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# A contribution to the physicochemical characterization of *Eugenia involucrata* DC. fruits to estimate genetic variability

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**Abstract**

Brazil has one of the greatest diversities in native fruit trees, but many species, despite the great environmental and economic potential for small farms, are little studied, such as the cherry-of-the-rio-grande (Eugenia involucrata DC.). The hypothesis of this research was that there is a high genetic diversity due to the propagation by seeds, occurring genotypes that produce better quality fruits which can be used to implement genetic improvement programs and the production of seedlings with better productive performance. So, this study aimed to characterize fruits of this genotypes and to evaluate the genetic divergence applying multivariate analysis techniques. Genotypes of different ages found in municipality of Serafina Corrêa, Rio Grande do Sul, were evaluated, with 50 genotypes in 2018 and 38 genotypes in 2019, since 12 did not bear fruit. Data were submitted to determine the mean and standard deviation. To assess genetic diversity, the relative contribution of characters was determined by the Singh method; the average Euclidean distance standardized matrix (UPGMA) and dendrograms were generated; and Tocher's optimization method was applied. Results showed that UPGMA and Tocher clustering methods are more efficient in representing the diversity between genotypes. Fruits characteristics varied from one year to another, due to the combination of biotic and abiotic factors (water regime), resulting in changes of characters with greater contribution in the divergence and formation of similar groups. The content of total soluble solids (TSS) in 2018 and fruit mass in 2019 harvest were characters that most contributed to the genetic divergence. It was concluded that the physicochemical characters of fruits revealed the existence of genetic divergence among genotypes, allowing the selection of agronomically superior plants.

**Keywords:** Acidity, Cherry-of-the-rio-grande, Fruit size, Genetic divergence, Sugars

## Introduction

Brazil has one of the largest biological diversities, however, many plant species despite its great potential, are not studied or have little information. The concern is accentuated by the constant deforestation and degradation of the environment, with irreversible losses of genetic diversity (Sarmento et al., 2012). Among the little studied species the native fruit trees are noteworthy, having high value and economic interest, which can add and contribute to new production chains emergence, to sustainable development and also diversity maintenance (Odalia-Rímoli et al., 2000; Pereira et al., 2012). The economic potential is also justified by the fact that they are different, tasty and nutritious fruits, and by the consumer market desire for new products (Sarmento et al., 2012). Yet, the vast majority of species remains unknown or do not have enough production to make commercialization feasible, either fresh or derived products (Pereira et al., 2014). The most well-known and commercialized native fruits in Brazil are cashew (Anacardium occidentale L.), açaí berry (Euterpe oleracea Mart.), passion fruit (Passiflora edulis Sims) and guava (Psidium guajava L.) (Santos, 2018; Vanin, 2015).

A significant number of native fruit species belongs to the Myrtaceae family, which houses more than 5.500 species, distributed in approximately 130 genera (Govaerts et al., 2019), considered one of the largest botanical families, with several genera considered of great economic and ecological importance (Grattapaglia et al., 2012). In Brazil, the genus Eugenia has more than 400 species (Flora do Brasil, 2020), sometimes so similar to each other that can result in taxonomic mistakes (Mazine et al., 2016).

The fleshy fruits produced by species of this genus are considered an important source of food for fauna. In addition to ecological importance, this genus has


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potential for pharmacological industry, once antioxidant, antibacterial, antifungal, diuretic and anti-inflammatory activities have been described (Magina et al., 2009; Pietrovski et al., 2008; Queiroz et al., 2015; Voss-Rech et al., 2011). Among species of this genus is the Eugenia involucrata DC., found in the Seasonal Semideciduous Forest as well in the Mixed Ombrophilous Forest (Lorenzi, 2002, Carvalho, 2009). The common denominations for the species are cherry-of-the-rio-grande, cerejeira-do-mato and cereja-da-terra (Lorenzi, 2002).

The species has a shrubby to arboreal habit, evergreen, heliophyte and hygrophyte, being able to reach approximately 15 m in height. The leaves are simple, glabrous on both sides, leathery and bright green. The flowers are hermaphrodites, occurring in pairs of two to four or isolated, pedunculated, white in color, with many stamens (Carvalho, 2009; Degenhardt et al., 2007, Lorenzi, 2002; Lorenzi et al., 2006). The fruits are globose drupe type, of varied shape, glabrous and shiny, red to black-violet in color, crowned by sepals. The pulp is fleshy, sweet to acidic flavor, with a maturation period that varies from 35 to 45 days, between the months of November and January (Degenhardt et al., 2007; Lorenzi et al., 2006).

The cherry-of-the-rio-grande fruit-bearing can be an option for cultivation and sustainable exploitation, generating income, mainly in small rural properties. It can be introduced in the recovery of Permanent Preservation Areas (APPs), in Legal Reserves (RL), commercial plantations in the Agroforestry System (SAFs) or in monoculture. Currently, cultivation is intended for consumption, planted in areas close to residences and in urban forestry. This behavior has led, over time, to a mass selection by the communities, when collecting seeds for the production of plant seedlings that produce better quality fruits. For many native fruit-bearing, this justifies the fact that we find agronomically superior plants more often in urbanized areas than in native forests.

Despite its high potential, researches on the species are more focused on its phytochemical characteristics, with very few studies on agronomic characteristics, important for the management and conservation of the species. Identification of individuals with superior characteristics can implement genetic improvement programs and the production of seedlings with better fruit quality and productive performance. Once genotypes are selected, genetic rescue can be performed via sexual (seeds), but commercially, vegetative propagation would be more recommended, in order to maintain the characteristics of the mother plants and to obtain precocity at the beginning of production.

Therefore, this study attempted to verify if there is variability in the characteristics of the fruits of Eugenia involucrata. The hypothesis was that diversity exists, since the propagation method used by the populations is the seminal one, with genotypes producing better quality fruits. Thus, the work had as objectives: to characterize Eugenia involucrata's seminal origin genotypes fruits and to evaluate genetic divergence using multivariate analysis techniques.

**Material and Methods**

Eugenia involucrata genotypes present in the municipality of Serafina Corrêa, Rio Grande do Sul state (RS), Brazil (latitude 28°42'43"S; longitude 51°56'06"W and average altitude of 509 m), were selected and characterized. Genotypes of different ages found in rural and urban areas, in the areas close to the residences and in the afforestation of public sidewalks were sampled.

The region's climate is classified as Cfa, according to the Köppen climate classification, humid in all seasons, with well-distributed rainfalls and hot summer (Kuinchtner & Burial, 2001). The soil is Luvic Chernozem type with Red-Brown (BV5) characteristic (Streck, 2008).

The evaluations were carried out for two years, in 2018 and 2019. In 2018, the precipitation regime of Serafina Corrêa in August, September, October and November, which correspond to the period of pre-flowering, flowering and fruit maturation, was 190, 200, 250 and 245 mm respectively, totalizing 885 mm, and for the year of 2019: 22, 20, 100 and 67 mm, totalizing 209 mm. The average temperature varied between 15°C and 21°C (INMET, 2020).

