**Genetic diversity of a native population of *Myrcia ovata* Cambess. using ISSR molecular markers**

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Genetic diversity of *Myrcia ovata*

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**ABSTRACT**

*Myrcia ovata* Cambess. (Myrtaceae) is a medicinal and aromatic plant that has analgesic, bactericidal and fungicidal properties. Even though the plant has economic potential, nothing is known about the variability and genetic diversity of this species. This information is necessary to establish conservation strategies and prospection of natural resources. The aim of this study was to evaluate the genetic diversity of *M. ovata* individuals of a native population in the municipality of Japaratuba, Sergipe State, Brazil, using Inter-Simple Sequence Repeat molecular markers (ISSR). Nine primers were tested, resulting in 99 polymorphic bands. The 24 individuals evaluated were clustered in 2 groups by the software Structure. The Jaccard similarity ranged from 0.21 (MYRO-034 and MYRO-159) to 0.82 (MYRO-178.1 and MYRO-178.2), with an average of 0.38. The genetic diversity of *M. ovata* was considered medium with low tendency. The individuals MYRO-154, MYRO-175 and MYRO-175.1 presented the most variability.

**Key words:** Myrtaceae; Medicinal plant; Genetic variability

**INTRODUCTION**

The *Myrcia* genus is composed of more than 300 species that belongs to the subtribe Myrciinae of the Myrtaceae family. *Myrcia ovata* is an aromatic and medicinal specie originally from South American tropics. In Brazil, it is popularly known as laranjinha-do-mato and is used in traditional medicine against diseases such as gastritis and diarrhea (Limberger et al., 2004; Lucas et al., 2007, Cândido et al, 2010). *M. ovata* has already been registered in the Brazilian states of Alagoas, Bahia, Ceará, Espírito Santo, Minas Gerais, Pará, Paraná, Pernambuco, Rio de Janeiro and São Paulo (SpeciesLink, 2016). In Sergipe, plants were found in an area exposed to human disturbance and fire, therefore, at risk of becoming locally extinct.

Works involving the study of *M. ovata* are quite recent, mainly after 2010 when it was proven that its essential oil has antibacterial activity, thus generating economic interest (Cândido et al., 2010). Since then, new studies discovered that it also has analgesic (Santos et al., 2014) and fungicidal properties (Sampaio et al., 2016).

Several species from the Brazilian flora have already been studied with the aim of obtaining bioactive molecules potentially useful to man. Unfortunately, many others species are already extinct before their capabilities were evaluated. The anthropic destruction of forests and natural ecosystems, habitat of several medicinal species, justifies the need to conduct research that aims for the conservation of these resources currently in risk of genetic erosion. 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 (Lima et al., 2015a).

A study of the chemical diversity in *M. ovata* essential oil was already done in plants from the Brazilian state of Sergipe (Sampaio et al., 2016). However, this is the first study that characterizes the genetic diversity of the specie. This characterization identifies the degree of polymorphism between individuals and populations, regardless of the phenotypic variation and the stage of development (Grattaplagia and Ferreira, 1998).

The analysis of genetic variation of individuals can be obtained by molecular characterization using molecular markers. Developed in the 90s, the molecular marker Inter Simple Sequence Repeats (ISSR) is a dominant marker (binary) that performs an amplification of the DNA chain by Polymerase Chain Reaction (PCR), without the need of prior knowledge of the gene sequence, generating polymorphic standards (Zietkiewicz et al., 1994). The ISSR marker has already been used in studies of variability and genetic diversity in species of plants from the Myrtaceae family, such as *Eucalyptus* spp. (Ballesta et al., 2015), *Psidium* spp. (Oliveira et al., 2014), *Eugenia* spp. (Brunchault et al., 2014) and *Myrcia* spp. (Brandão et al., 2015; Alves et al., 2016).

Since the assessment of genetic variability can be used for conservation and use of genetic resources programs, the aim of this study was to determine the genetic diversity of 24 individuals from the *M. ovata* found in the municipality of Japaratuba-SE, using ISSR molecular marker.

