Genetic diversity and selection of heirloom tomato accessions based on the physical and biochemical fruit-related traits

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ABSTRACT: Heirloom tomatoes are open-pollinated varieties bearing a wide diversity of colors and shapes that may be used by breeders aiming to improve physical and biochemical fruit traits. Hence, in this work heirloom tomato accessions were characterized, gathering information to genetic breeding programs focusing on human food. For that, 67 heirloom tomato accessions were evaluated for fruit size, fruit mass, fruit volume, color, vitamin C, titratable acidity, soluble solids content, phenolic compounds content, total flavonoid content, and antioxidant activity. The experiment was conducted in a randomized complete block design with three repetitions. Linear mixed model, Pearson's correlation and hierarchical clustering were applied to data. Five groups were formed by Ward's clustering method. The accession UEL 300 constituted group A, which had the greatest mass and volume fruit. Eight accessions formed group B and showed mostly yellow fruits. Group C was comprised of 13 accessions, which had the highest levels of carotenoids, vitamin C, and antioxidant activity. Thirty-three accessions that constituted group D did not stand out for any of the attributes, while 12 accessions into group E had the highest content of phenolic compounds and flavonoids, along with the highest ratio of soluble solids and acidity. Five accessions in this collection (UEL 296, UEL 146, UEL 238, UEL 231, and UEL 217) stood out for their biochemical traits. The wide diversity for physical and biochemical fruit traits can be explored in tomato breeding programs, seeking to develop new cultivars, and strengthening family farming.

Key words: *Solanum lycopersicum* L., gene bank, tomato breeding, post-harvest quality, horticulture.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a vegetable of great importance for human consumption (Menda et al. 2013, Rothan et al. 2019). The fruit is an excellent source of nutrients and bioactive antioxidant compounds that are essential for human health, including minerals, vitamins C and E, carotenoids, organic acids, and phenolic and flavonoid compounds (Chaudhary et al. 2018).

There is a growing search to improve the world population's life quality. These efforts include acquiring healthy habits such as a diet rich in fruits and vegetables. Thus, the availability of tomatoes enriched with nutritional factors and sensory characteristics desirable to consumers is especially interesting for commercialization, which can increase the producer's profitability by adding more value to the final product (Rocha et al. 2013a, Rocha et al. 2013b, Kyriacou and Rouphael 2018).

The narrow genetic base of modern tomato cultivars has limited the genetic gain for several attributes, such as yield, tolerance to biotic and abiotic stresses, and nutritional and sensory quality of the fruits (Tieman et al. 2017, Gao et al. 2019). The exploration of the genetic variability of accessions from a germplasm bank consists of a promising strategy to increase desirable agronomic, nutritional, and sensory traits (Patil et al. 2014). Therefore, the use of heirloom tomatoes, open-pollinated varieties which have been preserved by family farmers for generations, has drawn the interest of breeders focusing on developing new cultivars that meet consumer expectations (Joseph et al. 2017, Lázaro 2018).

In Europe and the United States of America, heirloom tomatoes are frequently sold in the vegetable market (Flores et al. 2017, Joseph et al. 2017, Fresh Trends 2020) and characterized by sweeter and more succulent fruits. They also display an exuberant appearance with colors and formats typically different from the fruits of cultivars currently marketed in Brazil (Barrett et al. 2012). According to the magazine *The Packer* (Fresh Trends 2020), this group of tomatoes represents 8% of consumer preference in the United States of America, indicating that there is a niche market that has been explored – a trend that may influence the Brazilian market.

Heirloom tomatoes may be highly valuable to tomato breeding programs for being sources of useful genes to expand the genetic base of modern cultivars (Dwivedi et al. 2019). In this study, 67 accessions of heirloom tomatoes evaluated belong to germplasm bank of Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil. Each accession was characterized based on physical and biochemical fruit traits and selected the promising genotype, aiming to contribute with tomato breeding programs focusing on human food, besides to strengthen family farming.

