academicJournals

Vol. 6(4), pp. 39-49, July, 2014
DOI 10.5897/JABSD2014.223
ISSN 2141-2340
Article Number: AAB9B8E46566

http://www.academicjournals.org/JABSD

Journal of Agricultural Biotechnology and Sustainable Development

Full Length Research Paper

Characterization of wild genotypes of Aroeira: Subsidy for plant breeding

Danilla Cristina Lemos Souza, Laura Jane Gomes, Arie Fitzgerald Blank, Itamara Bomfim Gois, Glauber Santos Pereira, Péricles Barreto Alves, Renata Silva-Mann

Federal University of Sergipe, Brazil.

Received 4 May, 2014; Accepted 22 July, 2014

The selection of genetically superior materials of different forest species with economic potential involves studies of germplasm characterization. Facing the exploitation of aroeira (*Schinus terebinthifolius* Raddi.) in areas of natural occurrence the genetic and chemical characterization of individuals is extremely important to suit the selection. The work was carried out to identify genetic and chemical differences in individuals of aroeira located in different forest fragments. The genetic polymorphism was accessed using 19 inter-simple sequence repeat (ISSR) primers and the fragments generated were used to evaluate the Nei's gene diversity, percentage of polymorphic loci, Shannon index, number of alleles observed and effective number of alleles. The fruits of all individuals were submitted to hydro distillation of essential oils. The oil chemistry composition was analyzed by Gas chromatography/mass spectrometry (GC/ME) and the averages of the compounds were compared by Scott-Knott ($p<0.05$). The correlations using genetic, geographic distances and chemical composition were analyzed by the Mantel test. There are considerable levels of genetic and chemistry variability among the individuals studied, which allows markers assisted selection aiming to identify potential genitors for use in plant breeding.

Key words: Aroeira, genetic polymorphism, inter-simple sequence repeat (ISSR), poivre-rouge, volatile chemicals.

INTRODUCTION

The potential of native species with agro-industrial use is endless. Brazil is considered to have the greatest number of higher plant species identified, and majority of these native plants contain essential oils (Mittermeier and Werner, 1990) with potential industrial use. However, Brazil's biodiversity is being destroyed at an accelerated pace due to city expansions and human developments. Reduction of genetic diversity implies reduction of opportunity to identify natural products with economic use, as many native species are important sources of biologically active natural compounds. For example, along the Basin of Lower São Francisco river in Sergipe, grows aroeira (*Schinus terebinthifolius* Raddi.), a native plant species extensively explored due to the chemical properties of its essential oils that are used in medicine, cosmetics and food. Studies on the essential oil of aroeira collected in different regions of the globe, has shown different chemotypes, with prevalence of different chemical compounds (Silva et al., 2010a). Plants in India, for example, higher content of α-pinene has been found

*E-mail: renatamann@hotmail.com

(51%) (Singh et al., 1998; Chowdhury and Tripani, 2001), whereas in plants of Egypt the highlight was α-phellandrene (24 %) (Ibrahim et al., 2004).

In Brazil, studying the chemical composition of the essential oil, Barbosa et al. (2007), observed mainly, monoterpenes (90%), with a higher concentration of δ-3-carene (29%), α-pinene (13 %), α-phellandrene (13%) e β-phellandrene (18%), but also the occurrence of sesquiterpenes was observed, as germacrene-D (3%). It is important to analyze different chemotypes and genotypes, as well as variation in essential oil yield obtained among genotypes, the presence of different compounds in a product in concentrations above or below, directly affects their quality (Brilho, 1967).

Genetic diversity within a species like *S. terbinthifolius*, risks being depleted due to extensive development of cities without appropriate management practices, which may lead to the loss of the genotypes with the most favorable characteristics, and possibly only plants of lower agro-industrial value.

Modern breeding relies on associating phenotypic differences with genetic characterization using molecular markers based on the analysis of nucleotide sequence differences and the identification of specific sequence differences between populations (Telles et al., 2010). Therefore, the identification of phenotypic differences of the chemical makeup of essential oils allows the selection of characters and individuals of economic importance that can then be analyzed genetically in breeding programs. The basic premise that this study proposes for aroeira breeding of is to estimate the genetic variation present in the species that could be estimated using ISSR molecular markers (Julio et al., 2008; Brandão et al., 2011). Thus, the aim of the present work was to phenotypically and genotypically characterize individuals of aroeira located in riparian forest fragments along the region of Lower São Francisco river in Sergipe, Brazil.

MATERIALS AND METHODS

Plant sampling

The study was performed in riparian forest fragments of different phytogeographic regions of the State of Sergipe, Brazil, located in the municipalities of Brejo Grande, Neópolis and Propriá.

Collecting plant material

For molecular analysis, tender leaves of individual aroeira plants were collected and stored at -20°C until DNA isolation. Mature fruits were also collected from the same individual plants for chemical analysis of essential oil composition.

Reactions of DNA-ISSR

DNA isolation

The isolation of genomic DNA was performed from approximately 2 g of tissue according to the protocol described by Nienhuis et al. (1995). The quality and concentration of DNA from each sample was determined by spectrophotometry (CARY 50 PROBE UV-VISIBLE), based on absorbance at 260 and 280 nm.

Amplifications of ISSR

The DNA was submitted to polymerase chain reaction (PCR) using 22 inter-simple sequence repeat (ISSR) primers and 19 primers were selected for analyses. The amplification reactions were performed on a Biometra thermocycler Uniscience Tpersonal in a volume of 13 µl containing: genomic DNA (200 ng); Green Gotaq 5X buffer (Promega); dNTPs (2.5 mM); $MgCl_2$ (50 mM); BSA (1 µg $µl^{-1}$); *Taq* polimerase (5U $µl^{-1}$, Promega), primers (2 µM) and ultrapure water. PCR reactions were carried over 40 amplification cycles after the initial denaturation at 94°C for 3 min. Each cycle consisted of: 94°C for 1 min., 46°C for 45 s., and finally 72°C for 2 min for extension of new DNA strands. After the cycles, the process the temperature was set to 72°C for 7 min.

The PCR products were separated by horizontal electrophoresis, using 1% agarose gel in 0.5X TBE buffer, at 100V for 90 min. Subsequently, the gels were stained with ethidium bromide (0.5 µg ml^{-1}) for 30 min, visualized and photographed under exposure to ultraviolet light. The size of the amplified fragments was estimated by comparison with molecular markers within a 100 bp DNA ladder.