**MATERIAL E METHODS**

**Plant material**

For the extraction of the DNA, fresh leaves of 24 individuals of *M. ovata* were collected in 09/15/2016 in silica from the municipality of Japaratuba, in the State of Sergipe, Brazil (Figure 1 and Table 1). This region has rain forest and dune vegetation. The average of annual rainfall is 1400 mm (Sergipe, Semarh/SRH, 2014) and the rainy season is from March to August. The average annual temperature is 25,3°C and the climate is dry sub-humid mesothermal type (Sergipe, Seplantec/Supes, 2000).

Insert Figure 1 here

**Figure 1.** Map where individuals of *Myrcia ovata* were collected from natural population located at the municipality of Japaratuba, in the state of Sergipe, Brazil.

**Table 1.** Identification of 24 *Myrcia ovata* individuals from a native population located at the municipally of Japaratuba, in the state of Sergipe, Brazil.

|  |  |  |  |
| --- | --- | --- | --- |
| Individuals | Classification | Longitude | Latitude |
| MYRO-154 | Shrub | 10°37’38.1”S | 36°53’17.0”W |
| MYRO-155 | Shrub | 10°37’37.9”S | 36°53’17.4”W |
| MYRO-178  | Shrub | 10°37’38.8”S | 36°53’19.7”W |
| MYRO-178.1  | Shrub | 10°37’38.8”S | 36°53’19.7”W |
| MYRO-178.2  | Shrub | 10°37’38.8”S | 36°53’19.7”W |
| MYRO-156 | Shrub | 10°37’38.6”S | 36°53’19.7”W |
| MYRO-157 | Shrub | 10°37’39.0”S | 36°53’19.7”W |
| MYRO-160 | Shrub | 10°37’37.7”S | 36°53’18.0”W |
| MYRO-813 | Shrub | 10°37’37.7”S | 36°53’18.2”W |
| MYRO-159.1 | Shrub | 10°37’37.3”S | 36°53’17.4”W |
| MYRO-159 | Shrub | 10°37’37.2”S | 36°53’17.4”W |
| MYRO-159.2 | Shrub | 10°37’37.2”S | 36°53’17.4”W |
| MYRO-093 | Shrub | 10°38’45.2”S | 36°52’17.5”W |
| MYRO-162 | Shrub | 10°38’45.4”S | 36°52’16.4”W |
| MYRO-174 | Shrub | 10°38’45.3”S | 36°52’17.0”W |
| MYRO-175 | Shrub | 10°38’45.2”S | 36°52’17.8”W |
| MYRO-175.1 | Shrub | 10°38’45.2”S | 36°52’17.8”W |
| MYRO-176 | Tree | 10°38’44.1”S | 36°52’19.4”W |
| MYRO-029 | Shrub | 10°37’37.8”S | 36°53’17.3”W |
| MYRO-030 | Shrub | 10°37’37.8”S | 36°53’17.3”W |
| MYRO-032 | Shrub | 10°37’38.7”S | 36°53’20.1”W |
| MYRO-033 | Shrub | 10°37’38.7”S | 36°53’20.2”W |
| MYRO-034 | Shrub | 10°37’38.8”S | 36°53’20.4”W |
| MYRO-036 | Tree | 10°38’45.3”S | 36°52’16.3”W |

**DNA extraction and ISSR amplification**

DNA extraction was carried out as described by Nienhuis et al. (1995) with modifications. For PCR-ISSR reaction, 9 primers were used from Invitrogen brand (Thermo Fisher Scientific, Carlsbad, CA, USA) (Table 2).