MATERIAL AND METHODS

Plant material

From a collection in a germplasm bank of UEL, 67 accessions of heirloom tomatoes were characterized and evaluated in this study. The experiment was carried out from January 2019 to April 2020 under greenhouse conditions in the experimental area at UEL (23°19'44" S, 51°12'11" W, 592 m). The experiment was conducted in a randomized complete block design with three repetitions and two plants per plot (pot with two plants). The plants were conducted with two stems and cultivated in 8-L pots containing organomineral substrate (Plantmax HT®). Recommended tomato cultivation practices were used, and fertirrigation was performed with Hoagland and Arnon's (1950) nutrient solution. The fruits were mature harvested and from the bunches in the middle third of plants to obtain a representative size. Then, the samples (a mixture of fruits) were stored at 8-10°C until further use.

Physical characterization

The mean mass of ten fruits (M, in g) was measured on a semi-analytical balance, while the volume (V, in cm^3) was measured in volumetric test tubes according to the water displaced by the immersion of the fruits. The color of the fruits was characterized by luminosity (L*), hue angle or hue (h*), and chroma or saturation (C*), using a colorimeter (Minolta Co., Japan, model CR-13) with the standard illuminant D65.

Biochemical characterization

The levels of soluble solids (SS, in °Brix) were obtained using a portable digital refractometer (PAL-1, Atago®). The titratable acidity (TA, in % of citric acid) was quantified by the Association of Official Agricultural Chemists (AOAC) titration method 942.15 (AOAC 2000). The vitamin C content (VitC, in mg of ascorbic acid·100 g^{-1}) was measured using the AOAC titration method (AOAC 1984) modified by Benassi and Antunes (1988). The extraction of beta-carotene (Beta, in mg·kg^{-1}) and lycopene (Lyco, in mg·kg^{-1}) was adapted from Adalid et al. (2010), modifying the extracting solution to ethanol and hexane (3:2, v/v). The carotenoids were quantified according to Rodriguez-Amaya (2001) and Rodriguez-Amaya and Kimura (2004),

and the data were obtained upon reading with a spectrophotometer (Genesys 10, Thermo) at 450 and 479 nm for Beta and Lyco, respectively.

The extraction of total phenolic content (TPC, mg of Gallic acid equivalents per 100 g of fresh mass), total flavonoid content (TFC, mg of Quercetin equivalents per 100 g of fresh mass), and antioxidant activity (DPPH, % of free radical scavenging) were performed according to Vázquez et al. (2008). The quantification of TPC was based on Swain and Hillis (1959), in which Gallic acid was used as a standard compound, ranging from 10 to 100 mg·L^{-1} (r = 0.9960). The quantification of TFC was based on Gurnani et al. (2016), with Quercetin as the standard, ranging from 50 to 500 mg·L^{-1} (r = 0,9942). The antioxidant activity was measured using the 2,2-Diphenyl-1-picryl-hydrazyl (DPPH·) free radical method according to Brand-Williams et al. (1995). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the analytical standard, ranging from 0.20 to 1.00 mmol·L^{-1} (r = 0.9992).

Data analysis

Linear mixed model to estimate predicted genotypic values

To estimate the predicted genotypic values for each trait that facilitates the selection of promising genotypes from a germplasm bank based on diversity, a linear mixed model was applied. Hence, the data were analyzed based on restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP) using the Selegen-REML/BLUP software (Resende 2016). The predicted genotypic means were calculated after verifying data normality and homogeneity by the Shapiro-Wilk and Hartley's tests ($p<0.05$), respectively. Deviance analysis (ANADEV) was performed based on the following statistical model (Eq. 1):

$$y = Xu + Zg + e \qquad (1)$$

In which: y: the data vector; u: the scale for the general mean (fixed effect); g: the genotypic effect vector (random effect); e: the vector of errors or residues (random effect); X and Z: the incidence matrices for u and g, respectively.

The significance of the genotypic effect from the ANADEV was verified by the likelihood ratio test at 5% of probability. All analyses were performed using the predicted genotypic values (BLUP values), and their distribution was presented in boxplots.

