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Characterization of *Eugenia uniflora* accessions: a native species with great commercial potential in America

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ABSTRACT - Surinam cherry (*Eugenia uniflora* L.) is a fruit species with the potential for economic exploration. The objective of this study was to evaluate the genetic variability and cytological stability in 40 accessions of Surinam cherry in the State of Rio Grande do Sul. We used 18 molecular markers of RAPD (Random Amplified Polymorphic DNA) to access the genetic variability. The cytological analysis included meiotic behavior, viability, and *in vitro* germination of pollen and counting the anthers number. The analysis of genetic variability allowed the separation of the accessions into three groups with an average of 9.5 bands amplified by the primer; 8.39 polymorphic bands; 88.57% of polymorphism and 86% genetic similarity. In the cytological analysis, the average normal meiotic cells were 85.07%; average pollen viability was 90.47%; *in vitro* germination of 44.33%; and the anther/flower average of 54.53. Therefore, the accessions have high genetic similarity and cytological stability.

Keywords: cytological analysis, genetic diversity, Myrtaceae

RESUMO - Caracterização de acessos de *Eugenia uniflora*: uma espécie nativa com elevado potencial comercial. A pitangueira (*Eugenia uniflora* L.) é uma frutífera com potencial para exploração econômica. O objetivo deste trabalho foi avaliar a variabilidade genética e a estabilidade citológica em 40 acessos de pitangueira no Rio Grande do Sul. A determinação da variabilidade genética foi feita com 18 marcadores moleculares RAPD (Random Amplified Polymorphic DNA). As análises citológicas consistiram na determinação do comportamento meiótico, viabilidade e germinação *in vitro* do pólen e contagem do número de anteras. A análise da variabilidade genética permitiu a separação dos acessos em três grupos com uma média de 9,5 bandas amplificadas por primer; 8,39 bandas polimórficas; 88,57% de polimorfismo e 86% de similaridade genética. Na análise citológica, a média de células meióticas normais foi de 85,07%; a viabilidade média do pólen foi de 90,47%; a germinação *in vitro* de 44,33% e a média de anteras/flor de 54,53. Portanto, os acessos possuem alta similaridade genética e estabilidade citológica.

Palavras-chave: análise citológica, diversidade genética, Myrtaceae

INTRODUCTION

The Myrtaceae family has 3.800 at 5.800 species, distributed in 150 genera, occurring mainly in tropical and subtropical regions of the world, with diversity centers in Australia, Southeast Asia, and America. Their species are taxonomically complex, have many components, and scarcity of studies (Seraglio *et al*. 2018, Rodrigues *et al*. 2020, Souza *et al*. 2020). This family has some fruits with economic potential but still unimpressive because they remain appreciated as wild plants or commercialized on a small scale (Seraglio *et al*. 2018).

A large number of Myrtaceae fruits are used for human consumption, often fresh or processed as the fruits are rich in iron, calcium, phosphorus, and vitamin A. Some fruit chemical such as vitamins, flavonoids, anthocyanins, and other phenolic compounds can act reducing the harmful effects of oxidative stress and preventing the deterioration of physiological functions, and decrease the chances of occurrence of some types of cancer, neurodegenerative and cardiovascular diseases (Chang, *et al*. 2016, Ferrari *et al.* 2019). In medicine, they are used as anesthetics, diuretics, antipyretics, and in hypertension and diarrhea treatments (Wilson *et al*. 2001, Romagnolo & Souza 2004, Rodrigues *et al*. 2020).

In this family, the genus *Eugenia* stands out as one of the largest one, composed of about 1000 species with distribution from Mexico to northern Argentina; in Brazil, there are more than 400 species (Marchiori & Sobral 1997, Guollo *et al*. 2020). Those native fruits are popular

and known because of the largest number of species with economic potential (Guollo *et al*. 2020, Souza *et al*. 2020). They are often found in domestic orchards and occupy a prominent place in natural ecosystems (Veit *et al*. 2019, Guollo *et al*. 2020).

In the genus *Eugenia*, one of the main species is the Surinam cherry or Brazilian cherry (*Eugenia uniflora*), which originates from the region that extends from Central Brazil to northern Argentina, Paraguay, and Uruguay. In Brazil, the species is distributed throughout most of the territory, from Pernambuco (PE) to Rio Grande do Sul (RS), and in this state, it occurs in all vegetation formations (Marchiori & Sobral 1997, Bezerra *et al*. 2004).