In 2018, fruits of 50 genotypes were characterized and, in 2019, fruits of 38 genotypes, since 12 of them did not bear fruit. The physicochemical characterization was performed from samples of 30 ripe fruits per plant, collected randomly in the four quadrants of the plant. After collected, fruits were placed in polyethylene bags, closed and transported in styrofoam collers with ice for evaluation in the laboratory of the Faculty of Agronomy and Veterinary Medicine (FAMV) from the University of Passo Fundo (UPF).

The fruits were submitted to the following evaluations: longitudinal and transversal diameter, using a digital caliper; mean fresh mass of fruits, number and fresh mass of seeds, using a digital scale; pulp percentage; hydrogenionic potential (pH), determined in pH meter; total soluble solids (TSS) content, determined with a manual refractometer; total titratable acidity (TTA);

and TSS/TTA ratio. TTA was determined from a sample of 10 mL of pulp diluted in 90 mL of distilled water, adding three drops of phenolphthalein and titration with 0,1 N sodium hydroxide (NaOH). The values were expressed as a percentage of citric acid, using the formula: V x 0,64 / P, where V is the volume of NaOH spent to neutralize acids and P the sample volume.

The data were submitted to analysis by descriptive statistics, determining the mean and standard deviation of the mean. To evaluate genetic diversity among the genotypes, multivariate techniques of principal component analysis and grouping methods were used. The relative contribution of characters to the genetic divergence was determined by the method of Singh (1981); correlations were tested using hierarchical grouping method of Single-linkage clustering (nearest neighbor clustering), Complete-linkage clustering (farthest neighbor clustering), Ward's method and Unweighted Pair Group Method with Arithmetic Mean (UPGMA); the average Euclidean distance standardized matrix by the UPGMA method was generated and the dendrograms were generated; and Tocher's optimization method was applied. The analyzes were performed using the Genes program (Cruz, 2013).

**Results and Discussion**

The results revealed the existence of variability between genotypes regarding characteristics of the fruits, demonstrating feasibility of selecting superior genotypes, either in plants of native occurrence as those cultivated in urban and rural areas.

The variation in fruits' characteristics was verified not only between genotypes in the same harvest but also for the same genotype from one year to another. The most extreme example was in the second year (2019). Despite the flowering, there was no fruiting for twelve of the fifty genotypes evaluated in 2018, a fact that was not possible to confirm with certainty its causes, but that can possibly be attributed to the genetic characteristics combined with the negative response to water deficit that occurred in that referred period.

The average longitudinal diameter of the fruits was 22.2 mm in 2018 (Table 1), ranging from 17.1 to 29.5 mm, with emphasis on the G23 and G29 genotypes. The transversal diameter was, on average, 19.4 mm, ranging from 15.3 to 25.1 mm, with an emphasis on G19, G20 and G30. In 2019, the average of the two diameters were similar to the previous harvest, with an average longitudinal diameter of 22.9 mm, ranging from 14.1 mm to 27.7 mm, with G26, G37, G39 and G48 showing longer fruits. The mean cross-sectional diameter was 19.7 mm, ranging from 12.5 to 23.3 mm, with emphasis on G1, G20, G26, G37 and G44.

The results show that genotypes with longer fruits did not necessarily have a larger cross-sectional diameter. Also, that the size of fruits varied from one harvest to another, to the point that only G20 had a larger cross-sectional diameter in both harvests. Genotype G26 was the only one in the same period (2019) to produce fruits with two diameters larger than the others, unlike what happened in the previous period, which this genotype did not stand out.

Lopes (2009), in Eldorado do Sul, Rio Grande do Sul (RS) state, when performing evaluation during three harvests (2006, 2007 and 2008), obtained fruits that varied in the longitudinal diameter from 20.1 to 22.1mm, and from 19.3 to 20.8 mm in transversal diameter, without differing between harvests. Camlofski (2008), in a study carried out in Ponta Grossa, Paraná (PR) state, found fruits with an average of 20.6 mm for longitudinal diameter and 18.8 mm for transversal diameter. These results were within the range of variation present in this study, which was broader, with genotypes producing larger and smaller fruits.

The fresh mass of fruits is a result of the combination of transversal and longitudinal diameters. Thus, it would be expected that genotypes that stood out by one or other diameter, or even both, would present fruits of greater mass.

In the 2018 harvest, the average mass of fruits was 5.3 g, with a range from 2.5 to 10.9 g, with genotypes G19, G23 and G37 standing out (10.9; 10.4 and 9.1 g, respectively), followed by G20, G24, G30 and G50, with more than 7.6 g (Table 2). In the 2019 harvest, the average was practically the same (5.2 g), but the variation was from 1.7 to 8.7 g, revealing the production of smaller fruits. The highlight was for genotype G26 (8.7 g), followed by G16, G20, G44 and G48, with more than 6.7 g each. Lopes (2009) found similar average values, showing fruits with 5.0 to 5.5 g in the three evaluated harvests.

**Table 1.** Longitudinal and transversal diameter of fruits of *Eugenia involucrata* genotypes in the 2018 and 2019 harvests. Serafina Corrêa, RS.

