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Genotyping and phenotyping of grape tomato hybrids aiming at possible genitors for breeding program

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ABSTRACT

Grape and cherry mini tomatoes have been conquering Brazilian consumers due to their sweet taste, practicality of consumption, and versatility in culinary use. Therefore, the present work aimed to characterize ten grape tomato hybrids based on genetic diversity and physical and biochemical traits to provide information for the development of new genotypes focused on the appearance and enrichment of nutritional factors. The evaluated hybrids were Aiko, BRSIG, BRS Zamir, Carolina, Dolcetto, Dulce, Guaraci, Santa West, SCI-023 and Sweet Heaven. The experiment was conducted in 2019 in a greenhouse at the State University of Londrina, using a completely randomized design with four replications and six plants in each replication. The plants were grown following the practices recommended for cultivating tomatoes. The physical and biochemical characterization data were subjected to analysis of variance by the F test ($p<0.05$) and Scott & Knott means cluster test ($p<0.05$), and the molecular characterization data were subjected to analysis of principal coordinates (PCoA) and Bayesian clustering. Aiko presented the highest fruit mass and size as opposed to that observed on SCI-023, while BRSIG was the firmest fruit with the largest pericarp thickness. SCI-023, Dolcetto, and Sweet Heaven had higher sugar content. The soluble solids content ranged from 6.04 to 9.66 °Brix among the genotypes. Hybrids SCI-023, BRSIG, Sweet Heaven, BRS Zamir, and Dulce had the highest antioxidant activity levels. In the phenotypic characterization two groups were formed. The genotypic characterization showed seven groups in which high genetic similarity was verified among BRS Zamir, SCI-023, and Guaraci, as well as between Carolina and Dulce. The greatest dissimilarity was observed between Sweet Heaven and BRSIG. Both characterizations were useful to evaluate the aptitude of these genotypes as outstanding genitors for breeding programs for the Brazilian market.

Keywords: *Solanum lycopersicum*, horticulture, post-harvest, genetic diversity, functional food, mini vegetable.

RESUMO

Genotipagem e fenotipagem de híbridos de tomate grape visando possíveis genitores para programas de melhoramento

Os minitomates tipo grape e cereja vêm conquistando o consumidor brasileiro pelo sabor adocicado, praticidade de consumo e versatilidade no uso culinário. Nesse contexto, o presente trabalho teve como objetivo caracterizar dez híbridos de tomate grape com base na diversidade genética e em descritores físicos e bioquímicos, afim de fornecer subsídios para o desenvolvimento de novas cultivares com foco no surgimento e enriquecimento de fatores nutricionais. Os híbridos avaliados foram Aiko, BRSIG, BRS Zamir, Carolina, Dolcetto, Dulce, Guaraci, Santa West, SCI-023 e Sweet Heaven. O experimento foi conduzido em 2019 na área experimental da Universidade Estadual de Londrina, utilizando o delineamento experimental em blocos completos ao acaso, com quatro repetições e seis plantas por repetição. As plantas foram cultivadas seguindo as práticas recomendadas para o cultivo do tomate. Os dados da caracterização física e bioquímica foram submetidos à análise de variância pelo teste F ($p<0{,}05$) e teste de agrupamento de médias de Scott & Knott ($p<0{,}05$); os dados da caracterização molecular foram submetidos à análise de coordenadas principais (PCoA) e agrupamento Bayesiano. O genótipo Aiko apresentou a maior massa e tamanho de fruto em relação ao observado para SCI-023, enquanto BRSIG apresentou o fruto mais firme e a maior espessura de pericarpo. SCI-023, Dolcetto e Sweet Heaven apresentaram maiores teores de açúcares totais. O teor de sólidos solúveis totais variou de 6,04 a 9,66 °Brix entre os genótipos. Os híbridos SCI-023, BRSIG, Sweet Heaven, BRS Zamir e Dulce apresentaram os maiores níveis de atividade antioxidante. Foram formados dois grupos na caracterização fenotípica. A caracterização genotípica mostrou sete grupos nos quais foi verificada alta similaridade genética entre BRS Zamir, SCI-023 e Guaraci, assim como Carolina e Dulce, enquanto a maior dissimilaridade foi observada entre Sweet Heaven e BRSIG. Ambas as caracterizações foram úteis para avaliar a aptidão desses genótipos como notáveis genitores para programas de melhoramento visando o desenvolvimento de novas cultivares para o mercado brasileiro.

Palavras-chave: *Solanum lycopersicum*, horticultura, pós-colheita, diversidade genética, alimento funcional, mini hortaliça.