Chemical composition

Extraction of essential oils

The essential oils from 200 g of aroeira fruits were hydro distilled in a Clevenger apparatus during 180 min. The recovered oils were stored at -20°C in amber bottles. The essential oils content, expressed as a percentage of total volume, was obtained based on 100 $ml.g^{-1}$ weight of matter.

Gas chromatography-mass spectrometry (GC/ME)

The chemical composition was determined by gas chromatography coupled to a mass spectrometer (GC/ME, Shimadzu, modelo QP 5050A), equipped with a fused silica capillary column DB-5 of 30 m x 0.25 mm i.d., 0.25 µm coating, using He as the carrier gas with a flow rate of 1.0 ml min^{-1}. The temperature was maintained at 50°C for 1.5 min, followed by an increase of 4°C min^{-1} until 200°C, and then 10°C min^{-1} to 250°C, keeping this temperature constant for 5 min. The injector temperature was 250°C and detector temperature (or interface) was 280°C. Forty milligram of the oil was dissolved in 1.5 ml of ethyl acetate and injected into a volume of 0.5 µl of the solution, at a partition volume ratio of 1:100 and injected on the column at a pressure of 64.20 kPa.

The conditions of ME were ion detector of the quadrupole, operating by electron impact and impact energy of 70 eV, scan speed 1.000; scan interval of 0.50 s^{-1} fragments and fragments detected in the range of 40-500 Da. The identification of oil components was based in MS data comparison followed by retention index and compared to the literature retention indices (Adams, 2007). For the retention rate, the van den Dool and Kratz (1963) equation was used in relation to a homologous series of *n*-alcanos (*n*C9- *n*C18). Were also used three libraries of the equipment WILEY8, NIST107 e NIST21, which allow the comparison of the spectral data with those contained in the libraries, using a similarity index of 80%.

Table 1. Geographic coordinates, latitude and longitude, for individuals of aroeira (*Schinus terebinthifolius* Raddi) in the Lower São Francisco (BG – Brejo Grande; N – Neópolis; P – Propriá).

Individual	Latitude (S)	Longitude (WO)
BG1	10°29'00,0"	36°28'04,9"
BG2	10°28'50,1"	36°28'07,8"
BG3	10°28'51,5"	36°28'09,4"
BG4	10°28'35,2"	36°27'04,8"
BG5	10°28'50,4"	36°28'12,4"
N1	10°18'33,4"	36°35'07,5''
N2	10°18'35,0"	36°35'00,2''
N3	10°18'37,4"	36°35'01,1''
N4	10°18'37,1"	36°35'00,0''
N5	10°18'38,4"	36°34'56,9''
P1	10°13'23,5''	36°48'07,0''
P2	10°13'22,6''	36°48'10,5''
P3	10°13'21,7''	36°48'13,8''
P4	10°13'21,8''	36°48'13,5''
P5	10°13'24,9''	36°48'06,7''

Data analysis

Molecular data

The ISSR fragments were used to obtain a binary array, considering the presence (1) or absence calculating the percentage of polymorphism obtained using each primer. The fragments with weak staining and low resolution were not considered. The genetic similarity index (*Sij*) between each pair of individual plants was estimated using the Jaccard coefficient, using the program NTSYS pc2.1 (Rohlf, 2000).

In order to verify the correlation between genetic and geographic distances of individuals analyzed, we used the Pearson correlation coefficient (r) (Pearson, 1892), by Mantel (Manly, 1997). Simplified representation of the similarity data was exposed in a dendrogram obtained by the clustering method Unweighted Pair-Group Method Arithmetic Average (UPGMA) (Sneath and Sokal, 1973), using the computer program NTSYS pc2.1, and the consistency of each group performed by *bootstrap* (Coelho, 2000).

Given the dominant nature of the data, the program required the calculation of allele frequencies, to verify the loci Hardy-Weinberg equilibrium. The percentage of polymorphic loci (P%), number of alleles observed, number of effective alleles, the genetic diversity of Nei (1973) ($\hat{H}_e$) and the Shannon Index (*I)* for individuals were estimated. To characterize the genetic diversity, the program POPGENE (Population Genetic Analysis) versão 1.32 (Yeh et al., 1999) was used, employing parameters for diploid dominant data.

Chemistry data

The performance data, content and major compounds of the essential oils were analyzed by ANOVA and means compared by Scott-Knott ($p<0.05$) (Ferreira, 2000). The presence (1) or absence (0) of the chemical components was used to obtain a binary matrix. The chemical similarity index (Sg_{ij}) between each pair of individuals was calculated using the Jaccard coefficient, and the simplified representation of the data was expressed in a dendrogram obtained by the UPGMA method, using the NTSYS pc2.1 (Rohlf, 2000). The chemical composition data were analyzed by the UPGMA method, considering the Euclidean distance as similarity coefficient (Cruz et al., 2004), and the principal components analysis (ACP), both using the program STATISTICA versão 6.0 (Statsoft, 2001). The correlation between the chemical profile and geographical distance, and genetic similarities were performed by Mantel (Manly, 1997) with 1,000 permutations.

RESULTS AND DISCUSSION

Five individuals of each fragment were selected, taking into account phenotypic characteristics such as height, trunk and branches with minimum levels of pests and pathogens and evident by abundant fruiting (Table 1).

Molecular characterization

Analyses of gel electrophoresis images identified 104 ISSR fragments showing polymorphism over an averaging of 76.92% of the total fragments obtained (Table 2). ISSR molecular markers have been successfully used to estimate of genetic variability in wild and cultivated species, including medicinal and aromatic species (Santana et al., 2011). The number of polymorphic fragments used in these analyzes is quite variable (Estopa et al., 2006). Using ISSRs primers, Brandão et al. (2011), detected 70 polymorphic fragments (91.10%) for genetic analysis of *Myrcia splendens*, a value very close to the values used in this study. At the same locus, 55 polymorphic fragments (82.08%) were used by Manica-Cattani et al. (2009) for analysis of *Lippia alba* Mill, while for *Pogostemon cabli*, Wu et al. (2010) obtained 194 polymorphic fragments

Table 2. Number of fragments (NF) for each ISSR primer and percentage of polymorphic fragments (%) for ISSR markers using individuals of aroeira (*S. terebinthifolius* Raddi.).