| Genotyopes | Diameter of fruits (mm) | | | |
|---|---|---|---|---|
| | Harvest 2018 | | Harvest 2019 | |
| | Longitudinal | Transversal | Longitudinal | Transversal |
| G1 | 20.4 ± 2.6 d | 18.2 ± 3.6 d | 24.3 ± 2.4 b | 22.8 ± 2.3 a |
| G2 | 22.3 ± 2.4 c | 20.5 ± 2.1 c | 24.5 ± 2.7 b | 19.6 ± 2.7 c |
| G3 | 19.1 ± 2.0 e | 17.3 ± 1.8 d | 19.8 ± 2.9 d | 17.7 ± 2.3 c |
| G4 | 17.1 ± 2.4 e | 15.3 ± 2.2 e | 20.4 ± 1.8 c | 16.5 ± 1.1 d |
| G5 | 18.8 ± 1.9 e | 16.3 ± 1.6 e | 20.0 ± 1.6 d | 16.0 ± 1.8 d |
| G6 | 18.1 ± 3.0 e | 17.6 ± 2.2 d | 22.0 ± 1.8 c | 20.9 ± 2.2 b |
| G7 | 20.4 ± 2.6 d | 17.9 ± 2.0 d | * | * |
| G8 | 18.6 ± 1.6 e | 17.1 ± 1.3 e | 14.1 ± 1.9 e | 12.5 ± 2.0 e |
| G9 | 21.1 ± 2.3 d | 18.6 ± 1.5 d | 20.8 ± 2.7 c | 17.7 ± 2.2 c |
| G10 | 21.3 ± 3.0 d | 19.1 ± 2.2 d | * | * |
| G11 | 19.4 ± 3.2 d | 17.4 ± 2.6 d | 23.8 ± 3.2 b | 19.3 ± 1.9 c |
| G12 | 22.9 ± 3.5 c | 18.7 ± 2.2 d | * | * |
| G13 | 18.9 ± 2.6 e | 17.2 ± 2.1 e | 21.7 ± 2.0 c | 19.4 ± 1.5 c |
| G14 | 20.5 ± 2.4 d | 17.8 ± 1.6 d | * | * |
| G15 | 21.3 ± 1.9 d | 18.9 ± 1.6 d | 22.9 ± 3.0 b | 19.7 ± 2.2 b |
| G16 | 24.4 ± 2.3 c | 21.5 ± 2.2 b | 25.1 ± 3.6 b | 21.4 ± 3.4 b |
| G17 | 23.3 ± 2.5 c | 21.8 ± 2.0 b | 22.6 ± 2.4 c | 20.3 ± 2.1 b |
| G18 | 22.3 ± 2.1 c | 18.7 ± 1.5 d | 24.7 ± 2.9 b | 20.2 ± 2.3 b |
| G19 | 27.1 ± 2.0 b | 25.1 ± 2.4 a | 23.4 ± 3.8 b | 20.7 ± 4.0 b |
| G20 | 25.5 ± 2.2 b | 23.7 ± 2.8 a | 24.3 ± 3.7 b | 23.0 ± 3.9 a |
| G21 | 22.7 ± 2.2 c | 18.9 ± 1.9 d | * | * |
| G22 | 21.2 ± 3.0 d | 16.5 ± 3.6 e | * | * |
| G23 | 29.3 ± 3.5 a | 20.9 ± 2.4 c | 23.9 ± 3.9 b | 17.5 ± 2.3 c |
| G24 | 22.2 ± 2.8 c | 20.9 ± 2.3 c | 24.7 ± 2.3 b | 21.4 ± 2.0 b |
| G25 | 20.8 ± 2.3 d | 18.2 ± 2.0 d | 25.0 ± 3.0 b | 21.3 ± 2.3 b |
| G26 | 20.9 ± 2.8 d | 17.2 ± 2.2 e | 27.7 ± 2.6 a | 23.3 ± 2.1 a |
| G27 | 19.9 ± 2.9 d | 18.2 ± 2.6 d | 25.0 ± 3.0 b | 20.9 ± 2.3 b |
| G28 | 19.7 ± 1.9 d | 18.6 ± 1.7 d | 23.5 ± 2.4 b | 20.5 ± 1.5 b |
| G29 | 29.5 ± 2.7 a | 19.3 ± 1.8 d | * | * |
| G30 | 23.8 ± 2.7 c | 23.9 ± 2.3 a | * | * |
| G31 | 23.8 ± 2.4 c | 20.6 ± 1.3 c | * | * |
| G32 | 17.8 ± 1.9 e | 17.7 ± 1.6 d | * | * |
| G33 | 23.0 ± 2.1 c | 18.5 ± 1.8 d | 24.2 ± 2.0 b | 18.8 ± 1.4 c |
| G34 | 19.1 ± 3.1 e | 18.0 ± 3.1 d | 20.3 ± 2.0 c | 18.2 ± 1.9 c |
| G35 | 19.5 ± 2.2 d | 19.7 ± 2.0 c | 18.3 ± 2.4 d | 19.4 ± 2.3 c |
| G36 | 22.2 ± 2.0 c | 18.5 ± 3.2 d | 25.2 ± 3.5 b | 20.4 ± 1.9 b |
| G37 | 27.0 ± 3.1 b | 22.7 ± 1.6 b | 27.2 ± 1.9 a | 23.3 ± 1.6 a |
| G38 | 24.5 ± 3.5 c | 20.2 ± 2.7 c | * | * |
| G39 | 24.3 ± 4,7 c | 18,1 ± 2,8 d | 27,2 ± 3,3 a | 20.1 ± 2.5 b |
| G40 | 22.8 ± 2.8 c | 21.6 ± 2.5 b | 23.5 ± 3.2 b | 20.3 ± 2.0 b |
| G41 | 22.3 ± 2.2 c | 21.7 ± 2.4 b | 21.4 ± 2.1 c | 20.3 ± 1.9 b |
| G42 | 26.6 ± 2.3 b | 22.2 ± 1.7 b | 22.2 ± 3.0 c | 19.8 ± 2.0 b |
| G43 | 19.2 ± 2.8 e | 19.5 ± 2.4 c | 21.5 ± 2.8 c | 20.0 ± 2.0 b |
| G44 | 21.6 ± 2.2 d | 20.5 ± 1.6 c | 23.2 ± 2.2 b | 22.6 ± 2.1 a |
| G45 | 22.2 ± 2.5 c | 17.8 ± 2.4 d | 24.1 ± 1.9 b | 21.1 ± 2.3 b |
| G46 | 23.8 ± 2.9 c | 19.4 ± 2.4 c | 19.9 ± 2.4 d | 15.3 ± 2.3 d |
| G47 | 25.3 ± 2.5 b | 19.0 ± 2.0 d | * | * |
| G48 | 26.8 ± 3.9 b | 21.2 ± 3.5 c | 27.6 ± 3.7 a | 21.9 ± 3.1 b |
| G49 | 19.4 ± 1.8 d | 17.5 ± 1.8 d | 19.1 ± 2.3 d | 16.8 ± 1.3 d |
| G50 | 25.0 ± 2.3 c | 22.7 ± 1.6 b | 21.5 ± 3.2 c | 19.1 ± 2.1 c |
| Mean | 22.2 | 19.4 | 22.9 | 19.7 |
| Standard deviation | 2.9 | 2.1 | 2.8 | 2.3 |

* There was no fruiting.

Means followed by the same letter do not differ considering the difference of 1 standard deviation from the mean.

**Table 2.** Fresh fruit mass, number and fresh mass of seeds per fruit, and percentage of pulp of *Eugenia involucrata* genotypes in the 2018 and 2019 harvests. Serafina Corrêa, RS.