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Tomato (*Solanum lycopersicum*) is one of the world's most consumed vegetables, producing more than 180 million tons in 2018 (FAO, 2020). The fruit is an important functional food due to its high nutritional value and source of fibers, sugars, vitamin C and E (Choi *et al.*, 2014). Furthermore, it is rich in bioactive compounds such as phenolic compounds and lycopene. These substances help to promote health by antioxidant capability due to free radical scavenging, preventing cellular oxidative processes (Salehi *et al.*, 2019).

According to FAO (2020), the daily consumption of vegetables in Brazil is 141 g/person, very low when compared to the value recommended by the WHO (World Health Organization), of 400 g/person/day, and lower than the consumption of some poorer countries in Asia and Latin America. Thus, the need to increase the consumption of vegetables such as tomatoes is clear, contributing to improving quality of Brazilians' live.

Miniature vegetables like mini tomatoes, have been attracting the attention of Brazilian consumers due to a sweet flavor, great practicality of consumption, and versatility in culinary use, given the diversity in color and format (Rocha, 2013). In the United States, a survey conducted among consumers by The Packer magazine (2020), indicated that grape tomatoes represent 7% of the consumer's preference and the most frequent forms of consumption are as salad, ingredients in recipes, and as accompaniments.

In addition, this segment has increased profitability for the grower due to the high yield of the crop, coupled to excellent security of marketing because it meets a specific market niche that pays for the added value that can be seven times higher than the value of traditional standard tomato size (Negrisoli *et al.*, 2015).

Despite the growing demand for mini grape tomatoes in Brazil and worldwide, and to supply the segment's expansion, it is essential to expand the options of cultivars that meet the interests of producers and consumers. Thus, genotype characterization is fundamental for identifying genes of interest in breeding programs (Preczenhak *et al.*, 2014). Therefore, this study aimed to characterize grape tomato hybrids based on physical, biochemical, and molecular descriptors to provide information to develop new cultivars focused on appearance and nutritional factors for the Brazilian market.

MATERIAL AND METHODS

Plant material

Ten grape tomato hybrids were evaluated in this study, acquired from the companies Feltrin (cv. Carolina), Agrocinco (cv. Dulce and BRS Zamir), Blue seeds (cv. Guaraci and BRSIG), Sakata (cv. Sweet Heaven, Aiko, and SCI-023), Seminis (cv. Santa West) and Isla (cv. Dolcetto). The experiment was conducted from April to September 2019 in a greenhouse at the Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil (23°17'34"S; 51°10'24"W; 550 m altitude). The completely randomized design was used, evaluating 10 tomato hybrids, in four replications with six plants each. The plants were conducted with one haulm.

The plants were grown following the practices recommended for tomato cultivation, with weekly pruning. Water supply and fertigation with nutrient solution, proposed by Hoagland & Arnon (1950), were made according to the needs at each development stage. The fruits were randomly harvested in the commercial maturity stage from the bunches in the middle third of plants to obtain a representative size.

Physical characterization

Ten fruits per replication from each genotype were measured in mass, length, diameter pericarp thickness, and fruit volume by water displacement after immersion in a graduated cylinder. From the longitudinal section of the fruit, the locular tissue (gel) was removed, and the volume was measured in a graduated cylinder. The color was characterized by luminosity (L*), chroma (C*), and hue angle (h*) using a colorimeter (Minolta Co. Japan, model CR-13) with illuminant standard D_{65}.

Fruit size classification was based on the mass and diameter according to Fernandes *et al.* (2007), such as giant (diameters 35-40 mm and mass >20 g), large (diameter 30-35 mm and mass 15-20 g), medium (diameter 25-30 mm and mass 10-15 g) and small (diameter <25 mm and mass 5-10 g).

The firmness of the fruit was expressed in Newtons (N) by applying the assays of resistance to puncture and resistance to compression using a texturometer (Model TA.XT Plus, Stable Micro System, United Kingdom). For the puncture test, a needle (P/2N) was inserted in the equatorial region of the fruit without removing the exocarp, piercing up to 5 mm. For the compression test, a flat aluminum probe with 35 mm diameter (P/35) was applied in the equatorial region of the fruit until it deformed 5 mm of the surface (Arazuri *et al*., 2007). In both tests, the conditions were: pre-test speed 5 mm/sec, speed test 0.5 mm/sec, post-test 10 mm/sec, distance 5 mm, and trigger force 0.5 N.