Surinam cherry can be characterized as multiple-purpose species because it can be consumed in its fresh form processed like jams, sweets, juices, ice cream, liqueurs and, good quality wines (Bezerra *et al*. 2004, Franzon *et al*. 2010). These fruits are rich in vitamins, as vitamin-C (26.3 mg/100g) and Vitamin-A (*i.e.*, 1200-2000 IU), and antioxidants; the leaves are used in traditional medicine, mainly in the treatment of wounds, infections, diarrhea, rheumatism, fever, and diabetes, and has antioxidant substances and essential oils with great economic value for the cosmetic industry (Marchiori & Sobral 1997, Santos *et al*. 2010, Pavithra *et al*. 2020). Besides, the species is regarded as an important ornamental and fruit tree, especially for the wild fauna, often included in recomposition projects in degraded or preserved areas, landscaping, and domestic orchards (Marchiori & Sobral 1997).

The Surinam cherry cultivation has been increasing every year and this expansion can be associated with its versatility, making this Myrtaceae species fruit one with the most potential for economic exploration in several segments and especially in market niches eager for novelties (Franzon *et al*. 2010). On the other hand, most of the existing orchards do not use defined cultivars, which usually come from seed-propagated plants, resulting in great variability due to gene recombination, being non-uniform, with low productivity, and fruits with low pulp yield and high perishability (Bezerra *et al*. 2004, Franzon *et al*. 2010). To obtain cultivars with desirable characteristics, germplasm characterization and evaluation studies are necessary to identify and select genotypes with meiotic stability, pollen grains with high viability and genetic variability, which may be indicated for commercial plantations as well as used in hybridizations. The objective of this study was to evaluate the genetic variability and cytological stability in 40 accesses of *Eugenia uniflora* collected in several regions of Rio Grande do Sul.

MATERIALS AND METHODS

Forty cherry free accessions (that is, individuals) from natural populations were collected for the evaluation of genetic diversity and cytological analyses. The sample places in the state of Rio Grande do Sul where the accessions were collected and the geographic coordinates are in Tab. 1.

Ten young leaves of each access were collected from all parts of the plant. The leaves were macerated in bulk for DNA extraction and genetic diversity evaluation using the protocol proposed by Ferreira & Grattapaglia (1998).

The quantity and quality of DNA were evaluated by applying the samples to 1% agarose gel, which was stained with ethidium bromide (0.5 ng/ml) and submitted to electrophoresis for an hour at 110 V. The quantification was performed by comparison with λ50, λ100, λ200 and λ500 Lambdas standards and the DNA quality was evaluated by the absence of DNA traces (Guerra *et al.* 2016).

The genetic diversity was obtained using RAPD ("Random Amplified Polymorphic DNA") molecular markers. Of the 20 RAPD primers tested, 18 were selected for polymorphism and good amplification profile. The reaction had a total volume of 25 uL containing: 10 mM of Tris-HCl, pH 8.3; 50 mM of KCl; 2.0 mM of MgCl2; 0.4 mM of dNTPs; 0.25 uM of primer; 5.0 ng of DNA; 1 unit of Taq DNA Polymerase and ultrapure water to complete the volume. The amplification of specific regions was performed using PCR technique in a thermocycler, being performed for 48 cycles: 92°C for 30 seconds; 37°C for 1 minute and 30 seconds; and 72°C for 1 minute and 30 seconds. Preceding the cycle, an initial denaturation was performed at 94°C for 5 minutes and, afterward, a final extension at 72°C for 5 minutes. After the amplification, the samples were applied to agarose gel (1.8%) and compared with the standard known size (Gibco BRL with 100 base pairs). The time for the electrophoretic separation was of two hours at 110 volts. The gels were photographed and the fragments were determined by comparison with the standard 100pb, using Kodak EDAS 290 (Electrophoresis Documentation and Analysis System) software. The results were analyzed by observing the gels and generating a binary matrix in which the individuals were genotyped regarding the presence (1) or absence (0) of bands, creating a matrix to calculate the genetic similarity and the dendrogram using the R program (The R core team 2020). These data were also evaluated regarding the total number of amplified bands, number of bands, and the percentage of polymorphic bands of each primer.

For the cytological analysis, flowers were collected and fixed for 24 hours in an ethanol and acetic acid solution/ in a ratio of 3:1, and were then transferred to 70% ethanol and stored in a freezer. For the evaluations, slides were prepared with the anthers of each flower, which are macerated and stained with propionic carmine dye (2%) (Guerra *et al*. 2013). For the analysis of meiotic behavior, ten flowers per accession and at least ten meiotic cells with good chromosome spreading per slide and at all stages of meiosis were evaluated. For the estimation of pollen viability, ten slides were analyzed, randomly counting 1,000 pollen grains, making a total of 10,000 grains per accession (Dahmer *et al*. 2013). Viability was estimated according to coloring capacity; the grains were considered viable when stained and non-viable when empty or colorless, and this was determined by dividing the number of stained pollen grains by the total number of grains observed and multiplying the result by 100 (Dahmer *et al*. 2013).