| Genotypes | Fresh fruit mass (g) | | Number of seeds per fruit | | Fresh mass of seeds per fruit (g) | | Percentage of pulp (%) | |
|---|---|---|---|---|---|---|---|---|
| | Harvest | | Harvest | | Harvest | | Harvest | |
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| G1 | 4.0 d | 6.3 c | 1.7 d | 1.4 d | 0.83 d | 1.08 b | 79.3 d | 82.7 c |
| G2 | 5.5 c | 5.5 c | 1.6 d | 1.3 d | 0.89 d | 0.58 d | 83.9 c | 89.6 b |
| G3 | 3.5 d | 3.3 e | 1.4 e | 1.6 c | 0.58 e | 0.56 d | 83.5 c | 82.8 c |
| G4 | 2.5 e | 3.3 e | 0.9 f | 1.5 d | 0.32 f | 0.56 d | 86.9 b | 86.9 b |
| G5 | 3.0 e | 3.2 e | 1.6 d | 1.7 c | 0.51 e | 0.70 d | 82.7 c | 77.7 d |
| G6 | 3.3 e | 5.2 c | 1.0 f | 1.8 c | 0.50 e | 0.88 c | 84.5 c | 82.9 c |
| G7 | 3.6 d | * | 1.3 e | * | 0.51 e | * | 86.0 b | * |
| G8 | 6.2 c | 1.7 f | 1.4 e | 1.0 e | 1.56 a | 0.17 e | 62.0 e | 90.6 a |
| G9 | 4.4 d | 3.9 d | 1.5 e | 1.5 d | 0.67 e | 0.56 d | 84.6 c | 85.6 b |
| G10 | 4.3 d | * | 1.8 d | * | 0.97 c | * | 77.5 d | * |
| G11 | 3.7 d | 5.5 c | 1.7 d | 2.2 b | 0.66 e | 0.85 c | 82.1 c | 84.5 c |
| G12 | 4.3 d | * | 2.6 b | * | 0.89 d | * | 79.3 d | * |
| G13 | 3.0 e | 4.2 d | 1.3 e | 1.5 d | 0.50 e | 0.60 d | 83.4 c | 85.7 b |
| G14 | 3.8 d | * | 1.2 e | * | 0.61 e | * | 83.9 c | * |
| G15 | 4.0 d | 4.5 d | 1.9 d | 1.3 d | 0.78 d | 0.74 c | 80.3 c | 83.8 c |
| G16 | 6.7 c | 7.2 b | 1.3 e | 1.3 d | 0.55 e | 0.63 d | 91.7 a | 91.2 a |
| G17 | 6.2 c | 5.2 c | 1.9 d | 2.3 b | 0.87 d | 0.88 c | 85.9 b | 83.1 c |
| G18 | 4.3 d | 6.3 c | 1.8 d | 1.9 c | 0.71 e | 0.67 d | 83.6 c | 89.3 b |
| G19 | 10.9 a | 6.5 c | 1.5 e | 1.0 e | 0.93 d | 0.38 e | 91.5 a | 94.2 a |
| G20 | 8.6 b | 7.4 b | 1.1 f | 1.2 d | 0.51 e | 0.56 d | 94.1 a | 92.3 a |
| G21 | 4.3 d | * | 1.0 f | * | 0.65 e | * | 84.8 c | * |
| G22 | 7.1 c | * | 1.4 e | * | 0.44 f | * | 93.8 a | * |
| G23 | 10.4 a | 4.4 d | 1.1 f | 0.9 e | 0.48 f | 0.20 e | 95.4 a | 95.5 a |
| G24 | 8.5 b | 6.0 c | 2.3 c | 2.4 b | 0.62 e | 0.62 d | 92.7 a | 89.7 b |
| G25 | 6.5 c | 5.9 c | 1.3 e | 1.8 c | 0.61 e | 1.11 b | 90.7 a | 81.1 c |
| G26 | 3.7 d | 8.7 a | 2.1 c | 1.1 d | 0.68 e | 0.39 e | 81.7 c | 95.5 a |
| G27 | 4.1 d | 5.6 c | 1.9 d | 1.7 c | 0.59 e | 0.92 c | 85.6 b | 83.5 c |
| G28 | 4.0 d | 5.3 c | 2.2 c | 2.6 a | 0.88 d | 1.65 a | 77.8 d | 68.9 e |
| G29 | 6.7 c | * | 2.3 c | * | 1.39 b | * | 79.4 d | * |
| G30 | 7.9 b | * | 1.2 e | * | 0.85 d | * | 89.2 b | * |
| G31 | 5.9 c | * | 1.4 e | * | 0.80 d | * | 86.4 b | * |
| G32 | 3.2 e | * | 1.1 f | * | 0.53 e | * | 79.5 d | * |
| G33 | 3.9 d | 4.6 d | 1.6 d | 1.3 d | 0.80 d | 0.88 c | 79.5 d | 80.7 c |
| G34 | 3.9 d | 3.7 d | 1.5 e | 2.8 a | 0.49 e | 0.79 c | 87.4 b | 78.5 d |
| G35 | 4.4 d | 3.9 d | 1.9 d | 1.8 c | 0.83 d | 0.69 d | 81.1 c | 82.0 c |
| G36 | 4.9 d | 6.3 c | 1.2 e | 1.4 d | 0.55 e | 0.68 d | 88.8 b | 89.3 b |
| G37 | 9.1 a | 5.7 c | 1.3 e | 0.8 e | 0.89 d | 0.55 d | 90.3 a | 90.6 a |
| G38 | 6 .0 c | * | 1.7 d | * | 0.74 d | * | 87.7 b | * |
| G39 | 4.8 d | 6.5 c | 1.6 d | 2.3 b | 0.66 e | 1.09 b | 86.3 b | 83.2 c |
| G40 | 5.4 c | 5.3 c | 2.1 c | 2.7 a | 0.90 d | 0.94 c | 83.4 c | 82.2 c |
| G41 | 5.7 c | 4.8 d | 1.3 e | 1.5 d | 0.84 d | 0.64 d | 85.3 b | 86.7 b |
| G42 | 4.7 d | 5.4 c | 1.1 f | 1.3 d | 0.56 e | 0.57 d | 88.0 b | 89.5 b |
| G43 | 3.9 d | 5.4 c | 1.2 e | 1.6 c | 0.59 e | 0.91 c | 84.8 c | 83.1 c |
| G44 | 5.7 c | 7.1 b | 1.9 d | 2.1 b | 0.89 d | 1.12 b | 84.4 c | 84.2 c |
| G45 | 4.2 d | 5.7 c | 1.2 e | 1.3 d | 0.50 e | 0.73 c | 88.1 b | 87.2 b |
| G46 | 5.3 c | 2.9 e | 3.1 a | 1.6 c | 1.04 c | 0.33 e | 80.4 c | 88.5 b |
| G47 | 4.9 d | * | 1.6 d | * | 0.78 d | * | 84.1 c | * |
| G48 | 7.0 c | 6.7 b | 1.0 f | 1.3 d | 0.54 e | 0.63 d | 92.3 a | 90.7 a |
| G49 | 3.4 d | 3.5 e | 1.3 e | 1.4 d | 0.49 e | 0.54 d | 85.5 b | 84.6 c |
| G50 | 7.6 b | 6.2 c | 1.4 e | 1.4 d | 0.89 d | 0.63 d | 88.3 b | 89.9 b |
| Média | 5.3 | 5.2 | 1.6 | 1.6 | 0.72 | 0.72 | 85.0 | 85.0 |
| Standard deviation | 1.9 | 1.5 | 0.4 | 0.5 | 0.23 | 0.28 | 5.5 | 5.3 |

* There was no fruiting.

Means followed by the same letter do not differ considering the difference of 1 standard deviation from the mean.

The different responses in size and mass of the fruits can be attributed to the genetic characteristics of each genotype and fruit load- which influences the production due to nutrients competition, but can also interfere in flowering and fruiting of the next harvest, by reducing the availability of nutritional reserves. Abiotic factors also interfere, such as soil fertility, location in terms of luminosity and annual changes in climatic events, like temperatures and rainfall. This work, in which flowering period occurred from September to the first ten days of October, and ripening from mid-October until November 20th, considering all genotypes, the fruits characteristics were greatly affected by the water regime. Between August and November 2019, accumulated rainfall was just 209 mm, while there was a record of 885 mm in 2018.