Primer	Sequence (5'-3')	NF	%
AW3 (GT) 6-RG	GTGT TGT GTG TGT RG	7	85.7
BECKY (CA) 7-YC	CAC ACA CAC ACA CAY C	5	60.0
CHRIS (CA) 7-YG	CAC ACA CAC ACA CAY G	4	100.0
DAT (GA) 7-RG	GAG AGA GAG AGA GAR G	4	100.0
GOOFY (GT) – YG	GTG TGT GTG TGT GTY G	4	50.0
JOHN (AG) 7-YG	AGA GAG AGA GAG AGY C	5	100.0
M1 CAA – (GA) 5	CAA GAG AGA GAG A	6	66.7
M2 GGGC – (GA) 8	GGGCAGAGAGAGAGAGAGA	4	100.0
MANNY (CAC) 4-RC	CAC CAC CAC CAC RC	6	33.3
MAO (CTC) 4-RC	CTC CTC CTC CTC RC	8	87.5
OMAR (GAG) 4-RC	GAG GAG GAG GAG RC	6	50.0
UBC 807 (AG) 8-T	AGA GAG AGA GAG AGA GT	4	75.0
UBC 808 (AG) 8-C	AGA GAG AGA GAG AGA GC	7	100.0
UBC 809 (AG) 8-G	AGA GAG AGA GAG AGA GG	6	50.0
UBC 811 (GA) 8-C	GAG GAG GAG GAG GAG AC	5	75.0
UBC 813 (CT) 8-T	CTC CTC CTC CTC CTC TT	6	83.3
UBC 825 (AC) 8-T	ACA CAC ACA CAC ACA CT	5	60.0
UBC 827 (AC) 8-G	ACA CAC ACA CAC ACA CG	7	100.0
UBC 834 (AG) 8-YT	AGA GAG AGA GAG AGA GY T	5	80.0
Total		104	-
Mean		5.47	76.92

R = Purine (A or G) and Y = pirimidine (C or T).

(80.50%). Genetic polymorphism observed among individuals of aroeira can be considered high, implying a low level of homozygosity among them, which can partly explain the phenotypic variation observed in the field. The existence of heterozygosity among individuals can be beneficial to a plant breeding program, and can be used as matrix trees for seed collection and would be the genotypes of choice for use in restoration of degraded areas as these plants would potentially add the maximum genetic diversity to an area.

With 80 polymorphic fragments, a dendrogram of genetic similarity was generated (Figure 1). The mean genetic similarity among individuals was 43.31% and the range of similarities ranged 15-72%, which confirms the data of genetic polymorphism. P1 and P2 are the most similar individuals, and the most divergent were BG3 and N2.

By dendrogram analysis of genetic similarities there is no clustering in accord with geographical distances among individuals. This lack of genetic differentiation among fragments can be explained because the species present cross-fertilization and pollen flow (Loveless and Hamrick, 1984) without a pattern of genetic structure. There is a fairly close genetic relationship (63.2%) among the majority of individuals situated in the Propriá fragment, which may be caused by isolation, as this fragment is sited in an island in the São Francisco River, and therefore has a barrier to gene flow.

On-the-other-hand, the explanation for imperfect grouping for each fragment cannot be explained by the species pollination, which is coved by bees moving a relatively short distance, but enough to improve gene flow. Loveless and Hamrick (1984) attest that the species pollinated by insects present low variability, due to the limited movement of the insects. Thus, an explanation for the reduced difference among the fragments could be supported by evolutionary occurrence of such events involving individuals. Populations from different regions, although geographically isolated by habitat fragmentation, contains similar allele frequencies and can be a result of ancient genetic exchanges, that is, until the recent past the populations constituted a metapopulation (Raposo et al., 2007).

The results obtained with the Mantel test prove the lack of association among the individuals when considering the genetic distances and geographical distances. The coefficient of correlation between these distances was negative (r=-0.26426) and significant (0.0028) (Figure 2). This indicates that the fragments are not isolated by distance, so the genetic diversity among them could not be explained by geographic distance (Loss et al., 2006). To evaluate the relationship between genetic diversity and location of individuals *Enterelobium contortisiliquum*, at Sergipean region of Lower São Francisco, Santana

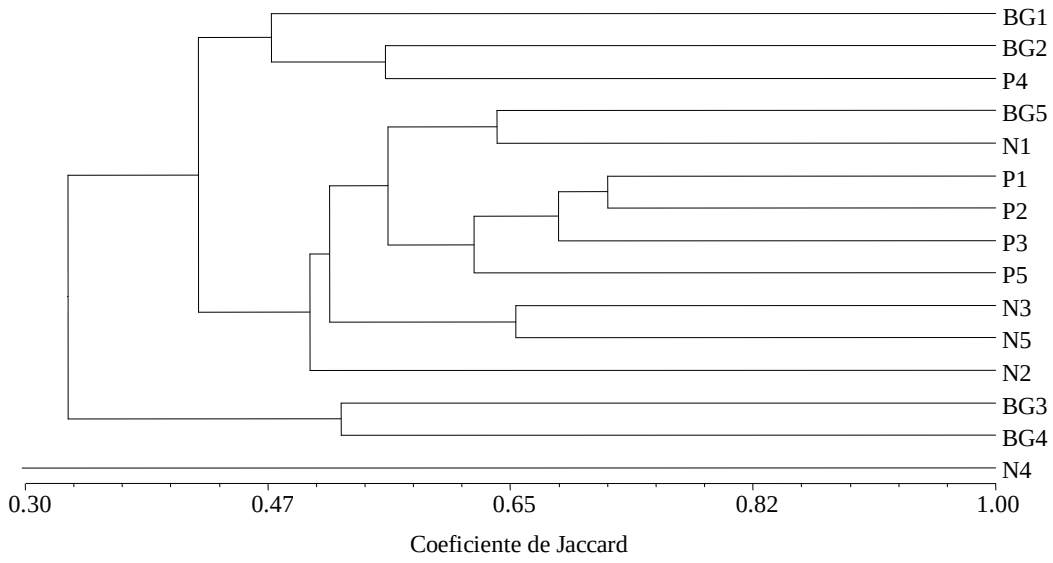

Figure 1. Cluster of similarities for individuals of aroeira (*S. terebinthifolius* Raddi.) analyzed by ISSR molecular markers.