Correlation and multivariate analysis

The correlations among the evaluated traits were verified by Pearson's correlation coefficients ($p<0.05$), using the R software (https://www.r-project.org/) with the 'corrplot' package (Wei et al. 2017). Ward's hierarchical clustering was performed using the standardized Euclidean distance, with the 'ape' package (Paradis and Schliep 2019). The selection index of Mulamba and Mock (1978) was used to select the five best genotypes (selection intensity of ~8%) based only on the biochemical characteristics (TPC, TFC, DPPH, Beta, Lyco, TA, VitC, and SS).

RESULTS

Deviance analysis

The deviance analysis showed significant effects ($p<0.05$) among accessions for all traits (Table 1). The contents of phenolic compounds and total flavonoids ranged from 22.01 to 54.34 and 21.07 to 77.21 mg·100 g^{-1}, respectively. The antioxidant activity values based on free radical scavenging ranged from 67.21 to 92.45%, and carotenoids varied from 30.73 to 104.59 mg·100 g^{-1} for Beta, and 27.86 to 122.75 mg·100 g^{-1} for Lyco. The highest and lowest acidity was 0.77 and 0.39%,

while the minimum and maximum levels of soluble solids were 5.68 and 9.39 °Brix and 27.63 to 84.3 mg·100 g⁻¹ for VitC. Among the accessions, mass and volume ranged from 11.86 to 77.56 g and 11.33 to 70.28 cm³, respectively, and the color varied from 34 to 48.85 for luminosity, 25.87 to 51.03 for saturation (chroma), and 51.15 to 96.88 for hue.

As it can be seen in the box-chart, the antioxidant activity, titratable acidity, and hue did not present discrepant averages among the accessions of the collection (Fig. 1). On the other hand, mass, volume, luminosity, and chroma showed the greatest number of accessions with discrepant averages.

Table 1. Genetic parameters for physical and biochemical traits of 67 heirloom tomato accessions.

Traits	Deviance	Maximum	Minimum	Mean
TPC	**	54.34	22.02	30.47
TFC	**	77.21	21.08	33.43
DPPH	**	92.45	67.21	84.25
Beta	**	104.59	30.73	53.58
Lyco	**	122.75	27.86	50.63
TA	**	0.77	0.39	0.55
SS	**	9.40	5.65	6.49
VitC	**	84.30	27.63	42.56
M	**	77.56	11.86	21.18
V	**	70.28	11.34	19.10
Luminosity	**	48.85	34.00	37.19
Chroma	**	51.03	25.88	29.87
Hue angle	**	96.88	51.15	66.98