The average number of seeds per fruit, in the two harvests, was 1.6 seeds, with variation between the genotypes from 0.9 to 3.1 seeds (2018) and from 0.8 to 2.8 seeds per fruit (2019) (Table 2). In 2018, genotype G46 had the highest number of seeds (3.1), followed by G12 (2.6 seeds) and G24, G26, G28, G29 and G40, with 2.1 to 2.3 seeds. These represented 14% of the evaluated genotypes. Another 30% of the genotypes produced fruits with 1.6 to 1.9 seeds, 40% with 1.2 to 1.5 seeds and 16% with 0.9 to 1.1 seeds. Only in G4 were verified some fruits without seeds. In 2019, genotypes G28, G34 and G48 were the ones that produced fruits with the largest number of seeds (2.6 to 2.8 seeds), followed by G11, G17, G24, G39 and G44 (2.1 to 2.4 seeds), representing 21% of the genotypes. Fruits with 1.6 to 1.8 seeds were verified in 24% of the genotypes, while 44% formed from 1.1 to 1.5 seeds per fruit and another 11% contained from 0.8 to 1.0 seeds, including G23 and G37, which showed some seedless fruits.

Seeds amount can influence the size of fruits, since they are responsible for producing hormones (auxins, gibberellins and cytokinins) that stimulate growth of the ovary (Taiz & Zeiger, 2002). However, the greater presence of seeds can reduce pulp yield and does not please the consumer. This was a positive aspect shown in the study, as the genotypes that stood out for fresh mass of fruits presented from 1.0 to 1.5 seeds, except G24, with 2.3 seeds in 2018, and G44 with 2.1 seeds in 2019.

Fresh mass of seeds was, on average, equal in the two harvests (0.72 g) (Table 2), varying in 2018 from 0.32 g to 1.56 g and in 2019 from 0.17 to 1.65 g. In 2018, genotype G8 presented fruits with the highest seed mass (1.56 g), although with an average of only 1.4 seeds per plant, followed by G29 (1.39 g), but in this case with more seeds (2.3 seeds). In 2019, the genotype with the highest seed mass was G28 (1.65 g), followed by G1, G25, G35 and G44, with 1.09 to 1.12 g. For the other genotypes, in both seasons, seed mass was less than 1.0 g, demonstrating that increasing the number, there is a tendency for each seed to present less mass. The average values found in this work agree with those confirmed by Lopes (2009).

Pulp percentage is a very important variable, as it determines yield both for fresh consumption and, mainly, for processing. The mean in both two harvests were identical (85%), varying from 62.0% to 95.4% in 2018 and from 68.9% to 95.5% in 2019 (Table 2). In 2018, the highest percentage of pulp was found in fruits of genotypes G16, G19, G20, G22, G23, G24, G25, G37 and G48, ranging from 90.3% to 95.4%. As expected, G8 showed the lowest percentage (62%), as it had the highest seed mass, followed by G10, G28, G29, G31 and G32, with 77.5 to 79.4% of pulp utilization. In 2019, G19, G20 and G23 again stood out, in addition to G26, with pulp yield between 90.6% and 95.5%. Genotype G28 repeated the low percentage of pulp (68.9%), followed by G5 (77.7%) and G34 (78.5%). The values found were, on average, higher than those verified by Camlofski (2008) with 76.6% of pulp and similar to those found by Lopes (2009), with percentages varying from 86.0% to 91.3%.

The mean content of total soluble solids in 2018 harvest was 9.2o Brix, ranging from 6.1 to 11.8oBrix (Table 3). The highest levels were obtained in twelve genotypes (G3, G4, G5, G9, G10, G11, G37, G39, G41, G46, G47 and G48), ranging from 10.9 to 11.8° Brix. In 2019 harvest, the sugar content was higher in practically all genotypes, with an average of 10.1oBrix and variation of 7.9 to 14.1oBrix, highlighting G4 and G39, and followed by G5, G33, G37 and G43, with more than 11.9oBrix. The reason for high sugar content in 2019 is (contrary to what happened in 2018) in the low occurrence of rainfalls, which increased photosynthesis rate and photoassimilates accumulation, due to longer time of light radiation. Also, water stress provided less dilution of the accumulated sugars. These values agree with those found by Camlofski (2008), with an average of 9.5° Brix, and Lopes (2009), with averages for the three collection periods ranging from 8.02 to 10.47° Brix.

**Table 3.** Total soluble solids (TSS), total titratable acidity (TTA), TSS/TTA ratio and pH of *Eugenia involucrata* genotypes fruits in 2018 and 2019 harvests. Serafina Corrêa, RS.