**Table 2.** Primers information and amplified products from the genetic diversity analysis of *Myrcia ovata* individuals from a native population located at the municipally of Japaratuba, in the state of Sergipe, Brazil.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Name | Sequence (5’-3’) | Length (bp) | Annealing temp. | Total bands | Polymorphic bands | Polymorphism (%) |
| UBC807 | (AG)8-T | 1500 ao 700 | 43°C | 10 | 10 | 100% |
| UBC808 | (AG)8-C | 2000 ao 400 | 47°C | 12 | 12 | 100% |
| UBC809 | (AG)8-G | 2000 ao 400 | 48°C | 14 | 14 | 100% |
| UBC810 | (GA)8-T | 1500 ao 400 | 45.4°C | 12 | 11 | 92% |
| UBC811 | (GA)8-C | 2000 ao 500 | 45°C | 9 | 9 | 100% |
| UBC813 | (CT)8-T | 2000 ao 500 | 47°C | 10 | 10 | 100% |
| UBC825 | (AC)8-T | 2000 ao 500 | 47°C | 13 | 13 | 100% |
| UBC827 | (AC)8-G | 2000 ao 500 | 47°C | 13 | 12 | 92% |
| UBC834 | (AG)8-YT | 2000 ao 700 | 46°C | 9 | 8 | 89% |

R = purine (A or G) e Y = pyrimidine (C or T)

The amplifications were carried out in a PTC-100 Thermocycler (MJ Research Inc., Quebec, Canada) programmed under the following protocol: initial denaturation for 5 min at 94°C; 35 cycles each comprising denaturation for 40 s at 94°C, 30 s for each primer annealing temperature (Table 2) and extension for 60 s at 72°C; and, a final extension for 7 min at 72°C.

The fragments were subjected to electrophoresis on a 1.5% agarose gel (1X TBE: 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) in a horizontal electrophoresis system (Loccus Biotecnologia LCH 20 x 25) at 120 V for 2 h. Each sample was stained with 2µL of GelRed® dye (Biotium) and the amplification products were visualized under UV light.

**Data analysis**

From the analysis and interpretation of the agarose gel, a binary matrix was constructed from the presence and absence of the fragments, represented by “1” and “0” respectively. The optimal number of fragments was estimated by the GENES software (Cruz, 2001), in order to obtain the correlation and stress value. The average values of Polymorphic Information Content (PIC) (Botstein et al., 1980) and Hardy-Weinberg Expected Heterozygosity (H*E*) (Nei, 1973) for dominant molecular markers, and the Jaccard similarity (Sneath and Sokal, 1973) and the Bootstrap analysis for 100 simulations analysis were also performed using GENES software. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was constructed by the NTSYS-pc 2.0 software (Rohlf, 2001). The percentage of polymorphic locus, the number of different alleles (N*a*), the number of effective alleles (N*e*) and the Shannon Index was calculated using the GeneAlEx 6.5 version (Peakall and Smouse, 2012).

Another cluster analysis, using Bayesian method, was performed in the STRUCTURE software, version 2.3.4 (Pritchard et al., 2012). The admixture model was used with correlated allele frequencies, and simulations were carried out with a burn-in period and a MCMC number of 104 each. The choice for the best fit clustering number (K) was evaluated using ΔK, from the Evanno et al. (2005) method, in the on-line STRUCTURE HAVERSTER software (Earl and vonHoldt, 2012).

**RESULTS**

**Primers analysis**

The 9 ISSR primers that were used generated 99 polymorphic bands. The fragments number varied from 9 to 13 per primer, with an average of 11.3 bands per primer (Figure 2).

Insert Figure 2 here

**Figure 2.** Electrophoretic profiles of the primers UBC807 (A) and UBC834 (B) amplified for 24 *Myrcia ovata* individuals from a native population located at the municipality of Japaratuba, in the state of Sergipe, Brazil.

The optimization analysis showed correlation and stress values of 0.9986 and 0.0127, respectively. These values confirm the stability among the number of primers and the number of fragments obtained. Furthermore, the content of PIC ranged from 0.1094 to 0.3469, with an average of 0.2594.

**Genetic variability**

The genetic variability for the population was estimated as moderate, but with low tendency. The average number of different alleles (N*a*) and number of effective alleles (N*e*) were 1.971 and 1.412, respectively. The Shannon Information Index (I*S*) was 0.4 and the Expected Heterozygosity (H*E*) ranged from 0.1162 to 0.4466, with an average of 0.3097. The percentage of polymorphic locus was 97.06%.