**Significant at 1% of probability by likelihood ratio test, respectively; TPC: total phenolic content (mg·100 g⁻¹); TFC: total flavonoid content (mg·100 g⁻¹); DPPH: antioxidant activity by DPPH assay (% free radical scavenging); Beta: beta-carotene content (mg·kg⁻¹); Lyco: lycopene content (mg kg⁻¹); TA: titratable acidity (% citric acid); SS: soluble solids content (°Brix); VitC: vitamin C (mg·100 g⁻¹); M: mass (g); V: volume (cm³).
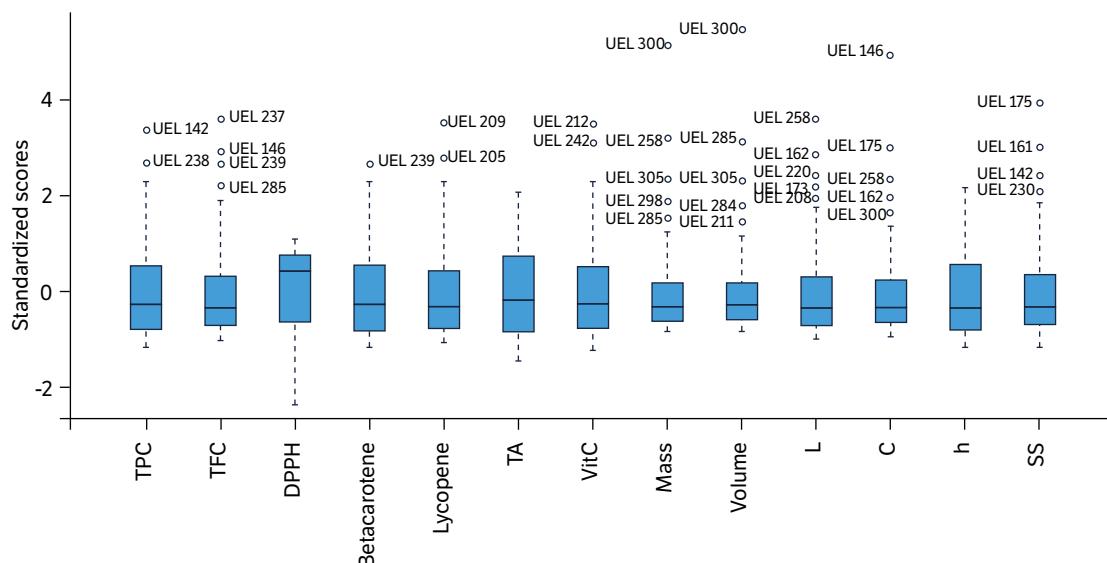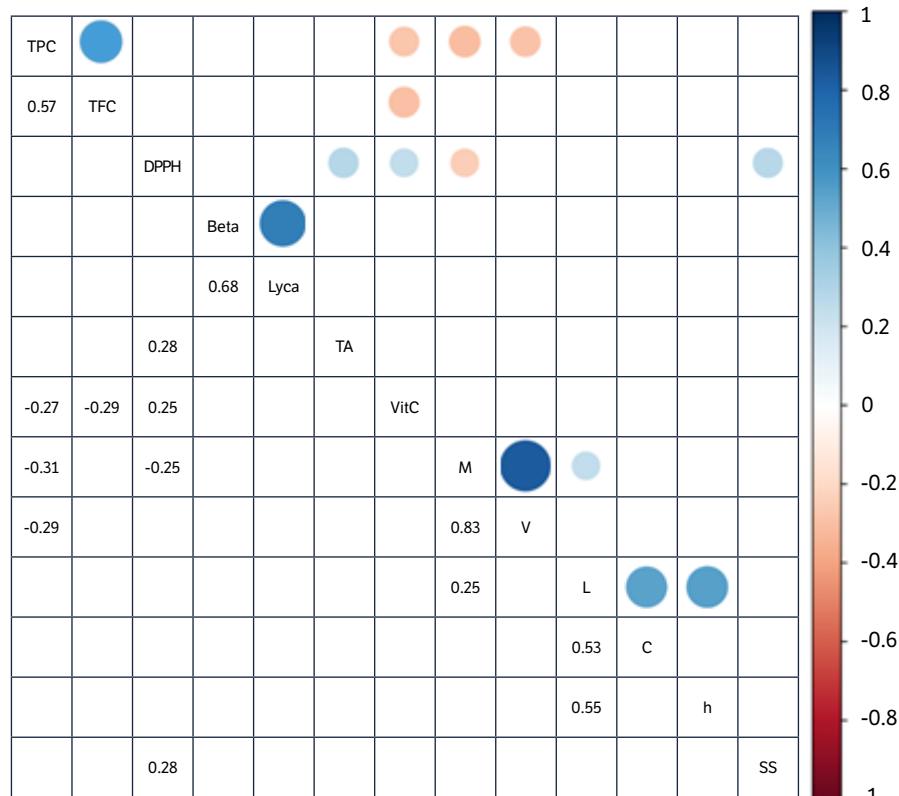

UEL: Universidade Estadual de Londrina; TPC: total phenolic content (mg·100 g⁻¹); TFC: total flavonoid content (mg·100 g⁻¹); DPPH: antioxidant activity by DPPH assay (% free radical scavenging); TA: titratable acidity (% citric acid); VitC: vitamin C (mg·100 g⁻¹), L: luminosity; C: chroma; h: hue angle; SS: soluble solids (°Brix).
Figure 1. Distribution of the estimated averages in boxplot regarding the physical and biochemical attributes of the 67 heirloom tomato accessions.

Correlation

The Pearson's correlation analysis showed a positive correlation between luminosity and chroma ($r = 0.53$), as well as for luminosity and hue ($r = 0.55$) (Fig. 2). Furthermore, positive correlations were observed between Beta and Lyco ($r = 0.68$), mass and luminosity ($r = 0.25$), and DPPH and VitC ($r = 0.25$), whereas mass and TPC and mass and DPPH were negatively correlated ($r = -0.31$ and -0.25, respectively). SS and TA were positively correlated with DPPH ($r = 0.28$ for both).