| Genotype | TSS (°Brix) | | TTA (% of citric acid) | | TSS/TTA | | pH | |
|---|---|---|---|---|---|---|---|---|
| | Harvest | | Harvest | | Harvest | | Harvest | |
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| G1 | 7.3 d | 9.3 d | 0.07 e | 0.07 d | 106.6 c | 125.3 b | 3.23 c | 3.18 e |
| G2 | 8.1 c | 9.2 d | 0.23 b | 0.12 a | 34.5 f | 75.7 d | 3.21 c | 3.21 e |
| G3 | 10.8 a | 11.3 c | 0.13 d | 0.09 c | 80.7 d | 124.3 b | 3.26 b | 3.46 c |
| G4 | 11.1 a | 13.8 a | 0.18 c | 0.12 a | 62.2 e | 117.2 c | 2.62 e | 3.50 c |
| G5 | 11.7 a | 13.1 b | 0.17 c | 0.09 c | 67.7 e | 146.2 b | 3.35 b | 3.34 d |
| G6 | 9.1 c | 10.7 c | 0.18 c | 0.08 c | 51.3 e | 131.6 b | 3.20 c | 3.47 c |
| G7 | 9.0 c | * | 0.16 c | * | 56.7 e | * | 3.26 b | * |
| G8 | 9.3 b | 8.0 e | 0.08 e | 0.09 c | 112.6 c | 88.0 d | 3.21 c | 3.41 c |
| G9 | 11.4 a | 11.0 c | 0.07 e | 0.09 c | 166.5 a | 119.4 c | 3.39 b | 3.43 c |
| G10 | 11.2 a | * | 0.21 b | * | 53.0 e | * | 3.20 c | * |
| G11 | 11.8 a | 9.5 d | 0.24 b | 0.07 d | 50.1 e | 131.4 b | 3.28 b | 3.28 d |
| G12 | 10.3 b | * | 0.20 b | * | 51.1 e | * | 3.30 b | * |
| G13 | 8.1 c | 9.0 d | 0.18 c | 0.08 c | 43.8 e | 109.9 c | 3.34 b | 3.46 c |
| G14 | 9.1 c | * | 0.16 c | * | 58.5 e | * | 3.18 c | * |
| G15 | 7.2 d | 9.0 d | 0.19 c | 0.07 d | 37.8 e | 129.0 b | 3.11 c | 3.30 d |
| G16 | 8.2 c | 6.9 e | 0.18 c | 0.13 a | 45.1 e | 53.6 e | 3.37 b | 3.37 d |
| G17 | 8.2 c | 8.3 e | 0.16 c | 0.08 c | 52.7 e | 109.0 c | 3.57 a | 3.64 b |
| G18 | 5.3 e | 11.0 c | 0.12 e | 0.07 d | 42.5 e | 149.5 b | 3.03 d | 3.20 e |
| G19 | 8.2 c | 9.4 d | 0.18 c | 0.08 c | 45.1 e | 116.6 c | 3.37 b | 3.40 c |
| G20 | 6.4 d | 8.3 e | 0.09 e | 0.06 d | 69.0 e | 129.7 b | 2.87 e | 3.20 e |
| G21 | 7.5 d | * | 0.15 c | * | 48.6 e | * | 3.51 a | * |
| G22 | 10.5 b | * | 0.16 c | * | 67.5 e | * | 3.26 b | * |
| G23 | 7.3 d | 9.7 d | 0.26 a | 0.08 c | 28.4 f | 121.3 c | 3.14 c | 3.47 c |
| G24 | 8.1 c | 7.9 e | 0.24 b | 0.10 b | 33.7 f | 78.1 d | 3.11 c | 3.26 d |
| G25 | 10.3 b | 9.5 d | 0.14 d | 0.07 d | 74.2 d | 133.7 b | 3.25 b | 3.47 c |
| G26 | 8.1 c | 9.1 d | 0.14 d | 0.09 c | 57.0 e | 105.3 c | 3.22 c | 3.14 e |
| G27 | 6.1 d | 9.8 d | 0.20 b | 0.07 d | 30.5 f | 148.7 b | 3.13 c | 3.12 e |
| G28 | 6.2 d | 8.0 e | 0.09 e | 0.07 d | 66.8 e | 122.6 c | 3.26 b | 3.40 c |
| G29 | 10.1 b | * | 0.09 e | * | 113.5 c | * | 3.25 b | * |
| G30 | 7.7 c | * | 0.16 c | * | 47.7 e | * | 3.29 b | * |
| G31 | 10.3 b | * | 0.29 a | * | 35.2 f | * | 3.19 c | * |
| G32 | 9.2 b | * | 0.23 b | * | 39.5 e | * | 3.15 c | * |
| G33 | 8.8 c | 11.9 b | 0.14 d | 0.08 c | 62.5 e | 146.4 b | 3.43 a | 3.39 c |
| G34 | 8.9 c | 11.2 c | 0.14 d | 0.08 c | 64.7 e | 145.8 b | 3.36 b | 3.63 b |
| G35 | 8.0 c | 9.9 d | 0.09 e | 0.07 d | 85.6 d | 148.7 b | 3.33 b | 3.43 c |
| G36 | 10.2 b | 9.8 d | 0.18 c | 0.07 d | 56.5 e | 136.7 b | 3.39 b | 3.57 b |
| G37 | 11.3 a | 12.4 b | 0.14 d | 0.07 d | 80.3 d | 170.0 a | 2.78 e | 3.44 c |
| G38 | 9.5 b | * | 0.09 e | * | 110.0 c | * | 3.57 a | * |
| G39 | 10.9 a | 14.1 a | 0.14 d | 0.09 c | 80.3 d | 160.8 a | 3.48 a | 3.50 c |
| G40 | 10.0 b | 10.9 c | 0.23 b | 0.08 c | 42.7 e | 129.0 b | 3.29 b | 3.56 b |
| G41 | 11.2 a | 9.2 d | 0.17 c | 0.07 d | 66.5 e | 135.6 b | 3.30 b | 3.24 d |
| G42 | 8.3 c | 9.8 d | 0.14 d | 0.11 b | 58.7 e | 90.6 d | 3.13 c | 3.38 d |
| G43 | 9.2 b | 13.0 b | 0.12 d | 0.10 b | 77.7 d | 135.4 b | 3.42 a | 3.57 b |
| G44 | 9.5 b | 9.7 d | 0.10 d | 0.07 d | 95.2 d | 137.8 b | 3.23 c | 3.28 d |
| G45 | 10.0 b | 10.2 c | 0.11 d | 0.07 d | 91.9 d | 153.3 a | 2.93 d | 3.28 d |
| G46 | 11.5 a | 8.2 e | 0.09 e | 0.10 b | 135.1 b | 78.6 d | 3.40 b | 3.54 b |
| G47 | 11.2 a | * | 0.11 d | * | 104.2 c | * | 3.32 b | * |
| G48 | 11.4 a | 10.4 c | 0.07 e | 0.07 d | 163.4 b | 159.3 a | 3.24 b | 3.36 d |
| G49 | 8.1 c | 11.0 c | 0.15 c | 0.09 c | 54.3 e | 127.3 b | 3.04 d | 3.30 d |
| G50 | 9.1 c | 10.0 d | 0.09 e | 0.10 b | 97.4 d | 98.9 c | 3.43 a | 3.75 a |
| Mean | 9.2 | 10.1 | 0.15 | 0.08 | 69.1 | 124.2 | 3.24 | 3.39 |
| Standard deviation | 1.6 | 1.7 | 0.05 | 0.02 | 31.6 | 26.1 | 0.18 | 0.15 |

* There was no fruiting.

Means followed by the same letter do not differ considering the difference of 1 standard deviation from the mean.

Comparing with other native fruits of the same family, TSS values found in cherry-of-the-rio-grande fruits were slightly lower than those determined in the jabuticaba fruit Plinia cauliflora (Mart.) Kausel species, with an average of 14.31° Brix (Semensato et al., 2020) and 13.4° Brix (Zerbielli et al., 2016); similar to that obtained in the species of jabuticaba fruit Myrciaria jabuticaba Berg., with an average of 10.6° Brix (Rufini et al., 2020), in the yellow fruit araçá (Psidium guineense Swartz) (11.0° Brix) (Melo et al., 2013) and in red (11.5° Brix) and orange (11.8° Brix) brazilian cherry (Eugenia uniflora L.), but slightly lower than in purple colored fruits (13.8° Brix) (Bagetti et al., 2011).

Cherry-of-the-rio-grande fruits are characterized by low acidity. Total titratable acidity (TTA), determined in 2018, ranged from 0.07 to 0.29% citric acid, with an average of 0.15%. Genotypes G23 (0.26%) and G31 (0.29%) showed greater acidity, followed by another eight genotypes with 0.20 to 0.24% (Table 3). The other genotypes (80%) showed values below 0.19%, considered a positive characteristic. In 2019, possibly due to the greater number of luminosity hours and water stress conditions, as already reported, TTA was much lower than the previous harvest, with an average of 0.08% citric acid, varying from 0.06 to 0.13%. Fruits with higher TTA were produced by genotypes G2, G4 and G16, accompanied by five other genotypes, with concentrations between 0.10 and 0.13%. Genotypes G1, G18, G20, G28, G35 and G48 showed the lowest TTA values for the second year. If compared to the research by Lopes (2009), which found an average of 0.93% to 1.63% of TTA, the values found were much lower.