Table 1. Locations and geographical coordinates of 40 Surinam Cherry accessions collected in the state of Rio Grande do Sul.

N° access	Municipality	Latitude (S)	Longitude (W)
1	Venâncio Aires	29° 36' 22''	52° 11' 40''
2	Porto Alegre	30° 03' 40''	51° 12' 27''
3	Gravataí	29° 56' 9''	51° 11' 54''
4	Gravataí	29° 56' 9''	51° 12' 04''
5	Cachoeirinha	29° 57' 04''	51° 05' 38''
6	Porto Alegre	30° 03' 40''	51° 12' 37''
7	Porto Alegre	30° 03' 40''	51° 13' 47''
8	Porto Alegre	30° 03' 40''	51° 14' 57''
9	Santa Cruz do Sul	29° 43' 03''	52° 25' 33''
10	Charqueadas	29° 57' 18''	51° 37' 31''
11	Eldorado do Sul	30° 05' 02''	51° 36' 58''
12	Eldorado do Sul	30° 05' 02''	51° 36' 18''
13	Eldorado do Sul	30° 05' 02''	51° 37' 08''
14	Eldorado do Sul	30° 05' 02''	51° 37' 18''
15	Eldorado do Sul	30° 05' 02''	51° 38' 12''
16	Eldorado do Sul	30° 05' 02''	51° 40' 18''
17	Porto Alegre	30° 03' 40''	51° 15' 17''
18	Porto Alegre	30° 03' 40''	51° 16' 37''
19	Canoas	29° 55' 8''	51° 18' 21''
20	Canoas	29° 55' 8''	51° 32' 04''
21	Esteio	29° 51' 40''	51° 10' 51''
22	Porto Alegre	30° 03' 40''	32° 08' 44''
23	Estrela	29° 30' 17''	51° 58' 11''
24	Campina das Missões	27° 59' 20''	54° 50' 22''
25	Venâncio Aires	29° 36' 22''	52° 11' 40''
26	Guaporé	28° 51' 03''	51° 53' 10''
27	Santa Cruz do Sul	29° 43' 03''	52° 25' 33''
28	Porto Alegre	30° 03' 40''	51° 12' 28''
29	General Câmara	29° 29' 22''	51° 21' 12''
30	Eldorado do Sul	30° 05' 02''	51° 36' 58''
31	Três Passos	27° 27' 31''	53° 55' 49''
32	Bom Progresso	27° 32' 37''	53° 51' 57''
33	São Luiz Gonzaga	28° 24' 31''	54° 57' 41''
34	São Borja	28° 40' 58''	55° 58' 39''
35	Tenente Portela	27° 22' 15''	53° 45' 28''
36	São Borja	27° 21' 29''	53° 59' 42''
37	Tenente Portela	28° 23' 19''	53° 54' 56''
38	Esperança do Sul	28° 55' 55''	52° 19' 19''
39	Ijuí	28° 40' 58''	55° 58' 39''
40	Campo Novo	27° 22' 15''	53° 45' 28''

For the analysis of in vitro pollen germination, flowers at the maturity stage and with closed petals were collected. The methodology of culture medium used was according to Guerra *et al*. (2016). Four slides per population were evaluated by counting the number of randomly selected germinated and non-germinated grains in 250 pollen grains per slide, totaling 1,000 grains per accession.

To count the number of anthers, ten flowers from each accession were selected, the petals and sepals were removed with the aid of needles and a scalpel under a magnifying glass and then separated and later counted.

The results obtained with cytological analysis, in vitro pollen germination and number of anthers the 40 Surinam cherry accessions were compared by the t test, according to Beiguelman (2002).

RESULTS AND DISCUSSION

The fragments ranged from 100 to 1300 base pairs, obtaining a total of 171 bands. The primers OPA-03, OPN-06, and OPN-08 produced the largest number of bands (12), whereas the primer OPA-08 produced only six bands. The mean of bands per primer was 9.5 and the mean of polymorphic bands was 8.39. The primers OPA-02, OPA-03, OPA-04, OPA-09, OPA-10, OPA-11, OPN-04, OPN-05, OPN-06, OPN-08, and OPN-09 were the most efficient in the evaluation, producing from ten to twelve bands each (Tab. 2).