**Clustering analysis**

The similarity coefficient of Jaccard between each pair of individuals ranged from 0.21 to 0.82, with an average of 0.38 (Table 3). The pair formed by the individuals MYRO-178.1 and MYRO-178.2 (0.82), followed by the pairs MYRO-032 and MYRO-033 (0.71), and MYRO-159 and MYRO-159.2 (0.69) presented higher genetic similarity. Moreover, the pairs of individuals MYRO-034 and MYRO-159 (0.21), MYRO-029 and MYRO-093 (0.24), and MYRO-030 and MYRO-162 (0.25) presented the lower genetic similarity.

From the UPGMA dendrogram was separated 2 groups (I and II) of individuals. According to this analysis, the group I was formed by six individuals representing 25% of the population (MYRO-159, MYRO-159.1, MYRO-159.2, MYRO-093 and MYRO-162), and the group II was formed by 18 individuals representing 75% of the population. The Bootstrap repeatability analysis showed a range from 19 to 100%. The junctions between MYRO-154 and MYRO-155 (100%); MYRO-178.1 and MYRO-178.2 (100%); and, MYRO-159 and MYRO-159.1 (97%) individuals showed higher consistencies (Figure 3).

**Table 3.** Jaccard similarity coefficient of 24 *Myrcia ovata* individuals from a native population located at the municipally of Japaratuba, in the state of Sergipe, Brazil.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | MYRO-154 | MYRO-155 | MYRO-178 | MYRO-178.1 | MYRO-178.2 | MYRO-156 | MYRO-157 | MYRO-160 | MYRO-813 | MYRO-159.1 | MYRO-159 | MYRO-159.2 | MYRO-093 | MYRO-162 | MYRO-174 | MYRO-175 | MYRO-175-1 | MYRO-176 | MYRO-029 | MYRO-030 | MYRO-032 | MYRO-033 | MYRO-034 |
| MYRO-155 | 0.43 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-178 | 0.45 | 0.59 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-178.1 | 0.47 | 0.59 | 0.65 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-178.2 | 0.48 | 0.55 | 0.57 | 0.82 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-156 | 0.44 | 0.46 | 0.51 | 0.47 | 0.53 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-157 | 0.38 | 0.41 | 0.49 | 0.41 | 0.51 | 0.58 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-160 | 0.35 | 0.52 | 0.51 | 0.53 | 0.63 | 0.59 | 0.51 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-813 | 0.37 | 0.48 | 0.50 | 0.40 | 0.43 | 0.46 | 0.41 | 0.41 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-159.1 | 0.35 | 0.30 | 0.33 | 0.31 | 0.36 | 0.32 | 0.27 | 0.33 | 0.41 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-159 | 0.33 | 0.30 | 0.31 | 0.35 | 0.38 | 0.26 | 0.27 | 0.29 | 0.28 | 0.67 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-159.2 | 0.40 | 0.33 | 0.37 | 0.38 | 0.44 | 0.31 | 0.33 | 0.36 | 0.33 | 0.67 | 0.69 | 1 |  |  |  |  |  |  |  |  |  |  |  |
| MYRO-093 | 0.28 | 0.26 | 0.27 | 0.28 | 0.34 | 0.32 | 0.28 | 0.30 | 0.29 | 0.43 | 0.36 | 0.50 | 1 |  |  |  |  |  |  |  |  |  |  |
| MYRO-162 | 0.36 | 0.40 | 0.39 | 0.40 | 0.43 | 0.33 | 0.32 | 0.33 | 0.35 | 0.50 | 0.40 | 0.50 | 0.54 | 1 |  |  |  |  |  |  |  |  |  |
| MYRO-174 | 0.29 | 0.31 | 0.30 | 0.33 | 0.38 | 0.36 | 0.31 | 0.32 | 0.38 | 0.60 | 0.51 | 0.51 | 0.51 | 0.51 | 1 |  |  |  |  |  |  |  |  |
| MYRO-175 | 0.35 | 0.31 | 0.33 | 0.30 | 0.34 | 0.40 | 0.28 | 0.33 | 0.37 | 0.38 | 0.28 | 0.36 | 0.32 | 0.39 | 0.37 | 1 |  |  |  |  |  |  |  |
| MYRO-175-1 | 0.30 | 0.38 | 0.40 | 0.35 | 0.35 | 0.36 | 0.36 | 0.37 | 0.35 | 0.33 | 0.32 | 0.38 | 0.33 | 0.37 | 0.30 | 0.37 | 1 |  |  |  |  |  |  |
| MYRO-176 | 0.37 | 0.38 | 0.42 | 0.41 | 0.44 | 0.34 | 0.36 | 0.37 | 0.36 | 0.34 | 0.38 | 0.44 | 0.31 | 0.29 | 0.33 | 0.34 | 0.38 | 1 |  |  |  |  |  |
| MYRO-029 | 0.33 | 0.32 | 0.33 | 0.34 | 0.35 | 0.30 | 0.29 | 0.33 | 0.32 | 0.35 | 0.34 | 0.31 | 0.24 | 0.36 | 0.30 | 0.33 | 0.34 | 0.37 | 1 |  |  |  |  |
| MYRO-030 | 0.37 | 0.46 | 0.42 | 0.38 | 0.41 | 0.44 | 0.36 | 0.44 | 0.38 | 0.29 | 0.30 | 0.30 | 0.29 | 0.25 | 0.29 | 0.37 | 0.33 | 0.50 | 0.37 | 1 |  |  |  |
| MYRO-032 | 0.29 | 0.43 | 0.39 | 0.33 | 0.36 | 0.41 | 0.38 | 0.41 | 0.51 | 0.41 | 0.33 | 0.30 | 0.31 | 0.32 | 0.40 | 0.40 | 0.41 | 0.41 | 0.57 | 0.46 | 1 |  |  |
| MYRO-033 | 0.25 | 0.38 | 0.36 | 0.27 | 0.30 | 0.41 | 0.38 | 0.39 | 0.48 | 0.36 | 0.27 | 0.27 | 0.33 | 0.37 | 0.35 | 0.39 | 0.38 | 0.43 | 0.45 | 0.43 | 0.71 | 1 |  |
| MYRO-034 | 0.32 | 0.34 | 0.38 | 0.39 | 0.39 | 0.40 | 0.31 | 0.35 | 0.42 | 0.30 | 0.21 | 0.31 | 0.29 | 0.30 | 0.32 | 0.38 | 0.33 | 0.37 | 0.41 | 0.39 | 0.44 | 0.47 | 1 |
| MYRO-036 | 0.32 | 0.36 | 0.40 | 0.38 | 0.36 | 0.34 | 0.25 | 0.35 | 0.36 | 0.30 | 0.25 | 0.31 | 0.27 | 0.33 | 0.28 | 0.37 | 0.36 | 0.46 | 0.43 | 0.44 | 0.38 | 0.40 | 0.42 |