TPC: total phenolic content (mg·100 g⁻¹); TFC: total flavonoid content (mg·100 g⁻¹); DPPH: antioxidant activity by DPPH assay (% free radical scavenging); Beta: beta-carotene content (mg·100 g⁻¹); Lyco: lycopene content (mg·100 g⁻¹); TA: titratable acidity (% citric acid); VitC: vitamin C (mg·100 g⁻¹); M: mass (g); V: volume (cm³); L: luminosity; C: chroma; h: hue angle; SS: soluble solids (ºBrix).
**Figure 2.** Diagram of Pearson's linear correlation matrix among physical and biochemical traits, indicating significant correlation by the t-test ($P<0.05$). Positive or direct correlations are displayed in blue and negative or inverse correlations are shown in red.

Multivariate analysis

Ward's cluster analysis using the Euclidean distance formed five distinct groups (Fig. 3):
- Group A consisted of only one accession, UEL 300, showing greater mass and volume;
- Group B was represented by eight accessions, mostly with yellow fruits;
- Group C was comprised by 13 accessions, in which bright red fruits predominate;
- Group D was composed of dark red or brown fruits, with 33 accessions;
- Group E was composed of dark red or brown fruits, with 12 accessions;

The box-chart revealed that the accession UEL 300 (Group A) had greater mass and volume, a lower content of phenolics and flavonoids, and less antioxidant activity (Fig. 4). Cluster B accesses stood out for the highest values of h and L, typical traits of light-yellow fruits. In group C, the accessions had greater antioxidant activity, levels of Beta and Lyco, and concentration of VitC. Fruits of group D, which included the largest number of accessions, did not stand out for any of the traits evaluated, only presenting intermediate results. Finally, group E genotypes had a high content of phenolics, flavonoids, and soluble
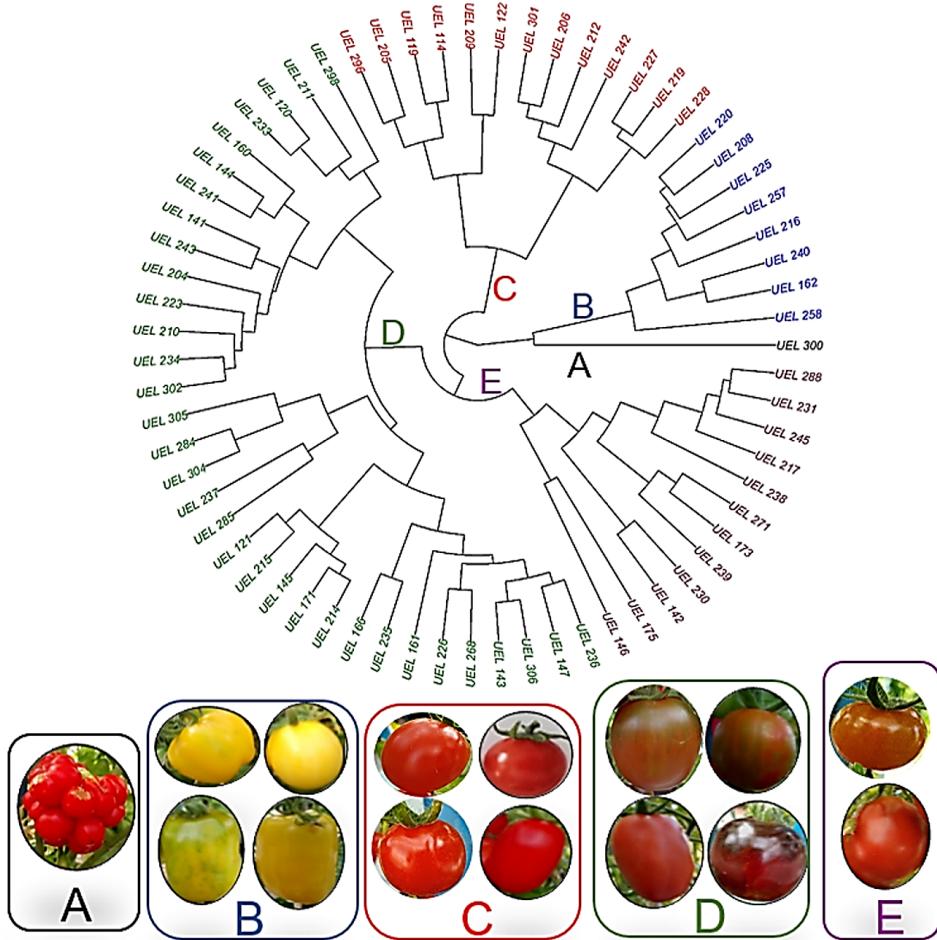
UEL: Universidade Estadual de Londrina.
Figure 3. Circular hierarchical clustering of 67 heirloom tomato accessions.