The mean polymorphism of all primers was 88.57% (Tab. 2). This result is very significant in terms of the contribution of the study, as well as in the reliability of the results and according to Areias *et al*. (2006) the higher the polymorphism observed in molecular analyzes, the higher the degree of contribution and reliability of the study. Furthermore, the high polymorphism values are in agreement with the results observed by Aguiar *et al*. (2013) who used RAPD-type markers and obtained 79% polymorphism with accessions in an area at an advanced stage of succession and 70% in an area at an initial stage of succession of Surinam cherry populations. They also corroborate with other authors, who evaluated the genetic diversity of Surinam cherry populations with AFLP (Amplified Fragment Length Polymorphisms) markers and obtained similar values, such as Margis *et al*. (2002) with 91% polymorphism among populations of Surinam cherry in Rio de Janeiro. On the other hand, Salgueiro *et al*. (2004), obtained 78.05% in fragments of tropical forest and Franzon *et al*. (2008) 64% of polymorphic loci in two populations in Pelotas, Rio Grande do Sul.

Several types of molecular markers have been used for the analysis of genetic diversity in different species, but there are few records for native species of the South of Brazil (Franzon & Raseira 2004), mainly species of the Myrtaceae family; which may be associated with the presence of polyphenols that hinder DNA extraction (Almeida *et al*. 2012). In this study, RAPD markers were used because it is a simple, fast, and relatively inexpensive technique with informative potential, although it presents repeatability problems (Areias *et al*. 2006).

The results obtained with the molecular analyses of the 40 accessions of Surinam cherry allowed the construction of a genetic similarity dendrogram (Fig. 1) which revealed the formation of three groups.

Group I consists of one accession (1), group II by seven (8, 36, 34, 3, 5, 2, and 6), and group III by the remaining 32 accessions. The groupings were not directly related to the origin of the materials, since Group I was constituted by only one accession from the city of Venâncio Aires (1), while the other one collected in this same city (accession 25) was in the third group. Group II, composed of seven accessions, seems to be related to the geographical distance since in this group there are three out of the seven accessions from Porto Alegre (2, 6 and 8), as well as two from the metropolitan area (Gravataí and Cachoeirinha, 3 and 5, respectively). On the other hand, also as part of this group, there are two accessions collected in distant areas like an accession from Tenente Portela (36) and another from São Borja (34). In group III are the other accessions collected in several locations of the state (Fig. 1).

In this study, the average genetic similarity between the accessions was 84% (Fig. 1). Values similar to those obtained by Aguiar *et al*. (2013) with 86% similarity in Surinam cherry accessions in an area the initial successional stage and 78% in an area of an advanced successional stage with RAPD markers and by Franzon *et al*. (2010) who obtained 60.4% similarity in Surinam cherry populations using AFLP markers. However, Margis *et al*. (2002) and Salgueiro *et al*. (2004) observed 88% and 78.9% of genetic diversity, respectively, within Surinam cherry populations using AFLP markers.

The molecular characterization is useful to evaluate the genetic similarity, to elaborate conservation strategies, and, when associated with botanical, chemical, and functional data, may be decisive in the incorporation of the species into the regional productive systems (Oliveira *et al*. 2018). Therefore, the genetic similarity among the studied access of Surinam cherry by RAPD molecular markers means demonstrated the efficiency of the technique.

In terms of the contribution of the study, genetic similarity analysis is very important because several species of the Brazilian flora have already been studied to obtain fruits and bioactive molecules potentially useful to the human population. Unfortunately, many other species became extinct before their capabilities were evaluated. Therefore, the establishment of conservation and management strategies to maximize the genetic variability within species is only possible through the measurement of the genetic variability in the populations (Donazzolo *et al*. 2020). Therefore, knowledge of genetic similarity is an important step for the efficient selection of species of commercial interest and in the process of domestication. Depending on the distribution of genetic

variability, strategies can be defined for *in situ* conservation of populations with greater variation, selection of mother plants for implantation and supplementation of *ex situ* collections, as well as identification of the existence of gene flow (White *et al*. 2018).

The collection points of the accessions, as well as the group arrangement according to genetic similarity obtained with the RAPD markers can be visualized in Fig. 2.

The arrangement of the accessions in groups (I, II, and III) allowed us to observe a wide distribution in the collection sites. These results are significant because they will allow the selection of more diverse genotypes to be used as parents in directed crosses. According to Ferreira & Grattaplaglia (1998), assisted selection with molecular markers in plant breeding programs is very advantageous, since it allows the identification and selection of genotypes with greater variability and which are superior in characteristics of interest. Besides, according to these authors, crosses using parents with greater variability allow to obtain new genetic combinations and greater heterosis, allowing for the selection of plants with superior agronomic characteristics afterward. According to these authors, the determination of genetic variability is essential to the pre-breeding and breeding of plants, which reinforces the importance of this work.