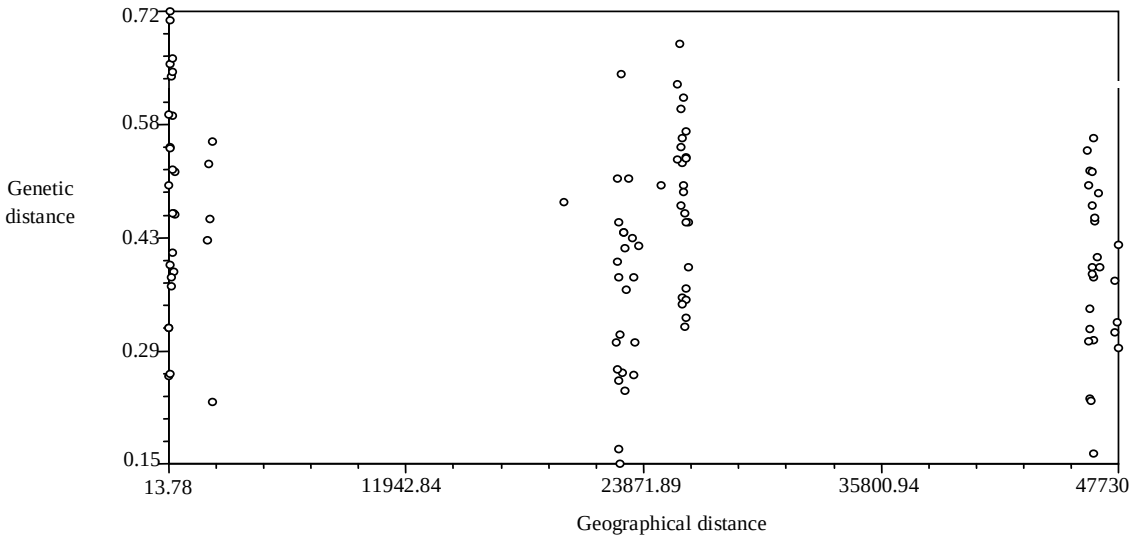

Figure 2. Correlation between genetic and geographic distances for individuals of aroeira (*S. terebinthifolius* Raddi.) by Mantel.

et al. (2008) obtained similar results to this work, verifying that, depending on the species traits (phenology, plant dispersal mechanisms etc.)., the recommended minimum distance between matrices for seed collection does not need to be strictly followed, since not always the genetic diversity between individuals can be explained by the geographical distance between them. The use of molecular markers enables the knowledge of spatial genetic structure of forest species aiming at the samples that are more significant with expressive variability in natural populations. The information is important for breeding purposes as well as for conservation and management, contributing thereby to the sustainability of genetic resources (Lacerda and Kageyama, 2003). The genetic parameters among the studied individuals are presented in Table 3.

The estimative of percentage of polymorphic loci was 76.92%. In contrast, the work of Yu et al. (2011) attested for individuals of *Magnolia officinalis* is 83.2%. The value is considered high for genetic variability among tree

Table 3. Genetic diversity among individuals of aroeira (*S. terebinthifolius* Raddi.) using ISSR markers.

Percentage of polymorphic loci (%)	76.92 %
Number of observed alleles (na)	1.77 (±0.42)
Number of effective allelles (ne)	1.48 (±0.38)
Genetic diversity ($\hat{H}_e$)	0.27 (±0.19)
Shannon of index (*I*)	0.41 (±0.27)

individuals. The number of alleles observed was 1.77 and the effective number of alleles was 1.48, which represents the number of alleles that effectively participate of gene flow. The index diversity by Nei, also called heterozygosity was used to assess the polymorphic content of each locus with a value of 0.27. Taking into account the stadium of fragmentation of the studied regions, the heterozygosity could be explained by a low or absent activity of genetic erosion processes in those fragments. Furthermore, the high levels of genetic variability among individuals of aroeira should be supported by self-incompatibility alleles and outcrossing.

Considerable levels of genetic variation and the absence of endogamy, due to incompatibility mechanisms, are indicative of long-term maintenance (Raposo et al., 2007). However, compared to the fast pace of exploration, only these types of conditions will not be sufficient to sustain populations of aroeira. Prioritization for pre-breeding actions aiming to establishment clones for commercial production and conservation of genetic variability is necessary with a focus on *ex situ* banks. Freitas et al. (2006) states that even populations with a high rate of genetic variability, which are not categorized as endangered, is important to conserve genetic resources for plant breeding and use of specific genes of interest.

Most indices of genetic diversity depends on the estimative of heterozygosity in the population, and, considering the dominant character of the ISSR, the estimation of these indices following this method is an approximation (Freire et al., 2007). The Shannon Index is not based on heterozygosity and has been used with dominant markers, such as RAPD and ISSR. The value obtained for this parameter was 0.41 indicating a relatively higher diversity (Botrel et al., 2006). Initially, the most divergent genetic materials are the best choice for starting a breeding program or establishing commercial plantations. However, recommendations should only be made after a careful analysis of their performance for commercial traits (Cruz et al., 2004). The fruits of this species have been extracted without optimal management, and therefore, they present great opportunity for enhanced profit for food industries. Therefore correlations with the organoleptic characteristics of fruit obtained from trees selected using chemical and molecular markers and identification of ideal genotypes should be used in breeding programs.

Chemical characterization

The content and yield of essential oils varied among individuals from 0.66 to 2.43 ml, and levels from 1.32 to 4.86% for individuals BG4 and N3, respectively (Table 4). The time of hydrodistillation (180 min) for fruits allowed obtaining optimal performance. According to Barbosa et al. (2007) the initial 20 min is possible to obtain a ratio of 78% of the total oil present in the fruit, and in the final minutes of 180 min only a small amount (2%) of the total oil is extracted. Barbosa et al. (2007) using individuals naturally from Viçosa-MG, found a similar value (4.65%). In comparison with our study, the maximum value obtained from fruits was 4.87. For the same species higher values were obtained by Roveda et al. (2010) (8.5%) using individuals in the Garden of Medicinal Plants of UFGD-MT.

Chemical compounds, obtained by extraction of essential oils from aroeira, were used for clustering analysis (Figure 3). The average of chemical similarity among individuals was 42.53%, and the similarities ranged from 13 to 90%. The most similar individuals were P4 and P5, and they both had high values. Low values were obtained for BG3 and P4.

The chemical similarity values of 79.45% was estimated for individuals located in Propriá. Given the observed genetic divergence between these individuals, as well as the relative distinction of this group, the values may be related to unique environmental conditions of Propriá, which, unlike the other fragments, is more characteristic of semiarid regions, and the synthesis of active medicinal, aromatic and spice compounds have been shown to be highly affected by temperature and soil type, as well as additional climatic conditions (Rosal et al., 2011). This direct influence of environmental factors on the composition and yield of essential oils is a challenge for farmers to establish stable and productive genotypes and maintain uniformity required by the chemical and food industries (Yamamoto et al., 2008).