Unlike TSS content, the results obtained from TTA are much lower than those presented by other fruits of Myrtaceae family. In jabuticabas, contents of 0.44% (Zerbielli et al., 2016), 0.93% (Rufini et al., 2020) and 2.24% (Semensato et al., 2020) were verified. In araçá from 1.02% to 1.21% (Melo et al., 2013), and in brazilian cherry of different colors from 1.63% to 1.87% (Bagetti et al., 2011).

The low TTA of fruits in relation to sugar content (TSS) determined high values for the TSS/TTA ratio, mainly in 2019 harvest (Table 3). In 2018, the average was 69.1; ranging from 28.4 to 166.5, with 14% of the genotypes reaching a ratio above 106.6. In 2019 it ranged from 53.6 to 170.0; with an average of 124.2; with 60.5% of the genotypes values above 124.3. In its turn, the low TTA also reflected in the pH of fruits, considered high. In 2018 harvest, with an average of 3.24; it ranged from 2.62 to 3.57; while in 2019 it ranged from 3.12 to 3.75; with an average of 3.39 (Table 3).

Genetic divergence evaluation between the genotypes was carried out for the two harvests, although in 2019 twelve genotypes were not evaluated, due to the lack of fruiting, and water regime has characterized the occurrence of a drought period during flowering and fruit development.

The relative contribution of the characters to divergence was well distributed. In 2018 it ranged from 7.68% to 15.95% (Table 4). However, three characters contributed more than 10% each to the divergence of genotypes, which were TSS (15.95%), followed by TTA (12.03%) and pH (10.73%), representing 38.71% (Table 4). On the other hand, in 2019 the contribution of characters ranged from 7.80% to 12.99%, but unlike the previous harvest, the largest contribution was the fresh mass of fruit (12.99%), followed by the transversal diameter (11.2%), pH (10.99%) and number of seeds per fruit (10.12%), totalizing 45.3%.

**Table 4.** Relative contribution to genetic divergence of fruits characters of 50 genotypes (in 2018) and 38 genotypes (in 2019) from *Eugenia involucrata* using the Singh method (1981). Serafina Corrêa, RS.

| Fruits characters | Relative contribution (%) | |
|---|---|---|
| | Harvest 2018 | Harvest 2019 |
| Longitudinal diameter of fruit | 8.78 | 7.89 |
| Transversal diameter of fruit | 8.98 | 11.20 |
| Fresh mass of fruits | 9.39 | 12.99 |
| Number of seeds per fruit | 9.37 | 10.12 |
| Fresh mass of seeds per fruit | 7.68 | 9.32 |
| Pulp percentage | 8.24 | 9.71 |
| Total titratable acidity (TTA) | 12.03 | 9.34 |
| Total soluble solids (TSS) | 15.95 | 9.93 |
| TSS/TTA ratio | 8.85 | 8.51 |
| pH | 10.73 | 10.99 |

The tested hierarchical clustering methods presented, for 2018 and 2019 harvests respectively, the following cophenetic correlations: 0.81 and 0.83 (Simple-linkage); 0.80 and 0.85 (Complete-linkage); 0.72 and 0.77 (Ward's method); and 0.84 and 0.86 (UPGMA). Due to the higher correlation values, the UPGMA method was considered more suitable for representing genetic divergence in both harvests.

Considering that characters with the greatest relative contribution differed between harvests (Table 4), there was a marked difference in the formation of groups regarding genetic similarity, demonstrating how much the biotic and abiotic conditions of each year can interfere in the results, despite the difference in the number of genotypes evaluated.

The measure of dissimilarity estimated by the average Euclidean distances (D), considering all the evaluated characteristics, revealed that in 2018: G10 and

G42 genotypes were more divergent (0.61); followed by G50 in relation to G1 and G10 (0.58); G4 with G41 and G50 (0.57); G7 and G50; and G10 in relation to G1 and G9 (0.56). The genotypes G8 and G19; and G33 and G44 were the most similar (0.03), followed by: G2 and G13; G5 and G16; G12 and G23; G11 and G44 (0.04). From the generated matrix, the dendrogram was elaborated, which considering its cutoff point at 42%, created 13 groups total, being five groups of five genotypes, five groups of four genotypes, one group of three genotypes and two groups of one genotype (G1 and G10), as shown in Figure 1.

In the 2019 harvest, values of the average Euclidean distance revealed as the most similar genotypes: G2 and G17; G4 and G45; G13 and G27; G15 and G28; G27 and G43; G37 and G50 (0.03). The most divergent were G39 and G40; G40 and G41 (0.52); followed by G13 and G42; G36 and G48; G41 and G42 (0.51). The matrix formed 15 groups, considering its cutoff point at 28%, with three groups consisting of four genotypes, five groups by three genotypes, four groups of two genotypes and three groups of one genotype (G41, G42 and G45) (Figure 2).