In this study, we found in group II accessions from Porto Alegre and the metropolitan region, as well as one accession collected in São Borja, which is located approximately 600 km from the capital (Fig. 2). This result is interesting but does not allow full clarification, since the origin of the accessions is unknown and may infer that they may have some degree of relationship, through anthropogenic seed propagation. According to Bezerra *et al*. (2004), most of the existing Surinam cherry orchards do not use defined cultivars and are generally from seed-propagated plants. Thus, the similarity observed between these accessions may be related to an anthropic factor, that is, the anthropogenic pre-selection of the plants with fruits of considerable size and good flavor, with subsequent seed propagation. Another factor that may be associated with the results obtained is the evolution of the genotypes due to the conditions under which they are submitted, which would be in agreement with Franzon *et al*. (2010), who consider that genetic variability is linked to ecological processes, which force plants to adapt to the different locations where they occur. Therefore, high genetic variation will support a population to adapt to changes that occur in the surrounding environment and biodiversity will be maintained (Barantal *et al*. 2019).

The molecular RAPD markers have some limitations due to low repeatability and reproducibility. However, other markers are still being tested for the Myrtaceae family, as SSR (simple sequence repeat) markers that can be more effective (Stefenon *et al*. 2019). Nogueira *et al*. (2016) evaluated the transferability of 158 SSR from *Psidium guajava* L. in 18 fruit trees of the genera *Eugenia* and obtained promising results, but further studies are needed to validate these markers in larger populations. Sarzi *et al*. (2019) using the methodology described by Lemos *et al*. (2018) used the new generation sequencing technology to identify microsatellite in *loci*, which were prospected using partial sequencing of the *E. uniflora* genome. The authors developed 69 SSR markers, of which, after in silico evaluation, 30 primers prospected, however, when tested in the laboratory, they were partially successful, with only 12 markers showing amplification and with reduced band quality (Beise 2018). For the authors, there was the possibility of using these markers in genetic studies, mainly to understand the species biology more clearly; however, more studies should be developed to recommend them for this species.

In the cytological analyses of the 40 Surinam cherry accessions, the value of the mean of normal meiotic cells was 85.07%, with a variation ranging from 82.17% to 87.32%. The average viability of the pollen was 90.47%, varying from 80.10% to 98.67%. The mean *in vitro* germination was 44.33%, ranging from 42.62% to 46.76%. The mean number of anthers was 54.53, ranging from 38.94 to 68.23 anthers/flower. In these analyses, the variations between the accessions were observed but without any statistical difference (Tab. 3).

Cytological analyses of species of the Myrtaceae family, particularly regarding meiotic behavior, are scarce. In this study, it was possible to determine the chromosome number of the accessions through the analysis of gametic cells, all of which were characterized as diploids with n = 11 and, by analogy, 2n = 22. This result corroborates with Costa & Forni-Martins (2007) that the Surinam cherry (*E. uniflora*) is a diploid species (2n = 22). In addition, Costa & Forni-Martins, (2006) observed the existence of polyploid plants at low frequencies in Myrtaceae family species. Nevertheless, plants with a distinct level of ploidy were not observed in this study, which allows to indicating them as male parents in crosses, since according to Guerra *et al*. (2013), hybridizations between plants with different levels of ploidy may impair progeny stability.

The meiotic behavior of the evaluated accessions can be considered regular and with few abnormalities such as delayed chromosomes and early disjunction (Tab. 3). Similar results were obtained by Forni-Martins & Martins (2000) who evaluated the meiotic behavior of *Campomanesia pubesce* O. Berg.; *Eugenia aurata* O. Berg., *Myrcia bella* Camb. and *Myrcia lingua* O. Berg., *Eugenia bimarginata* DC and *Psidium acutangulum* DC and observed a predominance of regular meiosis. Costa & Forni-Martins (2006) evaluated the meiotic behavior of four species of the genus *Campomanesia* (*C. adamantium* (Camb.) O. Berg*, C. guaviroba* (DC.) Kiaersk*, C. phaea* O. Berg Landrum and *C. pubesce*s DC O. Berg.) and four species of *Psidum* (*P. acutangulum* DC., *P. cattleyanum* Sabine, *P. cinereum* Mart. ex DC and *P. guajava* L.) and did not observe any meiotic abnormalities.

Table 2. Number of bands and percentage of polymorphism generated by 18 selected RAPD primers and used in 40 Surinam Cherry accessions collected in Rio Grande do Sul.

