further enunciated that total phenolic content had a range of 9.7–15.7 mg GAE/g DW, and the record-breakers in the accumulation of TPC were the tomato cultivars Paul Robeson (15.7 mg GAE/g DW), Shirley (15.7 mg GAE/g DW), and VS169-19 (13.9 mg GAE/g DW). Past studies also revealed the maximum values of the total content of polyphenols (0.456–0.818 mg GAE/g FW) depending on the growing conditions, and for dry weight, the provided values ranged from 7.6 to 13.6 mg GAE/g DW, which were also comparable to a previous data (Rapa *et al*., 2021).

Based on the studied biochemical parameters of the tomato fruits, a dendrogram construction used cluster analysis (Figure 1). Auto-scaling pre-treatment continued on the dataset to exclude the variances related to the different measurement units. The Agnes function in the Cluster package aided for the agglomerative hierarchical clustering with R. In this case, Ward's method gave the maximum value of the agglomeration coefficient. The optimal number of clusters determined employed the Silhouette method of the fviz_nbclust function in the factoextra R package (Figure 2). Thus, three clusters (groups) reached identification. Group 1 consisted of three tomato cultivars, VS167-19, VS166-19, and Garmonica. Group 2 included two cultivars, VS169-19 and Paul Robeson, and the third Group included the four tomato cultivars, Shirley, Black Jack, Christmas Blueberry, and Bosare Blue.

Principal component analysis (PCA) in R helped evaluate the biochemical data and highlight the natural sample grouping. In PCA (Figure 3), the first two principal components (Dim1 and Dim2) accounted for 78% of the total variance. The main contribution to the Dim1 component comes from β-carotene (bCar), lycopene (Lyc), and dry matter content (DMC), and the main contribution to the Dim2 component comes from total phenolic content (TPC) and ascorbic acid (AsA). Variables bCar and Lyc bore grouping, indicating that these parameters have a positive correlation, and similarly, TPC and AsA. Pearson's correlation coefficient indicated significant ($P \leq 0.01$) and significant ($P \leq 0.05$) degree of correlation for bCar and Lyc ($r = 0.94$) and TPC and AsA ($r = 0.80$) (Figure 4).

In the PCA scores plot (Figure 5), the Group 1 tomato accession blue circle plots in the lower-left quadrant, with the Group 2 accession yellow triangle in the upper-left quadrant. The Group 3 accession red square appeared in the right part of the diagram. The first principal component (Dim1) provided an apparent separation of Group 3 from Group 1 and Group 2 since Group 3 accessions plot more to the right than the tomato accessions of Group 1 and Group 2. The average values of bCar and Lyc were higher, and DMC was lower in Group 3 than the same characteristics in Group 1 and Group 2. According to Dim2, there was a clear separation of Group 1 from Group 2. The tomato accessions of Group 2 plotted higher than Group 1 accessions; therefore, the average values of TPC and AsA were higher in Group 2 than in Group 1 (Table 2).

Thus, a group with a higher content of pigments (Lyc, bCar) but with a slightly lower content of dry matter stood out, and this was Group 3. Samples of Group 2 have a high content of phenolic compounds and ascorbic acid. Moreover, part of Group 3, located in the

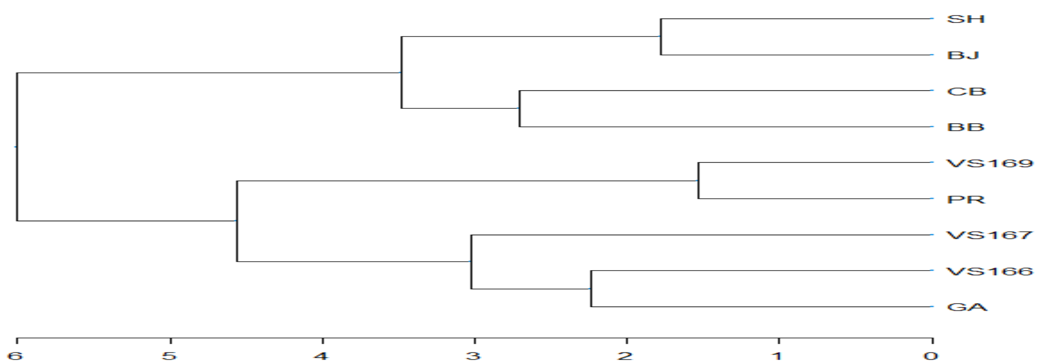

Figure 1. Dendrogram of cluster analysis using the Ward process of biochemical indicators of tomato fruits at biological ripeness.

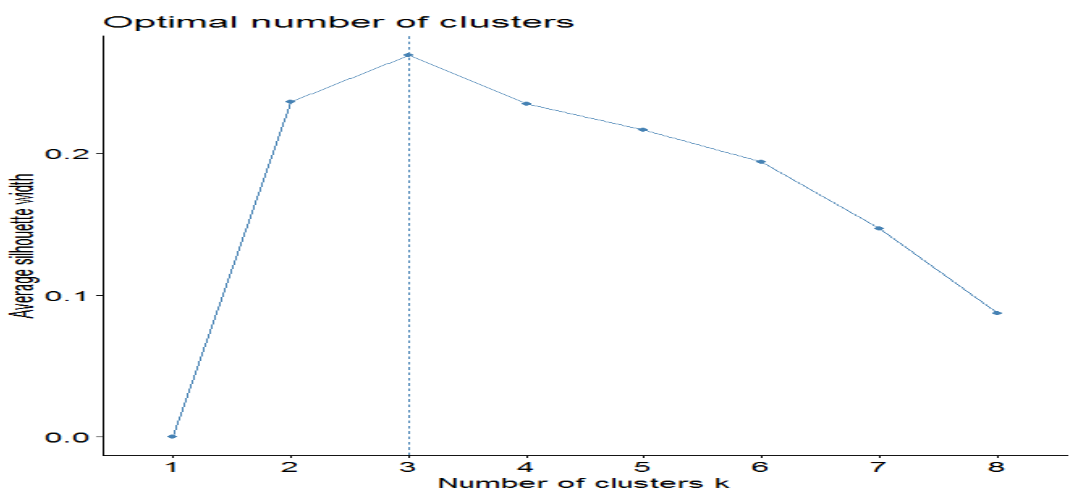

Figure 2. Optimization number of clusters by average silhouette method.