Biochemical characterization

Extraction of total phenolic content, total flavonoid content, and antioxidant activity was performed according to Vázquez *et al*. (2008). The quantification of total phenolic content was based on Swain & Hillis (1959), where gallic acid was used as an analytical standard ranging from 10 to 100 mg/L (r= 0,9960) and expressed as mg gallic acid equivalents (GAE) per 100 g. The quantification of total flavonoid was based on Gurnani *et al*. (2016) where Quercetin was used as an analytical standard ranging from 50 to 500 mg/L (r= 0,9942) and reported as mg Quercetin equivalents (QE) per 100 g.

The antioxidant activity by scavenging the 2,2-diphenyl-1-picryl-hydroxyl (DPPH·) radical was performed according to Williams *et al.* (1995). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard ranging from 0.20 to 1.00 mmol/L (r= 0,9992) and the result was expressed as % free radical scavenging.

The soluble solids content (SS) of fruits was measured by a portable digital refractometer (Atago®, with a corrected value for 25°C), expressed as ˚Brix. Titratable acidity (TA) was quantified by titration with 0.01 N sodium hydroxide (NaOH), according to the AOAC method 942.15 (2000), expressed as % citric acid. Vitamin C was quantified according to the standard AOAC method (AOAC, 1984) modified by Benassi & Antunes (1988) and expressed as mg ascorbic acid/100 g. The extraction and quantification of lycopene were adapted from Adalid *et al*. (2010). The extracts were read in the spectrophotometer at 470 nm, and the results were expressed as mg/kg.

The sugar was quantified using the methodology adapted from Constantino *et al*. (2020). Sugars were separated in a DIONEX AS-AP (Thermo Scientific) chromatography system using a DIONEX CaboPac PA10 4×250 mm column and an ICS-5000 electrochemical detector, the results being expressed in g/100 g. The ratio was calculated by total sugar content / titratable acidity.

Molecular characterization

Genomic DNA was isolated from young leaves of five plants per genotype using an automatic DNA extractor (RetchMM400), following the protocol of Doyle & Doyle (1987) with the replacement of cetyltrimethylammonium bromide (CTAB, Sigma-Aldrich, Missouri-USA) by alkyl trimethylammonium bromide (MATAB, Sigma-Aldrich, Missouri-USA) in the extraction buffer. The quality and integrity of the DNA were verified by electrophoresis, and the samples were quantified by spectrophotometer using Nanodrop 2000 / 2000c (Thermo Fisher Scientific, Waltham, MA, USA).

The AFLP (Amplified Fragment Length Polymorphism) technique was performed as described by Vos *et al*. (1995) with modifications. The digestion-binding pattern was visualized on a 1% agarose gel. Once the digestion was confirmed, the amplified product was diluted with ultrapure water four times (v/v). Pre-selective amplification was performed using 3.5 µL of Gotaq® Green Master Mix (Promega, Winchester-USA), 0.58 µL EcoRI + A and MseI + C (4.75 µM) pre-selective primers, 3.0 µL digestion-binding product and 2.92 µL ultrapure water. The result was confirmed on a 1% agarose gel, and the amplified product was diluted eight times (v/v) in ultra-pure water. For the selective amplification, four combinations of primers were chosen to develop a fluorescent multiplex assay, namely: i) EcoRI (FAM) / - ATC / MseI-CTCG, ii) EcoRI (NED) - AGC / MseI-CAA, iii) EcoRI (VIC) - ACT / MseI-GAG, and iv) EcoRI (PET) - AGC / MseI-CAC.

Selective amplification was performed in 10 µL reaction containing: 3.5 µL PCR Master Mix (Gotaq Green Master Mix, Promega, Winchester-USA); 0.54 µL of each MseI (5 µm) and EcoRI (1 µm) primer; 2.5 µL diluted pre-amplification reaction mixture and 2.92 µL ultra-pure water. The fragments were visualized by capillary electrophoresis using the automated genetic analyzer 3500XL (Applied Biosystems, California-USA). Only fragments with a fluorescence intensity >100 RFU (Relative Fluorescence Unit) were analyzed. The results were combined in a binary matrix with the GeneMapper® v software. 4.1 (Applied Biosystems).

Data analysis

The phenotypic characterization data (physical and biochemical) were subjected to analysis of variance by the F test ($p<0.05$) and Scott & Knott (1974) means cluster test ($p<0.05$). In addition to hierarchical clustering using the Euclidean distance and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, with visualization of normalized means on the heatmap.

The data of the genotypic (molecular) characterization were submitted to analysis of principal coordinates (PCoA), hierarchical clustering UPGMA using the Jaccard distance, and Bayesian clustering using the Structure software version 2.3.4 with 100,000 MCMC interactions (Markov Chain Monte Carlo) with 10,000 interactions burn-in, admixture model and correlated allele frequencies. The subgroup values (ΔK) were tested, varying between one and ten, with ten independent interactions for each K value. The ideal K number was determined using the Structure Harvester software version 0.6.92. The other analyzes were performed using the R program using the *easyanova, ade4,* and *pheatmap* packages.