Primer	Sequence (5'- 3')	Size pb	Total of amplified bands	Total of polymorphic bands	Polymorphism (%)
OPA-02	TGCCGAGCTG	220 – 1300	10	8	80.00
OPA-03	AGTCAGCCAC	120 – 1200	12	11	91.67
OPA-04	AATCGGGCTG	150 – 1300	10	8	80.00
OPA-07	GAAACGGGTG	180 – 1100	8	7	87,50
OPA-08	GTGACGTAGG	100 – 1200	6	6	100.00
OPA-09	GGGTAACGCC	150 – 1300	11	10	90.91
OPA-10	GTGATCGCAG	100 – 1200	10	9	90.00
OPA-11	ACGCGTCTGG	220 – 1100	10	8	80.00
OPA-12	GGGTAACGCC	200 – 1300	8	7	87.50
OPA-13	AGTCAGCCAC	180 – 1300	7	6	85.71
OPN-02	ACCAGGGGCA	150 – 1300	8	8	100.00
OPN-03	GGTACTCCCC	180 – 1200	8	7	87.50
OPN-04	GACCGACCCA	220 – 1200	10	9	90.00
OPN-05	ACTGAACGCC	180 – 1300	11	10	90.91
OPN-06	GAGACGCACA	200 – 1300	12	11	91.67
OPN-08	ACCTCAGCTC	180 – 1200	12	10	83.33
OPN-09	TGCCGGCTTG	100 – 1100	10	9	90.00
OPN-10	ACAACTGGGG	220 – 1300	8	7	87.50
Mean			9.5	8.39	88.57

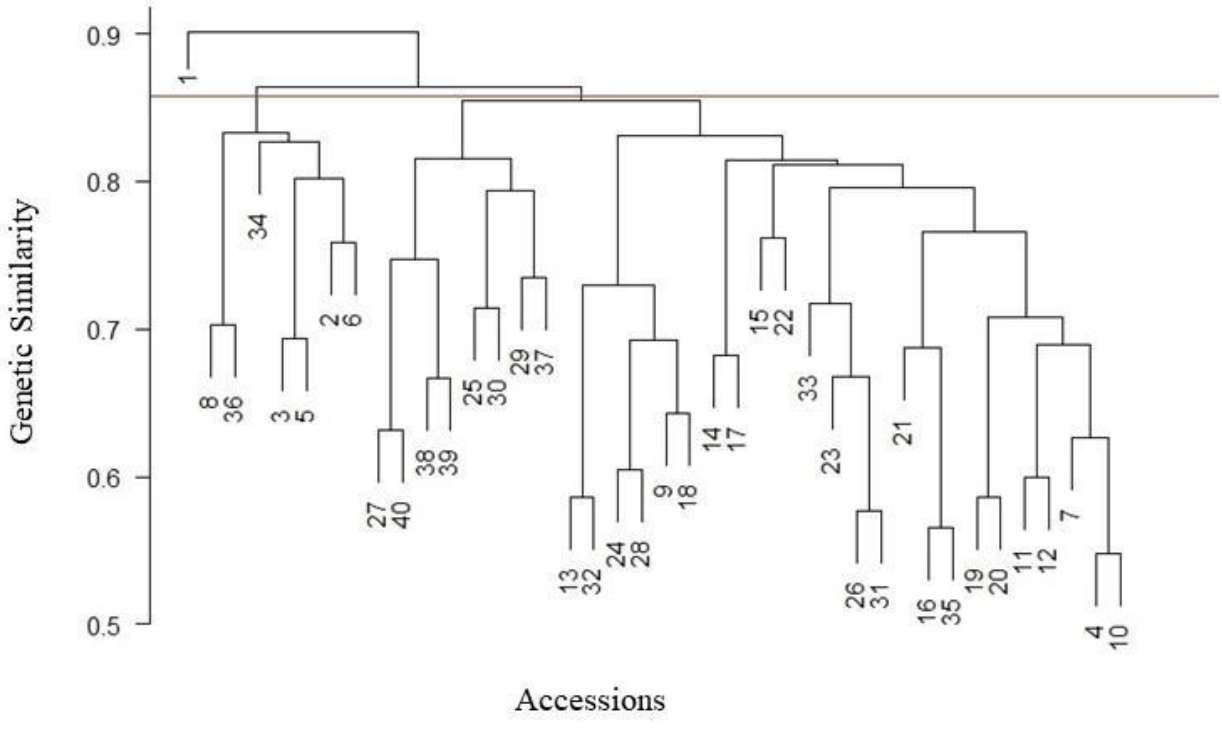

Figure 1. Dendrogram of genetic similarity between the 40 Surinam Cherry, obtained from RAPD markers. The line indicates the 84% cut-off point based on the average similarity between populations.