The results obtained with the Mantel test presented a negative correlation (r=-0.43) and high significance (0.00) using chemical and geographical distances (Figure 4). The chemical diversity of individuals could not be explained by geographical/ environmental conditions. Essential oils extraction from aroeira identified 51 chemical compounds. Major compounds of essential oils of aroeira were α-pinene, β-pinene, α-phellandrene, δ-

Table 4. Yield and content of essential oils of aroeira (*S. terebinthifolius* Raddi.) in the Lower São Francisco (BG – Brejo Grande; N – Neópolis; P – Propriá).

Individuals	Yield (ml)	Content (%)
BG1	1.10[d]	2.20[d]
BG2	0.95[d]	1.90[d]
BG3	1.03[d]	2.07[d]
BG4	0.66[e]	1.32[e]
BG5	1.10[d]	2.20[d]
N1	1.03[d]	2.07[d]
N2	1.00[d]	2.00[d]
N3	2.43[a]	4.87[a]
N4	1.38[c]	2.77[c]
N5	2.33[a]	4.67[a]
P1	1.77[c]	3.54[d]
P2	2.02[b]	4.03[b]
P3	1.30[c]	2.60[c]
P4	1.57[c]	2.93[c]
P5	1.50[c]	3.00[c]

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

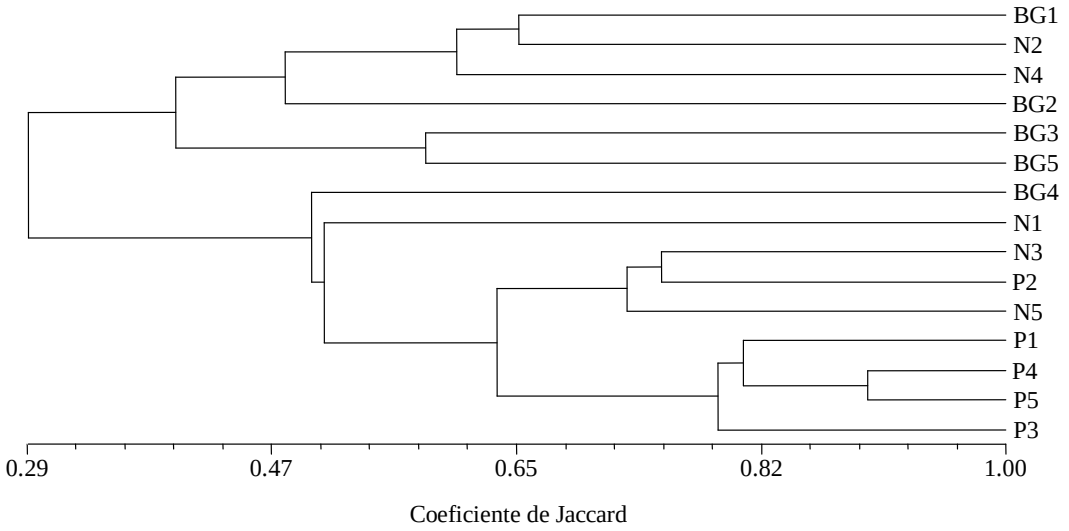

Figure 3. Chemistral similarities among individuals of aroeira (*S. terebinthifolius* Raddi.).

carene, o-cymene, limonene, β-phellandrene and myrtenol (Table 5). There is a significant difference between the levels of the chemical compounds present in the oils. This chemical variation, with prevalence of different compounds in the essential oils, obtained from the same species collected at different locations (ecotypes), is often explained by the high chemical complexity of essential oils. However, the genetic variability of plants should be considered, in addition to geographical factors (location) and ecological (habitat), which are closely related to the quality of essential oils and is expressed through chemotypes (Roveda et al., 2010).

As observed by Singh et al. (1998) using leaves and flowers of the species, and by Barbosa et al. (2007) using oils from leaves and fruits, that both analyses were characterized by the presence of mostly monoterpenes. Silva et al. (2010b) verifying chemical compounds present in the essential oils from fruit of aroeira, determined that α-pinene was the main constituent

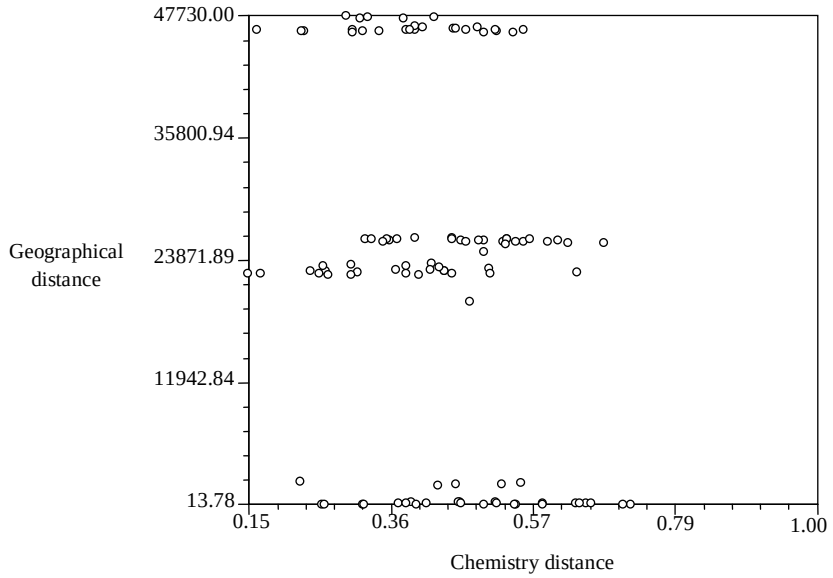

Figure 4. Chemical and geographic correlation for aroeira (*S. terebinthifolius* Raddi.).

Table 5. Comparison of the major compounds in the essential oils of aroeira (*S. terebinthifolius* Raddi.) in the Lower São Francisco (BG – Brejo Grande; N – Neópolis; P – Propriá).