RESULTS AND DISCUSSION

Physical characterization

The analysis of variance showed a significant effect of the genotypes *(*$p<0.05$) for all fruit phenotypic traits. The Aiko genotype presented the highest values of fruit mass, length, diameter, and volume and was classified as giant (Table 1). On the other hand, the genotype SCI-023 was classified as small fruit because it had the lowest levels, and the different genotypes were classified as intermediate (medium to large). Preczenhak *et al*. (2014), evaluating 64 mini tomato accessions, also found a higher number of intermediate-size genotypes. This is an essential feature because mini tomato are usually commercialized in packaging, and the consumer prefers intermediate and standardized fruits about color, shape, and size (Finzi *et al.*, 2017).

Applying the fruit firmness assay, genotypes BRSIG and Santa West were more resistant to puncture (penetration), with 1.35 to 1.19 N, respectively. At the same time, BRSIG and Sweet Heaven were more resistant to compression (surface deformation), with 28.66 and 28.54 N, respectively (Table 1). This important data allows us to relate the puncture with the bite by the canine teeth, in which there is disruption of the exocarp, followed by penetration into the mesocarp. In turn, the compression test is related to the action of molar teeth that press the fruit on the bite and to the act of tightening the fruit by consumers (Lucas *et al*. 2004). Constantino *et al*. (2021), evaluating BRS Iracema, BRS Zamir, Irai, Sweet Heaven, and UEL 238 mini tomato genotypes, showed that the puncture resistance ranged from 0.64 to 1.39 N, from 7.21 to 26.03 N, where the

Iraí and Sweet Heaven genotypes were the firmest fruits.

Genotypes Dolcetto and BRS Zamir obtained the lowest firmness values for compression and puncture, respectively (Table 1). Brashlyanova *et al.* (2014) analyzed the firmness of eight tomato cultivars at different storage temperatures, two of which being mini tomato, and obtained results similar to the ones of the present study. The authors found that mini tomatoes showed less variation in firmness throughout refrigerated storage compared to conventionally sized fruits.

It is also essential to highlight that cultivars Guaraci and Aiko have a firmer exocarp but a less resistant mesocarp, which may be undesirable by the consumer since more resistant peel may persist in the mouth even after chewing.

In addition to showing resistance to puncture and compression, the genotype BRSIG has greater pericarp thickness of fruits, promoting greater firmness, less water loss, greater physical barrier to pathogen entry, and greater resistance to postharvest damage, such as transportation and consumer handling (Moreira *et al.*, 2018).

Regarding luminosity, Santa West and Carolina were considered the lightest, while genotypes Dulce and Dolcetto were the least red according to the hue angle. Dulce, BRS Zamir, and Dolcetto have the most opaque fruits, while the others presented more intense red fruits (Table 2). Preczenhak *et al.* (2014) observed values for brightness (33.49 to 45.20) and hue (24.64 to 85.76), similar to those of the present study, although for chroma (1.47 to 4.99), the results were lower. Although a significant difference was found for the components of exocarp color, genotypes presented similar red color, even without naked eye difference. Consumers prefer red fruits (Oltman *et al.*, 2014), and the study by Adegbola *et al.* (2019) confirms the importance of color in the purchase decision, along with shelf life, flavor, and price.

Biochemical characterization

The BRS Zamir and Santa West fruits had the highest total phenolic contents, while the highest total flavonoid contents were observed for Dulce, BRSIG, Sweet Heaven, and SCI-023 (Table 3). Paula *et al.* (2015) verified the content of total phenolic compounds in salad-type hybrid tomatoes at different ripening stages, which ranged from 33.63 to 35.01 mg/100 g, similar to that found for Zamir and Santa West. In the same study, it is confirmed that fruits harvested at more advanced stages of maturation contain higher content of phenolic compounds because, fruits on the mother plant are longer exposed to radiation and temperatures, which are factors related to the production of phenolics.

For antioxidant activity, the highest values were observed in Dulce (91.19 mg/100 g), BRSIG (91.56 mg/100 g), Sweet Heaven (90.92 mg/100 g), and SCI-023 (90.50 mg/100 g). On the other hand, Maruyama *et al.* (2015) found that BRS Zamir showed higher antioxidant capacity contents compared to the different evaluated genotypes.