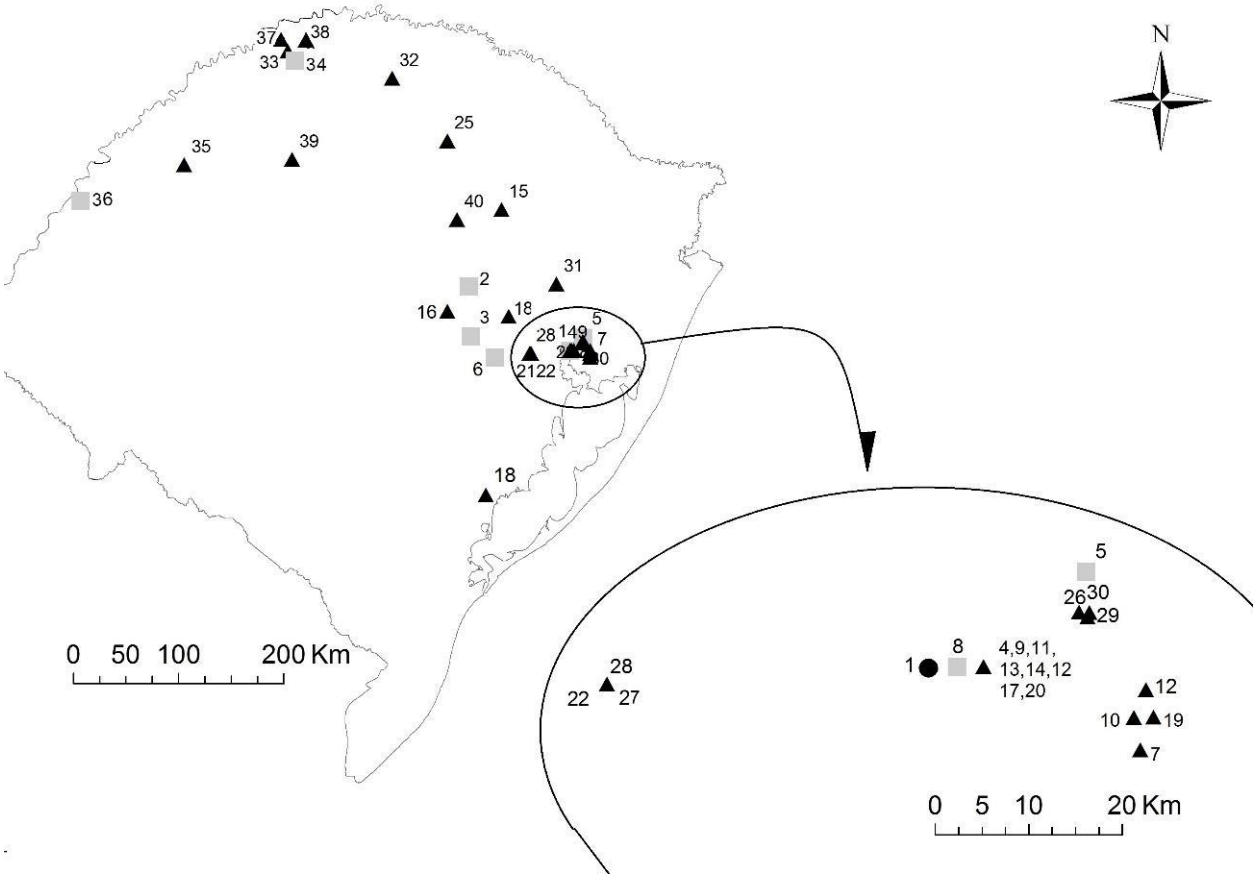

Figure 2. Collection sites of the 40 Surinam Cherry accessions in the state of Rio Grande do Sul with the cluster according to genetic similarity obtained with RAPD markers. Group I =; Group II =; Group III =).

Franzon & Raseira (2004) evaluated the meiotic index in two Surinam cherry accessions and observed considerable stability with values of 84.8% and 88.3%, respectively. These authors did not evaluate the meiotic behavior but inferred that it was regular, since according to Muñoz *et al*. (2006), the normal course of meiosis ensures the viability of the gamete, so the meiotic index and pollen viability are directly related to the normality of the microsporogenesis. This association was already observed by Guerra *et al*. (2011) in triticale (*Triticosecale* Wittmack) and by Guerra *et al*. (2016) in cherry trees (*Eugenia involucrate* DC).