Individuals	Major compounds							
	α-pinene	β-pinene	α- phellandrene	δ-carene	o-cymene	Limonene	β – phellandrene	Myrtenol
BG1	20.21^{b}	0.61^{g}	23.95^{a}	0.00^{d}	12.05^{c}	14.23^{a}	11.63^{a}	0.43^{h}
BG2	17.53^{c}	0.46^{g}	27.60^{a}	0.00^{d}	9.46^{d}	14.29^{a}	11.68^{a}	0.25^{h}
BG3	5.66^{d}	0.17^{g}	18.42^{b}	33.34^{a}	5.11^{e}	7.48^{d}	6.44^{d}	0.24^{h}
BG4	26.80^{a}	36.78^{a}	5.05^{c}	0.00^{d}	1.66^{h}	2.57^{f}	4.58^{e}	3.06^{e}
BG5	12.11^{c}	0.37^{g}	9.75^{c}	28.62^{a}	8.65^{d}	4.67^{e}	4.63^{e}	0.34^{h}
N1	21.99^{b}	15.66^{f}	7.04^{c}	16.32^{b}	03.73^{f}	4.33^{e}	3.62^{f}	1.54^{g}
N2	16.36^{c}	0.42 ^{g}	12.90^{b}	6.35^{c}	17.79^{b}	13.35^{b}	10.29^{b}	0.35^{h}
N3	20.45^{b}	29.37^{d}	0.28^{d}	9.11^{c}	00.83^{i}	1.95^{g}	1.43^{i}	6.82^{a}
N4	19.62^{b}	0.60 ^{g}	13.84^{b}	1.85^{d}	18.04^{a}	8.59^{c}	9.00^{c}	0.38^{h}
N5	26.44^{a}	35.41^{b}	0.89^{d}	0.00^{d}	1.04^{h}	2.10^{g}	2.04^{h}	6.84^{a}
P1	15.80^{c}	33.42^{c}	1.10^{d}	3.05^{d}	1.17^{h}	2.39^{f}	2.36^{h}	4.86^{c}
P2	23.67^{a}	25.45^{e}	1.20^{d}	5.83^{c}	2.70^{g}	2.57^{f}	1.55^{i}	6.32^{b}
P3	25.23^{a}	33.64^{c}	0.51^{d}	8.82^{c}	0.35^{i}	1.89^{g}	2.24^{h}	2.73^{f}
P4	27.41^{a}	37.84^{a}	1.01^{d}	2.59^{d}	0.67^{i}	2.38^{f}	2.83^{g}	4.17^{d}
P5	26.14^{a}	34.58^{b}	0.46^{d}	8.87^{c}	0.39^{i}	1.93^{g}	2.37^{h}	3.04^{e}

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

(29.39%) of the oils, followed by δ-carene (19.69 %), limonene (18.15%) and α-phellandrene (9.39%), in addition to a large concentration of monoterpenes. Barbosa et al. (2007) chemically analyzed a sample of commercial essential oils of aroeira (FLAVÉX) and obtained the same major compounds found here: α-pinene (18.82%), α-phellandrene (23.55%), δ-carene (6.32%), β-phellandrene (16.88%). They also found germacreno-D (11.89%), which in this study was detected at a much lower concentration (0.36%). This chemical similarity between the major compounds is an indication that the oil composition is consistent enough to be feasible for larger scale commercialization, and that breeders should select genetic materials that will reliably express these major compounds of interest.

Many samples of essential oils of aroeira present

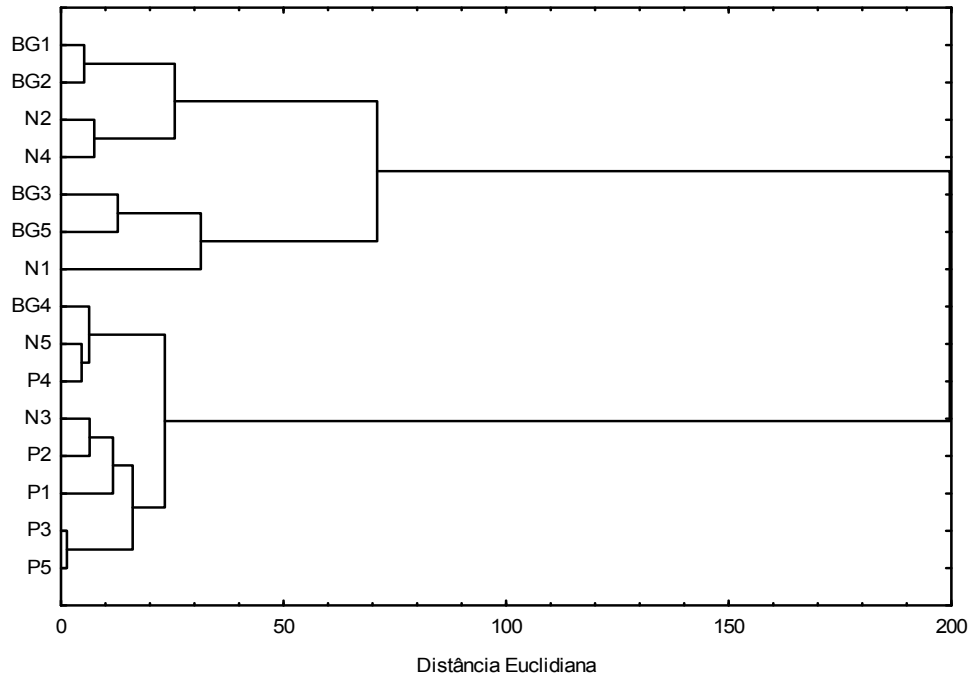

Figure 5. Cluster of individuals of aroeira (*S. terebinthifolius* Raddi.) for chemical diversity.

basically the same major compounds. However, there are other studies in which other compounds are the majority, as in the case of the work conducted by Ibrahim et al. (2004), which confirmed the presence of germacrene-D (14.31%) and elixene (15.18%). Santos et al. (2007) observed that sabinene (25.41%), β-caryophyllene (20.69%) and germacrene-D (25.05) majority found in leaves. Also analyzing leaf oils of aroeira, Silva et al. (2010a) found p-cymene-7ol (22.5%); 9-epi-(E)-caryophyllene (10.1%), carvone (7.5%) and verbenone (7.4%) at high concentrations. Some of these reported compounds were not identified in any of the essential oil extracts of this study. These variations in the chemical composition of the different samples supports the findings in this work showing that there are distinct chemotypes that occur in different regions.