Soluble solids content ranged from 6.04 to 9.66°Brix, with the highest values observed for Sweet Heaven, BRS Zamir, Dulce, SCI-023, Santa West, and Dolcetto (Table 3). Oliveira *et. al* (2014), studying the soluble solids content of fruits of cultivar Carolina, produced under organic management, ranged from 3.77 to 6.35°Brix. Soluble solids are mainly composed of sugars but also contain mineral compounds, organic acids, soluble pectins, and amino acids. In this context, high soluble solids levels do not necessarily indicate high sugar levels. Thus, a better option for breeding programs is to use more precise techniques for

Table 1. Mean physical attributes from ten grape tomato hybrids. Londrina, UEL, 2019.

Genotypes	Traits[1/]							
	Mass	Length	Diam	PT	FV	VLG	Compression	Puncture
Carolina	13.39 b	31.26 d	27.22 b	3.84 b	14 b	3.6 c	18.41 d	0.97 c
Dulce	15.31 b	35.86 c	27.02 b	3.48 b	14.5 b	4.6 b	17.07 e	0.95 c
BRS Zamir	14.49 b	35.12 c	26.64 b	4.06 b	13.5 b	4.0 b	19.52 d	0.62 d
Guaraci	15.36 b	32.9 d	28.26 b	4.06 b	15 b	3.6 c	16.40 e	1.10 b
BRSIG	12.41 b	35.66 c	24.8 c	5.6 a	13 b	0.16 e	28.66 a	1.35 a
Sweet Heaven	17.49 b	39.14 b	26.92 b	4.18 b	17.5 b	3.0 c	28.54 a	1.19 b
Santa West	13.37 b	33.64 d	24.9 c	3.76 b	12.5 b	2.6 d	23.67 c	1.41 a
Aiko	27.55 a	42.5 a	32.6 a	4.14 b	27 a	6.5 a	17.02 e	1.19 b
Dolcetto	16.04 b	32.78 d	28.4 b	3.92 b	15.5 b	4.4 b	14.94 f	1.02 c
SCI-023	7.36 c	27.62 e	20.9 d	3.96 b	7.0 c	0.54 e	25.46 b	0.92 c
CV (%)	14.40	5.13	17.1	13.49	17.1	26.13	4.23	6.82

[1/]Means followed by the same letter in the same column do not differ by the Scott & Knott (1974) test ($p<0.05$); Mass (g); Length (mm); Diameter (mm); PT= pericarp thickness (mm); FV= fruit volume (cm^3); VLG= volume of locular gel (cm^3); Compressive strength (N) and Puncture resistance (N).

quantifying sugars, for sweeter fruits. It is emphasized that the flavor and aroma are among the attributes that determine the purchase of tomatoes which are closely related to the concentration of molecules such as sugar and organic acids (Adegbola *et al*., 2019; Casals *et al*., 2019).

The highest levels of sugar were observed for SCI-023 (3.14%) and Sweet Heaven (2.83%), which consequently presented the highest levels for glucose and fructose, while Aiko showed the lowest levels for both sugars. It is noteworthy the fact that SCI-023 is also included in the group of genotypes with higher contents of total soluble solids along with low acidity, presenting a high ratio (Table 3), providing greater sweetness to the fruit, which is one of the most prominent characteristics in grape tomatoes.

Casals *et al*. (2019) evaluated the chemical and sensory characteristics of cherry tomato fruits with consumers' preference. They concluded that consumers are positively influenced by sweetness and flavor, and these attributes are correlated with fructose, but not glucose. According to the authors, for plant breeding, the selection of genotypes with higher fructose values or a higher proportion of fructose/glucose is suggested, aiming at greater acceptance by consumers. Our data shows that in the fruits, we found the highest fructose values; we also observed the highest glucose values, in which SCI-023 stands out as the fruit with the highest content of both sugars.

Two groups were formed based on the acidity; Group I (Caroline, Dulce, Sweet Heaven, and Guaraci) with values ranging from 0.29 to 0.35%, and group II (Dolcetto, Santa West, SCI-023, BRSIG, Aiko and BRS Zamir) with values ranging from 0.20 to 0.27%. Constantino *et al*. (2021) found values ranging from 0.21 to 0.39% for titratable acidity. The total acidity in tomato points out the amount of organic acids present and its astringency, influencing the taste of the fruit (Nascimento *et al*., 2013). The preference for sweeter or more acid cultivars depends on the consumer's eating habits and the cultural patterns

Table 2. Means of epicarp color parameters from ten grape tomato hybrids. Londrina, UEL, 2019.