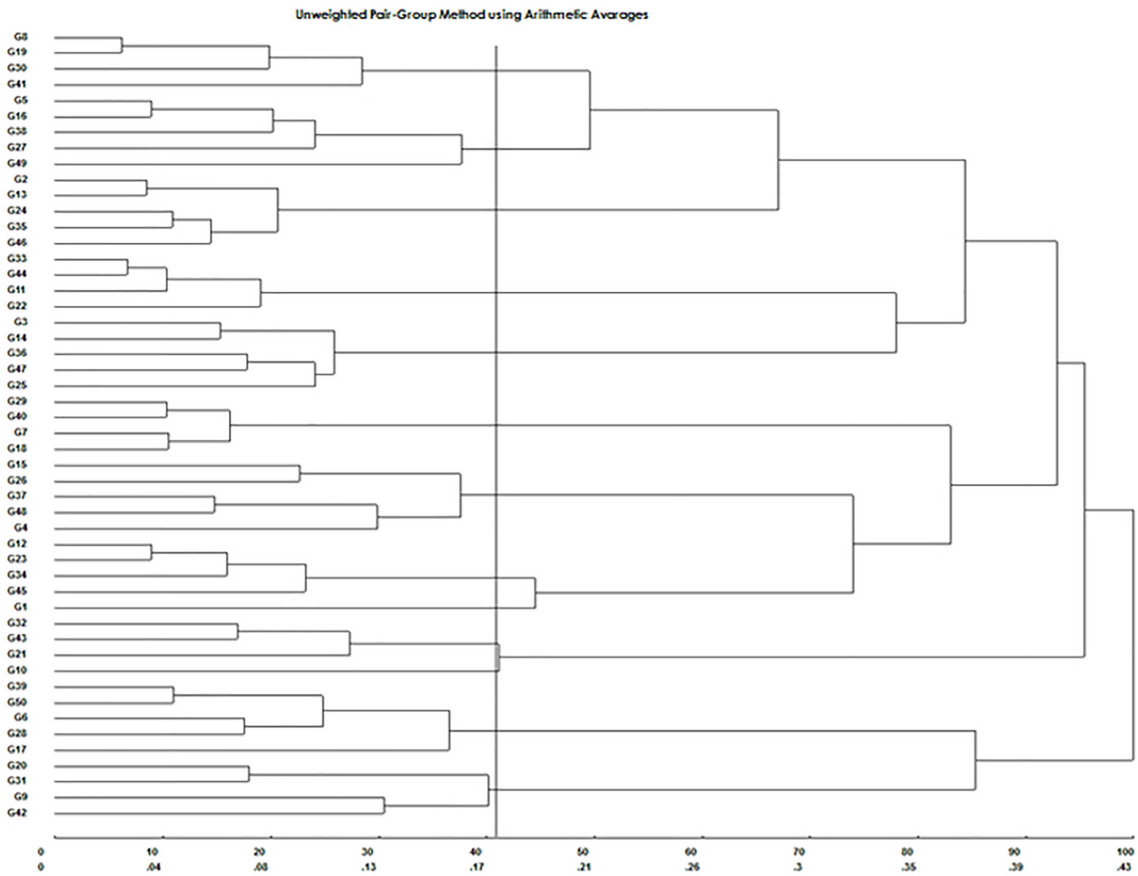


**Figure 1.** Dendrogram obtained from the average Euclidean distance matrix, considering ten physicochemical characters of fruits of 50 genotypes from *Eugenia involucrata*, harvest of 2018. Cophenetic correlation coefficient = 0.84. Serafina Corrêa, RS.

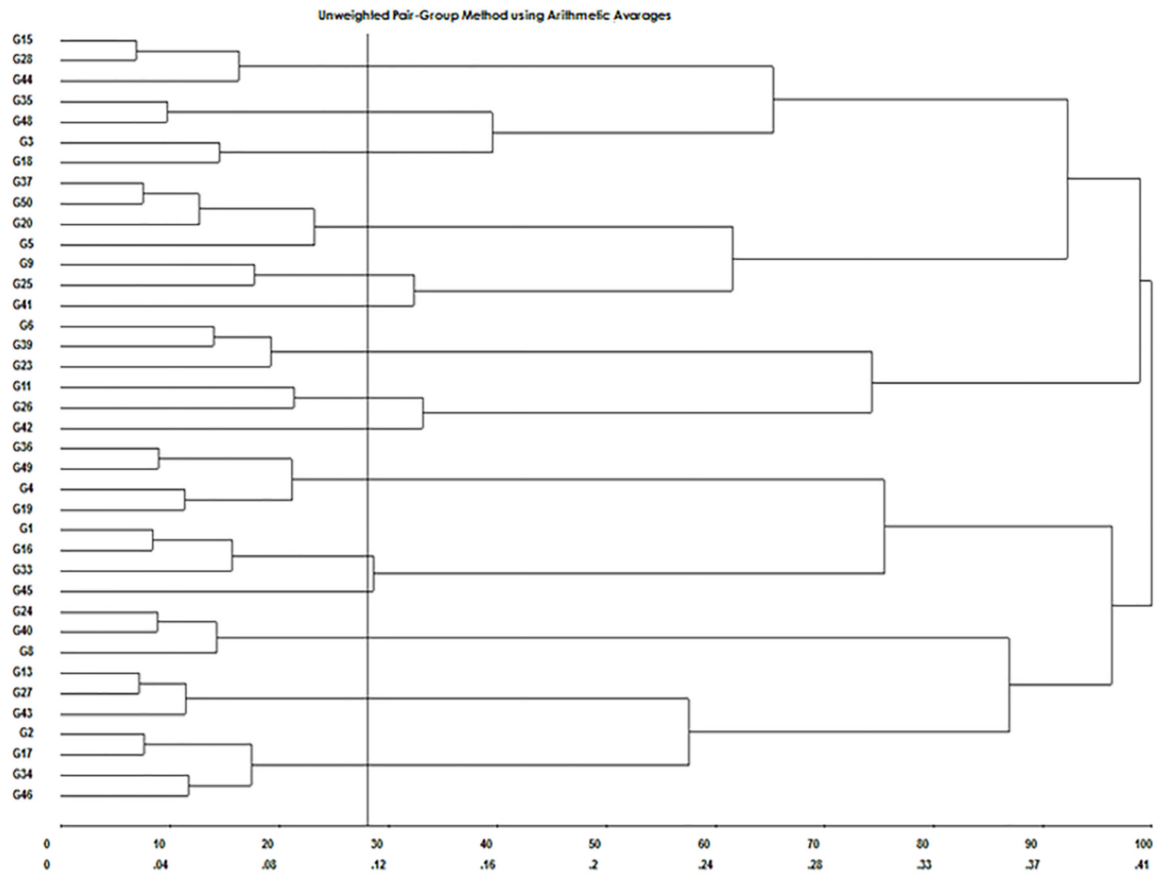


**Figure 2.** Dendrogram obtained from the average Euclidean distance matrix, considering ten physicochemical characters of fruits of 38 genotypes from *Eugenia involucrata*, harvest 2019. Cophenetic correlation coefficient = 0.86. Serafina Corrêa, RS.

The grouping by Tocher method agrees with that obtained by UPGMA, only with the addition of one more group in 2018, represented by G26, forming 14 groups (Table 5). In 2019 there is no change in the number and formation of groups.

The characteristics of fruits vary from one year to another, due to the combination of biotic and abiotic factors (water regime), resulting in changes in the characters that most contribute to the divergence and formation of similar genotype groups.

**Table 5.** Groups established by Tocher method considering ten physicochemical characters of genotype fruits from *Eugenia involucrata*, harvests 2018 and 2019. Serafina Corrêa, RS.

| Groups | Genotypes | |
|---|---|---|
| | Harvest 2018 | Harvest 2019 |
| 1 | 8, 19, 30, 41 | 15, 28, 44 |
| 2 | 33, 44, 11, 22 | 13, 27, 43 |
| 3 | 2, 13, 24, 35, 46 | 37, 50, 19, 5 |
| 4 | 12, 23, 34, 45 | 2, 17, 34, 46 |
| 5 | 5, 16, 38, 27, 49 | 1, 16, 33 |
| 6 | 29, 40, 18, 7 | 24, 40, 8 |
| 7 | 39, 50, 28, 6, 17 | 36, 49, 4, 19 |
| 8 | 37, 48, 15, 4 | 35, 48 |
| 9 | 3, 14, 36, 47, 25 | 6, 39, 23 |
| 10 | 32, 43, 21 | 3, 18 |
| 11 | 20, 31, 42, 9 | 9, 25 |
| 12 | 26 | 11, 26 |
| 13 | 1 | 42 |
| 14 | 10 | 45 |
| 15 | - | 41 |

**Conclusions**

The physicochemical characteristics of fruits demonstrate that there is genetic divergence between Eugenia involucrata genotypes, allowing the selection of agronomically superior plants.

The UPGMA and Tocher clustering methods are more efficient in representing the diversity between genotypes.

The content of total soluble solids (TSS), in 2018 harvest, and the fresh mass of fruits, in 2019 harvest, are the characters that most contribute to genetic divergence.

Considering the two harvests, the genotypes G19, G20 and G37 stand out due to their larger fruit size and pulp yield, while G4, G5, G37 and G39 due to the higher levels of TSS, with low TTA.

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