Analyzing the different concentrations of the major compounds in the oils samples analyzed, there was a cluster with six groups, two with individuals with higher concentration of α-pinene, α-phellandrene, o-cymene, limonene and β-phellandrene (BG1 and BG2, N2 and N4). The third group is characterized by a higher tenor of α-pinene, α-phellandrene, δ-carene and limonene (BG3 and BG5). N1 presented the highest concentrations of α-pinene, β-pinene, α-phellandrene, δ-carene and limonene. α-pinene and β-pinene clustered the individuals BG4, N5 and P4; and α-pinene, β-pinene, δ-carene and myrtenol clustered the individuals N3, P1, P2, P3 and P5 (Figure 5).

According to the principal component analysis, the main component of primary information represent 41.19% of the total and positively associated with triciclene (r = 0.012), γ-terpinene (r = 0.041), β-pinene (r = 0.033), sabinene (r = 0.042), α-terpinene (r = 0.041), germacrene-D (r=0.016), myrtenol (r=0.041) and E-cariofilene (r = 0.035); and negatively with perilla-aldehyde (r=-0.013), canphor (r=-0.037) and cis-p-ment-2-en-o1 (r=- 0.010). The secondary principal component represented 16.26% of the total information related positively to limonene (r=0.009), o-cimenene (r=0.052), α-phellandrene (r=0.028), carvacrol (r=0.033) and myrcene (r=0.037); and, as the main primary negative component relates to perilla-aldehyde (r=-0.077), canphor (r=-0.073) and to cis-p-ment-2-en-o1 (r=-0.008) (Figure 6). When analyzing the correlation between the genetic and chemical profiles, the method of Mantel revealed that there is a positive correlation (0.46) but no significance (0.99). The chemical profile of individuals could not be explained by the genetic profile (Figure 7).

Conclusions

There are genetic and chemical differences among individuals of aroeira, even those that are close geographically. The existence of variations in genetic and chemical profiles is useful for selection of potential individuals for commercial use, and therefore for plant breeding. The evidences for genotypes selection could include oil composition, and phenotype traits of fruits

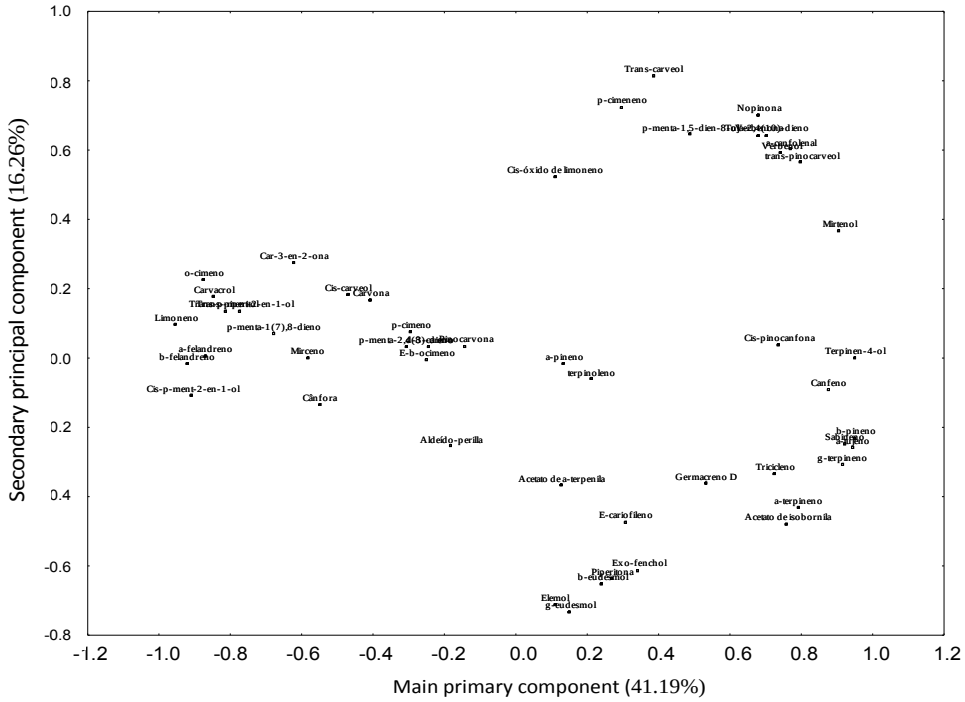

Figure 6. Principal component analysis of chemical compounds of the essential oils of aroeira (*S. terebinthifolius* Raddi.).

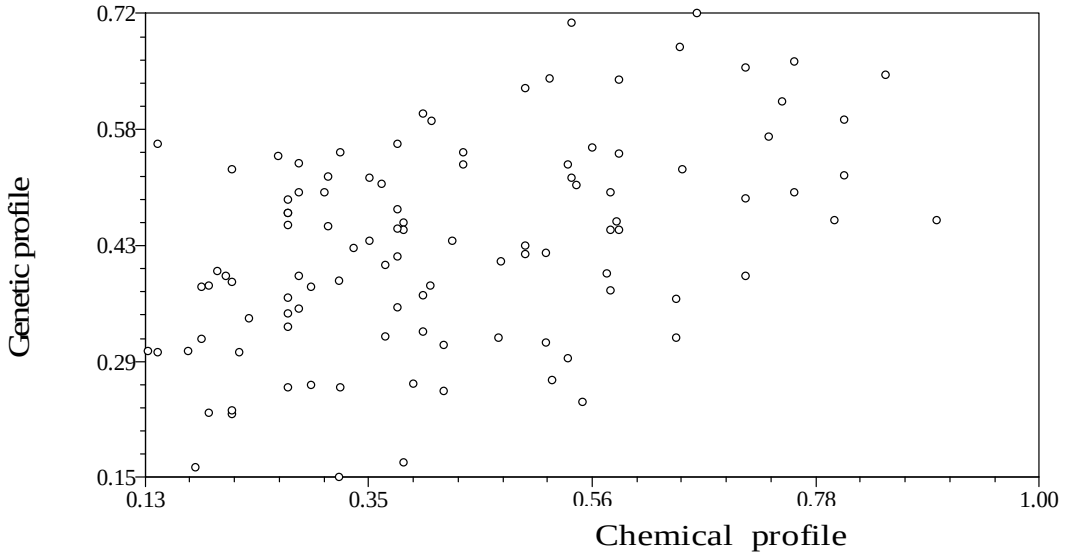

Figure 7. Correlation between genetic and chemical profiles of aroeira (*Schinus terebinthifolius* Raddi.).

production, and also fruits quality.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Adams RP (2007). Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation: Illinois.