Genotypes	Luminosity	Hue	Chroma
Carolina	33.84 a	42.40 b	22.02 a
Dulce	32.20 b	52.58 a	18.88 b
BRS Zamir	32.34 b	42.72 b	23.46 a
Guaraci	32.54 b	47.90 b	21.12 a
BRSIG	31.90 b	43.14 b	24.00 a
Sweet Heaven	31.72 b	45.08 b	23.00 a
Santa West	33.36 a	48.10 b	21.10 b
Aiko	32.72 b	46.52 b	21.76 a
Dolcetto	32.38 b	49.22 a	19.82 b
SCI-023	31.42 b	44.54 b	22.32 a
CV (%)	2.67	9.15	7.27

Means followed by the same letter in the same column do not differ by the Scott & Knott test ($p<0.05$).

Table 3. Means of biochemical attributes from ten grape tomato hybrids. Londrina, UEL, 2019.

Genotypes	Traits[1/]										
	SS	TA	Glucose	Fructose	TS	Ratio	TPC	TFC	VitC	Lycopene	DPPH
Carolina	6.04 c	0.29 a	0.68 f	0.53 e	1.22 e	4.20 e	20.02 c	14.95 b	12.69 e	76.09 b	45.86 b
Dulce	9.34 a	0.32 a	1.12 c	1.18 c	2.23 c	7.17 c	25.57 b	62.45 a	30.64 b	79.13 b	91.19 a
BRS Zamir	9.42 a	0.27 b	0.77 f	0.63 e	1.39 e	5.15 d	33.95 a	32.23 b	33.94 a	107.83 a	56.90 b
Guaraci	8.84 b	0.35 a	0.99 d	0.89 d	1.89 d	5.39 d	30.42 b	12.91 b	30.76 b	48.59 c	56.58 b
BRSIG	8.02 b	0.24 b	0.70 f	0.59 e	1.30 e	5.40 d	18.30 c	54.27 a	29.51 b	74.64 b	91.56 a
Sweet Heaven	9.66 a	0.33 a	1.39 b	1.47 b	2.85 b	8.65 b	25.82 b	60.64 a	26.10 c	46.72 c	90.92 a
Santa West	9.16 a	0.21 b	0.86 e	0.62 e	1.48 e	7.03 c	38.63 a	27.91 b	25.08 c	10.14 d	56.85 b
Aiko	7.86 b	0.26 b	0.520 g	0.36 f	0.88 f	3.37 e	30.48 b	25.41 b	24.51 c	11.74 d	54.83 b
Dolcetto	9.08 a	0.20 b	0.77 f	0.92 d	1.68 d	8.40 b	31.01 b	34.27 b	17.35 d	86.52 b	54.19 b
SCI-023	9.24 a	0.23 b	1.51 a	1.63 a	3.14 a	13.67 a	15.78 c	66.55 a	34.28 a	73.77 b	90.50 a
CV (%)	7.38	18.09	5.37	5.99	5.60	10.75	16.13	30.66	7.33	16.38	12.13

Means followed by the same letter in the same column do not differ by the Scott & Knott (1974) test ($p<0.05$); SS= total soluble solids (ºBrix); TA= titratable acidity (%); Glucose (%); Fructose (%); TS= total sugars (%); Ratio= (glucose + fructose)/TS; TPC= total phenolic compounds (mg GAE/g); TFC= total flavonoids (mg EQ/g); VitC= vitamin C (mg/100 g); Lycopene (mg/kg); DPPH= antioxidant activity by the DPPH assay (%).