Insert Figure 3 here

**Figure 3.** Dendrogram generated by the Unweighted Pair Group method with Arithmetic Mean (UPGMA) analysis of Jaccard similarity indices of 24 *Myrcia ovata* individuals from a native population located at the municipality of Japaratuba, in the state of Sergipe, Brazil.

The Bayesian cluster analysis from the STRUCTURE software determined the population in 2 groups. The individuals MYRO-154 and MYRO-175 were those that represented the most variability (Figure 4).

Insert Figure 4 here

**Figure 4.** STRUCTURE clustering results with K=2 of 24 *Myrcia ovata* individuals from a native population located at the municipality of Japaratuba, in the state of Sergipe, Brazil.

**DISCUSSION**

The ISSR marker was able to detect a relatively high level of polymorphism between the 24 plants of *M. ovata* of a native population located in the municipality of Japaratuba, in the state of Sergipe, Brazil. This is the first study to evaluate the genetic variability of *M. ovata*.

The number of fragments amplified by the ISSR markers was included in a lower range that it may be expected for the species of Myrteae tribe (Lima et al., 2015b). In addition, the 99 polymorphic bands obtained from the 9 primers was enough to find a reliable number of fragments to estimate the genetic variability (Dudley, 1994). Also, by the optimization analysis it was proved that the number of primers used were sufficient to evaluate the genetic variability of *M. ovata* individuals, with correlation and stress closer to 1.0 and below 0.05, respectively (Kruskal, 1964).