solids and presented less acidity, resulting in higher values of correlation between SS and TA and suggesting that their fruits may be sweeter and perceived as more interesting for the consumers due to higher sensorial quality.

The genotypes UEL 296, UEL 146, UEL 238, UEL 231, and UEL 217 were considered promising (Fig. 5), using the selection index of the rank sum, proposed by Mulamba and Mock (1978), considering only the biochemical attributes evaluated. It was noticed that only the first accession mentioned belonged to group C, while the others took part in Group E, indicating that these two groups had accessions with greater nutritional potential, and better palatability.

DISCUSSION

The present study found a wide genetic diversity among the accessions of heirloom tomatoes in the evaluated collection, which has also been found in other germplasm banks around the world (Cortés-Olmos et al. 2014, Figàs et al. 2015, Bhandari et al. 2016). This work confirms that the characterization and evaluation of germplasms for physical and biochemical characteristics represent a fantastic tool for breeding programs focusing on species cultivated for human consumption since the genetic variability available in these banks is essential to develop cultivars with greater nutritional quality and sensory acceptance.

It is well known that fruits with low hue values (around 0°) imply red fruits, while fruits with h* values close to 90° are yellow. The brightness ranges from 0 to 100 from dark to light, respectively. The chroma indicates how opaque the color is
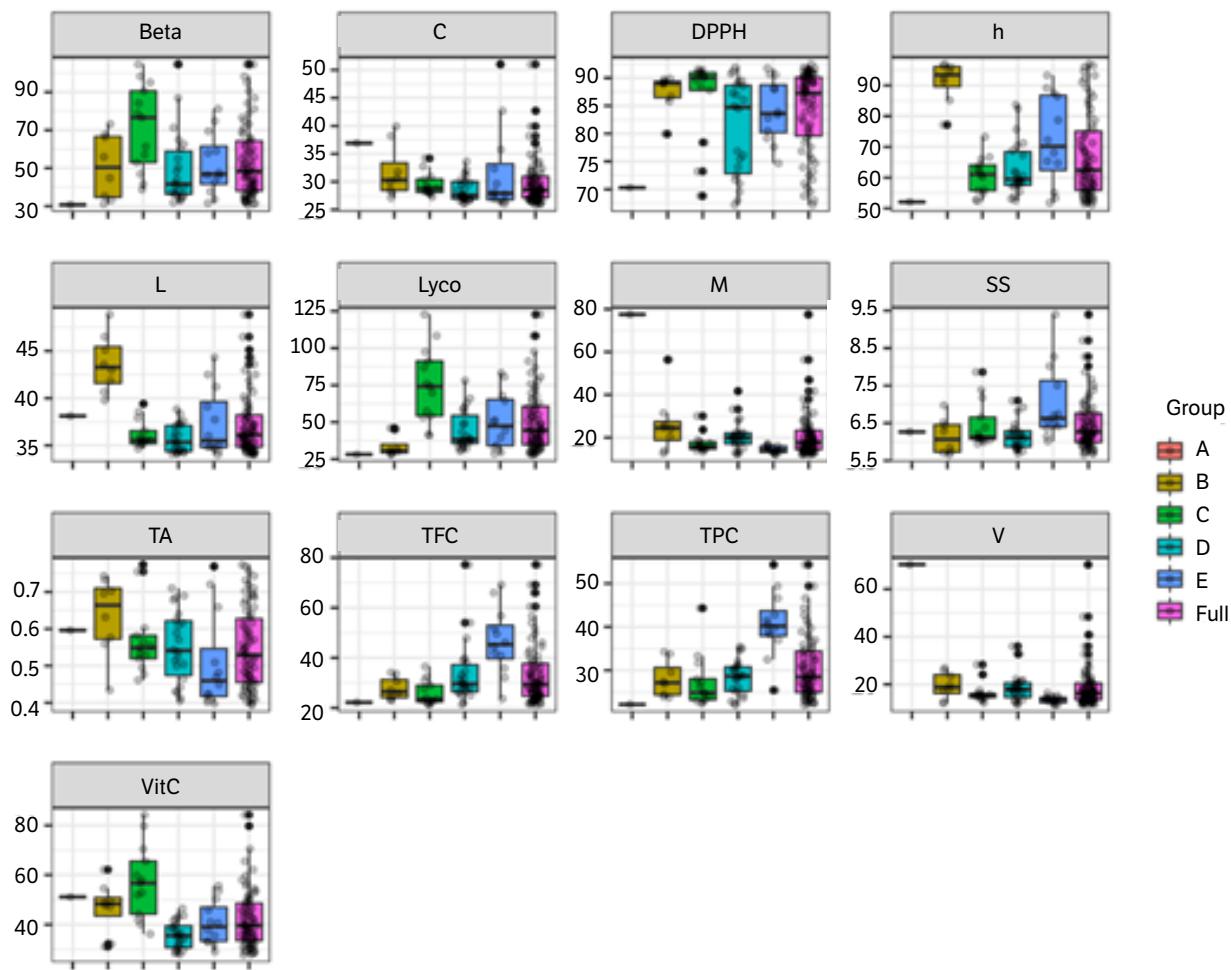
Beta: beta-carotene content (mg·100 g⁻¹); C: chroma; DPPH: antioxidant activity by DPPH assay (% free radical scavenging); h: hue angle; L: luminosity; Lyco: lycopene content (mg·100 g⁻¹); M: mass (g); SS: soluble solids (°Brix); TA: titratable acidity (% citric acid); TFC: total flavonoid content (mg·100 g⁻¹); TPC: total phenolic content (mg·100 g⁻¹); V: volume (cm³); VitC: vitamin C (mg·100 g⁻¹).

Figure 4. Distribution of the standardized means of 67 accessions in a boxplot regarding physical and biochemical attributes based on the five groups formed in the hierarchical cluster analysis.
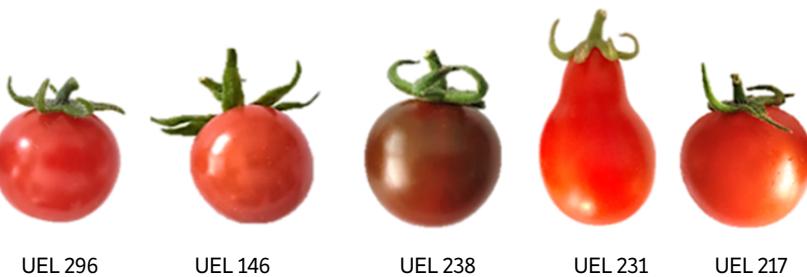
UEL: Universidade Estadual de Londrina.

Figure 5. Five genotypes of heirloom tomatoes chosen by of Mulamba and Mock's (1978) selection index based on the biochemical attributes of the fruits.

(values close to 0), highly influenced by the grayscale, or bright (values far from 0), with greater intensity of the pure color and far from the grayscale (López Camelo and Gómez 2004, Saad et al. 2016). The wide range detected for these parameters indicates that there are genotypes in this collection with epicarp colors that vary from dark and bright red to light and opaque greenish yellow, with the presence of orange fruits.