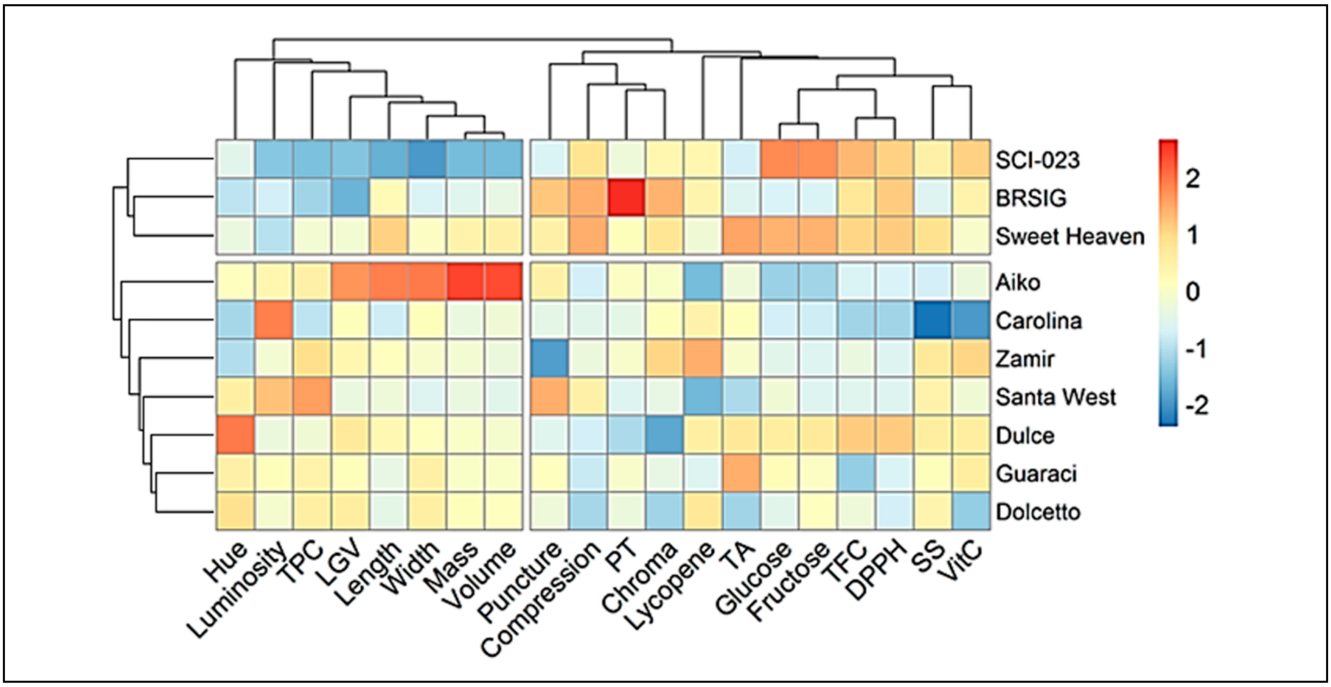

Figure 1. Ward hierarchical cluster analysis based on the standardized mean Euclidean distance of the physical and biochemical traits from ten grape tomato hybrids. Londrina, UEL, 2019.

of the region (Nascimento *et al*., 2013).

The highest vitamin C contents were observed for SCI-023 and BRS Zamir, with an average of 34.11 mg/100 g. Adalid *et al*. (2010) found vitamin C contents in tomatoes from the mini tomato group and traditional groups, ranging from 35 to 311 mg/L and 44 to 195 mg/L, respectively. The use of this attribute for selection is a great strategy because tomato is an important source of phenolic compounds and vitamin C, associated with antioxidant activity. So, it is excellent to choose genotypes with high levels to use as parents in crosses (Salehi *et al*., 2019).

For lycopene, BRS Zamir obtained the highest value (107.83 mg/kg), followed by Dolcetto, Dulce, Carolina BRSIG, and SCI-023 with 86.52, 79.13, 76.09, 74.64, and 73.77 mg/kg, respectively. BRS Zamir is a cultivar released by Embrapa Vegetables in 2013, focusing high lycopene content, presenting itself as a promising genetic material to be used in breeding programs focused on lycopene enrichment since it was superior to the other evaluated genotypes. However, the content of this attribute was lower than that found by Maruyama *et al*. (2015), which is 144 mg/kg. The same study states that the lycopene content is higher in fruits ripened outside the plant as a function of the exposure time of the tomato to temperature, which, even though it is an important factor for the development of the tomato plant, when found above 30°C, inhibits the synthesis of lycopene. Adalid *et al*. (2010) evaluated the lycopene content of different accessions of mini tomatoes ranging the averages from 0.2 to 169 mg/kg fruit; the accessions of the present study showed lycopene contents within this range. Lycopene is one of the most important antioxidants in tomato culture, and several studies have demonstrated this potential for the prevention of several chronic diseases (Rodrigues *et al*., 2018; Przybylska, 2020; Li *et al*., 2021).

Antioxidant compounds are molecules capable of neutralizing the effects of reactive oxygen species (ROS) originated by metabolic processes. ROS have groups of free radicals that make them highly reactive and, if not neutralized, can cause oxidative damage to proteins, lipids, and DNA. This damage can result in various neurodegenerative and cardiovascular diseases and even cancer (Çolak *et al*., 2020).

Multivariate analysis

Two distinct groups were observed by hierarchical cluster analysis using the physical and biochemical data (Figure 1). Group I (SCI-023, BRSIG, and Sweet Heaven) presented high mean values for sugars, fruit firmness, total flavonoids, and antioxidant activity. On the other hand, group II (Aiko, Carolina, Zamir, Santa West, Dulce, Guaraci, and Dolcetto) presented the highest averages for hue angle, luminosity, phenolics, mass, diameter, length, fruit volume, and locular gel. Generally, genotypes with higher physical attributes are less attractive than functional foods. In a breeding program focused on the enrichment of nutritional factors, these genotypes would not be interesting for selection.