In the pollen analysis, the mean viability was 90.47% (Tab. 3). These values are high, which allows inferring that the accessions can be used as male parents in crossings. These results are in agreement with the one suggested by Guerra *et al*. (2016), which considers that cytological stable plants are those which have a meiotic index and pollen viability greater than 90%. High pollen viability was also observed in four species of the genus *Campomanesia (C. adamantium, C. guaviroba, C. phaea* and *C. pubesce*) and four species of *Psidum* (*P. acutangulum, P. cattleyanum, P. cinereum and P. guajava*) with values greater than 86% (Costa & Forni-Martins 2006).

The result obtained in this study regarding *in vitro* germination of pollen was 44.33% (Tab. 3). This result was higher than that observed by Franzon & Raseira (2004), who obtained values of 35.3% and 33.5%, respectively, in two accessions of Surinam cherry. The results obtained in this study are also higher than those obtained by Franzon *et al*. (2007), who analyzed the *in vitro* germination capacity of Surinam cherry pollen using various types of culture and obtained germination results below 30%. The high viability and *in vitro* germination of the pollen grains (Tab. 3) may allow cross-pollination, which increases genetic variability and the obtaining of superior genotypes.

In this study a mean number of 54.53 anthers/flower was observed when counting the anthers, but with a variation of 38.94 to 68.23 anthers/flower (Tab. 3). The variation was considerable, although the factors responsible for this variation cannot be indicated. When comparing accessions with the highest numbers of anthers and the ones with the greatest genetic diversity we found that accessions 2 and 34 stood out and there may be a direct relation between them for these two variables. Therefore, the assessed accessions showed a great variation in the number of anthers which allows us to infer that the species produces a large amount of pollen for artificial crossings, as well as natural crossings.

Table 3. Cytological analysis of 40 Surinam Cherry accessions collected in the state of Rio Grande do Sul.

No.	Cells in meiosis considered normal (%)	Pollen Viability (%)	Pollen Germination (%)	Average No. Anthers
1	84.12	96.47	43.12	60.82
2	85.79	92.62	44.56	66.16
3	84.56	93.26	43.18	40.58
4	83.98	94.52	45.16	71.51
5	84.34	98.08	44.17	61.72
6	85.68	81.06	44.24	39.81
7	85.04	98.36	43.98	56.93
8	83.56	91.35	45.64	60.62
9	86.72	83.82	44.58	38.94
10	85.34	95.21	43.45	68.27
11	85.04	96.53	44.95	54.13
12	86.48	94.25	44.16	61.98
13	84.32	96.49	45.32	52.17
14	85.16	83.38	43.98	56.62
15	83.98	97.82	45.12	66.03
16	82.17	97.76	42.68	59.34
17	86.24	96.09	44.77	58.85
18	85.13	95.20	44.56	53.88
19	85.68	94.26	46.76	48.43
20	87.32	98.67	46.02	39.71
21	86.54	92.38	45.48	42.45
22	85.89	91.32	45.62	51.25
23	85.96	86.15	45.32	39.82
24	83.32	84.02	45.08	44.74
25	84.45	81.00	45.34	62.12
26	85.27	81.20	44.36	48.56
27	85.67	80.10	44.54	58.91
28	83.38	82.64	43.08	49.59
29	83.52	83.40	43.15	44.45
30	85.87	81.18	44.26	62.18
31	85.34	83.34	44.46	67.91
32	85.16	94.58	42.91	58.42
33	84.48	91.15	44.54	60.82
34	85.68	81.24	43.15	61.78
35	85.06	90.16	43.76	68.23
36	86.04	88.35	42.62	59.37
37	85.78	94.78	44.49	39.79
38	84.16	92.02	43.14	47.93
39	84.48	92.49	44.19	42.54
40	85.90	92.05	43.45	53.86
Mean	85.07	90.47	44.33	54.53

In the last years, several fruit trees such as like Surinam cherry have gained international notoriety due to their peculiar tastes and nutritional potentials. Also, their cultivation became a suitable alternative to the conservation of genetic resources as well as an important source of regional development (Donazzolo *et al*. 2020). Therefore, the accessions evaluated in the present study showed high cytological stability and genetic similarity and can be used in breeding programs as parents in crosses, or used directly in commercial plantations due to the genetic similarity, if they have suitable plant and fruit characteristics.

The evaluated accessions displayed a high level of polymorphism, genetic similarity, and cytological stability, making them viable for direct use in commercial orchards, or as male parents in directed crosses in the breeding program as long as they have suitable plant and fruit characteristics, such as: resistance to pests and diseases, fruits with morphological and chemical quality, as soluble solids, among others. Furthermore, the accessions evaluated showed a great variation in the number of anthers which allows us to infer that the species produces a large amount of pollen for artificial crossings, as well as natural crossings.

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