In general, the red color of the fruit correlates to low hue values and high levels of Lyco (Jarquín-Enríquez et al. 2013). In this study, the non-significant correlation between the color and the Lyco content may be since the color was measured in the epicarp, while the Lyco was quantified in the whole fruit. Thus, even though a strong positive correlation between color and Lyco content in tomatoes was expected, as already reported in Srivastava and Srivastava (2015) and Goisser et al. (2020) works, the type of measurements may explain the underestimation of correlation between these traits. Nevertheless, results similar to the ones of the present study were reported by Lázaro (2018), who also found no significant correlation between the two attributes. Therefore, the selection of heirloom tomato genotypes by color does not directly imply the Lyco content in the fruits, mainly because many heirloom tomatoes have a mixture of colors in the epicarp, justifying the importance of measuring both. It is noteworthy that the color of tomatoes is one of the most important factors for commercialization, especially in the consumer's purchase decision, which often chooses to buy red fruits (Oltman et al. 2014, Adegbola et al. 2019).

In the study by Cortés-Olmos et al. (2014), lower levels of Beta were observed compared to Lyco. In addition, a positive correlation between Beta and Lyco corroborates other studies in the literature (Arthanari and Dhanapalan 2019) since these carotenoids share common metabolic and precursor routes. Consumers prefer high levels of these compounds in fruits (Klee and Giovannoni 2011, Namitha et al. 2011), as they inhibit oxidative cell oxidation, a process that can lead to the incidence of several types of cancer (Kelkel et al. 2011, Friedman 2013, Siddiqui et al. 2015).

Higher levels of phenolic compounds and flavonoids and lower levels of VitC were also found by Vela-Hinojosa et al. (2019), who claim that fruits can synthesize polyphenols by decreasing the concentration of VitC in the cytosol. Also observed in the present study, Barros et al. (2012) found higher levels of phenolic compounds in yellow fruits, around 54.23 $\mu g \cdot g^{-1}$. Antioxidant activity is one of the parameters better related to the food capability of promoting health benefits, representing the ability to inhibit cellular oxidative stress by capturing free radicals and donating electrons to unstable molecules (Bhandari et al. 2016, Salehi et al. 2019). The results of the present study are similar to those found by Bhandari et al. (2016), who observed values between 32.7 to 82.3%.

The positive correlation between VitC and antioxidant activity aggress with other studies, since ascorbic acid has a reducing capacity, as well as carotenoids – phenolic compounds that include flavonoids (Ilahy et al. 2011, Kavitha et al. 2014, Mukherjee et al. 2020). In addition, the levels of VitC in the genotypes of this heirloom tomato collection, with an overall average of 27.63 mg·100 g^{-1}, were higher than those obtained by Bhandari et al. (2016) evaluating germplasms that contained cherry tomatoes and other commercial groups, ranging from 38.68 to 206.71 mg·100 g^{-1}.

The greatest SS/TA ratio is a desirable and important feature for tomato breeding programs, indirectly indicating the most pleasant flavor (Zhu et al. 2018). According to Costa et al. (2019), a high SS/TA determines a mild flavor, which is desired by consumers; on the other hand, low values of this ratio indicate less appreciable flavor. In tomato breeding programs, it is desirable to improve the nutritional and sensory quality of the fruits of commercial cultivars, while it can guarantee a greater financial return to the horticulturist by exploring new consumer market niches, which are increasingly demanding in functional food and beautiful vegetables (Bartoshuk and Klee 2013, Oltman et al. 2014, Patil et al. 2014).

CONCLUSION

The collection of 67 heirloom tomatoes characterized in this study showed a wide diversity for physical and biochemical fruit traits. The five promising accessions selected will allow the development of cultivars producing fruits enriched with bioactive compounds and more palatable, which can be explored in tomato breeding programs focusing on nutritional quality, besides to expand the genetic basis of modern cultivars, and strengthening family farming.

AUTHORS' CONTRIBUTION

Conceptualization: Gonçalves, L. S. A.; **Methodology:** Constantino L. V. and Zeffa, D. M.; **Investigation:** Macera, R., Fukuji, A. S. S.; **Writing – Original Draft:** Shimizu, G. D.; **Writing – Review and Editing:** Koltun, A.; **Funding Acquisition:** Gonçalves, L. S. A; **Resources:** Gonçalves, L. S. A; **Supervision:** Constantino, L. V.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

FUNDING

ACKNOWLEDGMENTS

Not applicable.

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