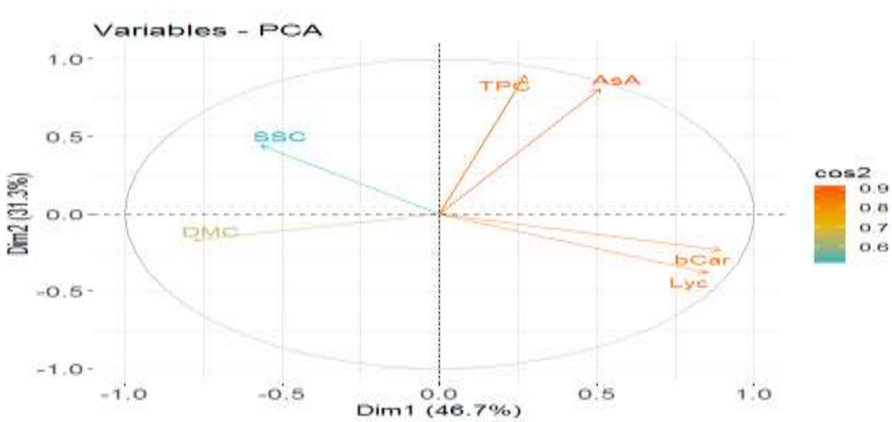

Figure 3. Variable correlation plot.

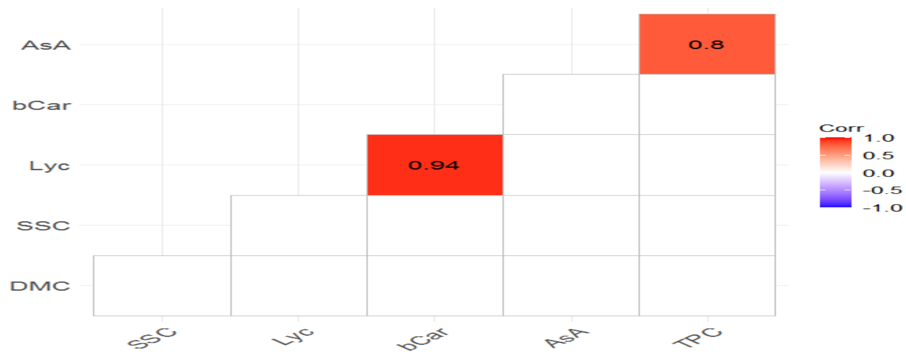

Figure 4. Pearson's correlation coefficients for biochemical indicators of tomato fruits at biological ripeness (Significant at $P \leq 0.05$).

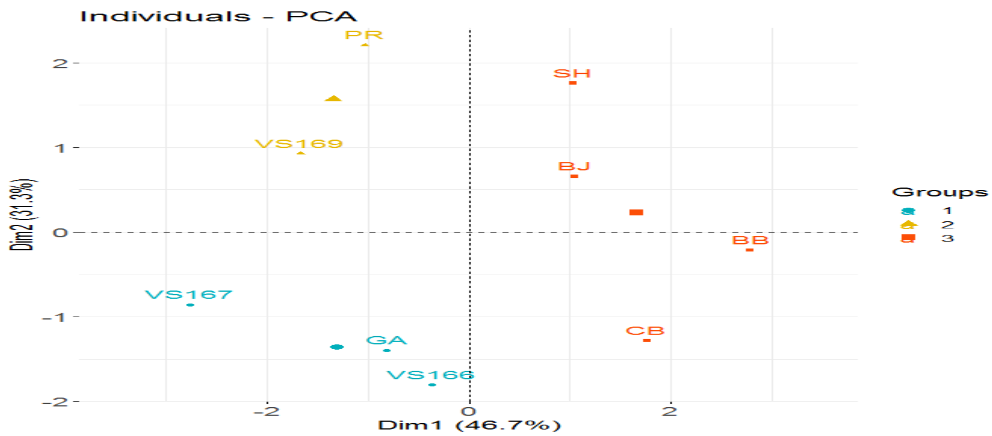

Figure 5. Clusters of tomato cultivars on PCA biplots.

upper-right quadrant, combines a high content of pigments, ascorbic acid, and polyphenols (Figure 5). It will be valuable to use both biochemical characteristics and accessions to broaden the base of the tomato-breeding program in the future.

CONCLUSIONS

Based on the presented results, the tomato cultivars Shirley and Black Jack were remarkable to recommend for obtaining genetic material with the most potential for antioxidant content. The high antioxidant status of these two tomato genotypes is explainable based on their high phenolics, carotenoids, and ascorbic acid contents. Cultivars Christmas Blueberry and Bosare blue deserve attention as a source of the highest pigment content. The tomato cultivars in Group 2 (VS169-19 and Paul Robeson) were also prominent due to the increased content of ascorbic acid, phenols, soluble sugars, and dry matter.

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