The PIC content represents the probability of finding each marker in present and/or absent on each band revealing allelic variation. It ranges from 0 to 0.5 and lower values can correspond to very rare or abundant markers (Roldan-Ruiz et al., 2000). In this paper, the PIC content (0.259) was considered with moderate discriminatory power. In addition, the PIC content was lower than the H*E*, as expected (Cruz, 2001).

Regarding the genetic variability, the number of different alleles (N*a*) obtained for dominant markers was (1.97), very closer as the highest as can be, and of this number 1.41 was considered as effective alleles (N*e*). This means that 71.57% of the alleles can contribute to the construction of the genetic information of this native population of *M. ovata* in the Sergipe state.

The Shannon Index (I*S*) measures the certitude in predicted genetic proximity between individuals, ranging from 0 to 1. The lower the number gets, the higher is the certainty degree and the lower is the diversity population (Estopa et al., 2006). The average H*E* is associated with lower diversity and consequently, reduced capacity of the remaining population for adaptation (Álvares-Carvalho et al., 2016).

The Shannon Index and the average H*E* found for this native population (0.40; 0.30), was lower than those found by Brandão et al. (2015) (0.48; 0.33) and by Alves et al. (2016) (0.46; 0.30) that worked with *M. splendes* and *M. lundiana*, respectively. Knowing that the range of H*E* is expected to be similar between species that present similar characters, such as biological reproductive and distribution (Lima et al., 2015b) the lower result for the Shannon Index and H*E* can be, in part, explained because of the origin of the plants used in their research, which was a conserved vegetation. This contrasts with the *M. ovata* location, an anthropized vegetation (Santana et al., 2012), which could imply a lower gene flow.

The Jaccard analysis showed a moderate similarity (0.38), which can be influenced by cross-pollination reproduction system (Kageyama et al., 2003; Sampaio et al., 2016) and the absent of domestication (Silva et al., 2011). Nevertheless, the existence of moderate genetic diversity does not justify the lack of conservation activities for this specie, mainly because it was not found in other locations of the state of Sergipe besides the study area, and the genetic variability shows a tendency to decay if no action is taken.

It’s important to emphasize that the most similar pairs of individuals are shrubs, each located side-by-side, and clustered by the same group in the UPGMA and STRUCTURE analysis, implying that they probably have the same progenitors. This observation is applied to other shrubs that are side-by-side, for example MYRO-159 and MYRO-159.1, MYRO-159.1 and MYRO-159.2 and MYRO-178 and MYRO-178.1. Furthermore, about the population genetic structure, the 24 individuals of *M. ovata* were clustered in two groups by the UPGMA and STRUCTURE analysis, which presented the same arrangement.

The choice of matrices to describe the variability and/or genetic diversity of individuals within and between populations is a prerequisite for genetic characterization of the species, being that this characterization is a common procedure in conservation of natural resource and genetic improvement activities. Based on this study, it was determined that the individuals MYRO-154, MYRO-175 and MYRO-175.1 of *M. ovata* present in the state of Sergipe are indicated as priorities for conservation of the species.

Regarding the comparison of chemical and genetic analysis, 12 individuals of *M. ovata* used in Sampaio et al. (2016) were also used in this paper. There wasn’t found a match of the chemical and genetics groups described in the clustering analysis. For example, each of these individuals: MYRO-174 and MYRO-176, and MYRO-159 and MYRO-160 were chemically grouped in the same clusters, but genetically clustered in different groups. This differentiation can be partially explained by the fact that different samples were used, from different collected periods for these studies. The chemical variation is commonly found in the chemical composition of plants, because it is influenced not only by gene composition, but by dynamic factors such as rainy season, drought, temperature and pests, as also by the extraction method (Scheffer, 1993; Ribeiro et al., 2016). Lastly, this comparison may be better with other molecular makers that also are influenced by dynamic factors, such as enzyme makers (Faleiro, 2007).

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**Conflicts of interest**

The authors declare no conflict of interest.

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