The genotypes SCI-023 and Aiko were the most contrasting concerning most physical characteristics. Cultivar Aiko stood out about the phytometric attributes, with the highest averages for diameter and width, besides mass, locular gel volume, and fruit volume. SCI-023 stood out among the most

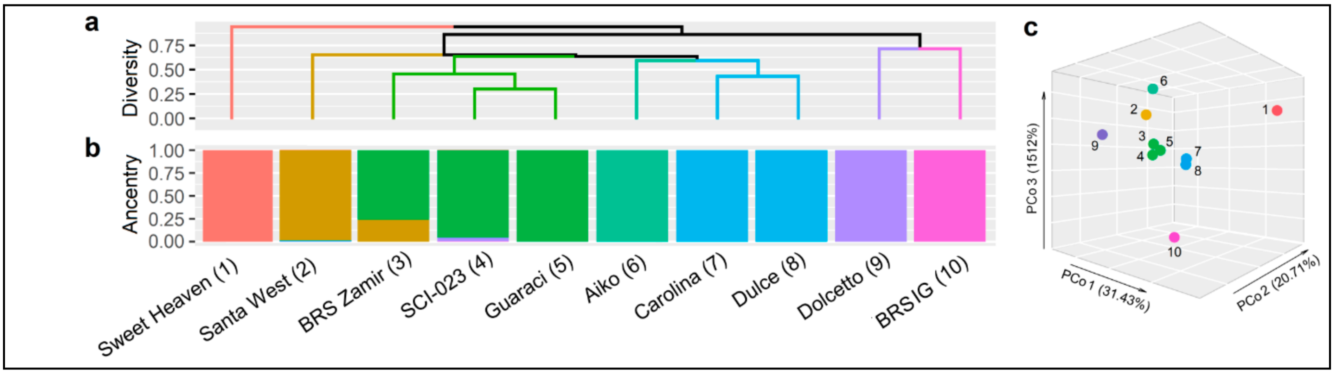

Figure 2. Dendrogram obtained by ward hierarchical clustering method (a), Bayesian cluster considering *K*= 7, and principal coordinate analysis (c) based on AFLP markers of ten grape tomato hybrids. Londrina, UEL, 2019.

nutritious, having higher levels for approximately 70% of the biochemical attributes; however, for the physical attributes, this cultivar presented lower levels in 90%, especially length and size.

Molecular characterization

The AFLP marker identified a wide genetic variability among grape tomato genotypes. Four selective EcoRI/MSeI primer combinations generated 792 bands, of which 686 were polymorphic (86.6%). The E-ATC/M-CTCG, E-AGC/M-CAA, E-ACT/M-GAG, and E-AGC/M-CAC generated 116, 222, 200 and 148 polymorphic fragments, respectively. AFLP marker by capillary electrophoresis in an automated system has been considered an important tool for exploring genetic divergence in several agricultural species (Giordani *et al*., 2019; Constantino *et al*., 2020; Massucato *et al*., 2020). Constantino *et al*. (2020), evaluating 22 accessions from *Capsicum baccatum*, found data similar to those of our work (92.48% of polymorphism).

Based on Jaccard distance, a dissimilarity range was observed between 0.30 and 0.87, with a mean distance of 0.63 (±0.11 DP). SCI-023 and Guaraci were the closest genetically, while Sweet Heaven and Dolcetto genotypes were the farthest. Based on simulations provided by Structure software and the methodology of the Δ*K* value, the optimal K was seven, which indicated a formation of seven distinct groups. Groups 1 (red) and 2 (brown) were constituted by Sweet Heaven and Santa West genotypes, respectively. Group 3 (dark green) was formed by the BRS Zamir, SCI-023, and Guaraci, while group 4 (light green) by the Aiko genotype. Carolina and Dulce were allocated in group 5 (blue) and the Dolcetto and BRSIG genotypes in groups 6 (purple) and 7 (pink), respectively (Figure 2).

By principal coordinate analysis (PCoA), the first three coordinates explained 67.26% of the total variation among genotypes. In general, the groups constituted by the Bayesian analysis were consistent with the groups formed by Ward and PCoA methods, indicating agreement in the results of these methodologies.

The physical, biochemical and molecular characterizations were useful tools in verifying genetic diversity among grape tomato genotypes, evidencing the aptitude of these genotypes to be used in mini tomato breeding programs that aim the development of new cultivars for the Brazilian market. In this sense, new options of mini grape tomatoes for consumers are highly desirable, focusing on fruits of medium to large sizes, intense and bright red color, enriched with bioactive compounds, and with higher antioxidant activity.

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