Barbosa LCA, Demuner AJ, Clemente AD, Paula VF, Ismail FMD (2007). Seasonal variation in the composition of volatile oils from Schinus terebinthifolius Raddi. Qui Nova. 30(8):1959-1965.

http://dx.doi.org/10.1590/S0100-40422007000800030
Botrel MCG, Souza AM, Carvalho D, Pinto SIC, Moura MCO, Estopa RA (2006). Caracterização genética de Calophyllum brasiliense camb. em duas populações de mata ciliar. Rev. Árvore. 30(5):821-827. http://dx.doi.org/10.1590/S0100-67622006000500016
Brandão MM, Vieira FA, Carvalho D (2011). Estrutura genética em microescala espacial de Myrcia splendens (Myrtaceae). Rev. Árvore. 35(5):957-964. http://dx.doi.org/10.1590/S0100-67622011000600001
Brilho RC (1967). A destilação de óleos essenciais. Campinas: CETREC, P. 20.
Chowdhury AR, Tripani S (2001). Essential oil from leaves of Schinus terebinthifolius Raddi. Ind. Perfumer. 45:257-259.
Coelho ASG (2000). BOOD: avaliação de dendrogramas baseados em estimativas de distâncias/similaridades genéticas através do procedimento de bootstrap. Goiânia: UFG.
Cruz CD, Regazzi AJ, Carneiro PCS (2004). Modelos biométricos aplicados ao melhoramento genético. Viçosa, MG, UFV. 1:480.
Estopa RA, Souza AM, Moura MCO, Botrel MCG, Mendonça EG, Carvalho D (2006). Diversidade genética em populações naturais de candeia (Eremanthus erythropappus (DC.) MacLeish). Sci. Forest (70):97-106.
Ferreira DF (2000). Manual do sistema Sisvar para análises estatísticas, Lavras, Universidade Federal de Lavras. P. 66.
Freire JM, Pi-a-Rodrigues FCM, Lima ER, Sodré SRC, Corrêa RX (2007). Estrutura genética de populações de Schizolobium parahyba (Vell.) Blake (guapuruvu) por meio de marcadores RAPD. Scientia Forestalis (74):27-35.
Freitas MLM, Aukar APA, Sebbenn AM, Moraes MLT, Lemos EGM (2006). Variação genética em progênies de *Myracrodruon urundeuva* F.F. & M.F. Allemão em três sistemas de cultivo. Rev. Árv. 30(3):319-329. http://dx.doi.org/10.1590/S0100-67622006000300001
Ibrahim M, Fobbe R, Nolte J (2004). Chemical composition and biological studies of Egyptian *Schinus molle* L. and *Schinus terebinthifolius* Raddi. oils. Bull Faculty of Pharmacy. (42):289-296.
Julio N, Sobral A, Duenas JR, Rienzo J, Renison D, Hensen I (2008). RAPD and ISSR markers indicate diminished gene flow due to recent fragmentation of Polylepis australis woodlands in central Argentina. Biochem. Syst. Ecol. 36:329-335. http://dx.doi.org/10.1016/j.bse.2007.10.007
Lacerda CMB, Kageyama PY (2003). Estrutura genética espacial de duas populações naturais de Myracrodruon urundeuva M. Allemão na região semi-árida, Brasil. Rev. Árv. 27(2):145-150.
Loss ACC, Leite YLR, Louro ID (2006). Diversidade genética de populações de maracujá-doce (Passiflora alata Curtis) no estado do Espírito Santo, Brasil. Natureza on line. 4(2):55-61.
Loveless MD, Hamrick JL (1984). Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. (15):65-95.
Manica-Cattani MF, Zacaria J, Pauletti G, Atti-Serafini L, Echeverrigaray S (2009). Genetic variation among South Brazilian accessions of Lippia alba Mill. (Verbenaceae) detected by ISSR and RAPD markers. Braz. J. Biol. 69(2):375-380. http://dx.doi.org/10.1590/S1519-69842009000200020 PMid:19675941
Manly BFJ (1997). Randomization, bootstrap and Monte Carlo methods in Biology. Chapman & Hall, London.
Mittermeier RA, Werner TB (1990). Wealth of plants and animals unites megadiversity" countries. Tropicus. 4(1):4-5.
Nei M (1973). Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the USA. 70(12):3321-3323.http://dx.doi.org/10.1073/pnas.70.12.3321 PMid:4519626 PMCid:PMC427228.
Nienhuis J, Tivang J, Sckroch P, Santos JB (1995). Genetic relationships among cultivars and lines of lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J. Am. Soc. Hortic. Sci. 120(2):300-306.
Pearson K (1982). The grammar of science. London, J. M. Dent and Company.
Raposo A, Martins K, Ciampi AY, Wadt LHO, Veasey EA (2007). Diversidade genética de populações de andiroba no Baixo Acre. Pesq. agropec. bras. 42(9):1291-1298.
Rohlf FJ (2000). Numerical taxonomy and multivariate analysis system. version 2.1. New York: Exeter Software. P. 38.
Rosal LF, Pinto JEBP, Bertolucci SKV, Brant RS, Niculau ES, Alves PB (2011). Produção vegetal e de óleo essencial de boldo pequeno em função de fontes de adubos orgânicos. Rev. Ceres. 58(5):670-678.
Roveda LM, Formagio ASN, Baldivia DS, Santos LAC, Vieira MC, Cardoso CAL, Foglio MA, Carvalho JE, Formagio Neto F (2010). Composição química e avaliação da atividade antitumoral do óleo essencial de *Schinus terebinthifolius* Raddi (Anacardiaceae). In: SIMPÓSIO BRASIL-JAPÃO, Campo Grande. Anais. Campo Grande, MS.P. 3.
Santana GC, Silva-Mann R, Ferreira RA, Gois IB, Oliveira AS, Boari A J, Carvalho SVA (2008). Diversidade genética de Enterolobium contortisiliquum (Vell.) Morong. no Baixo Rio São Francisco, por meio de marcadores RAPD. Rev. Árv. 32(3):427-433.
Santana IBB, Oliveira EJ, Soares Filho WS, Ritzinger R, Amorim EP, Costa MAPC, Moreira RFC (2011). Variabilidade genética entre acessos de Umbu-Cajazeira mediante análise de marcadores ISSR. Rev. Bras. Frutic. 33(3):868-876. http://dx.doi.org/10.1590/S0100-29452011005000090
Santos ACA, Rossato M, Agostini F, Santos PL, Serafini LA, Moyna P, Dellacassa E (2007). Avaliação química mensal de três exemplares de Schinus terebinthifolius Raddi. Rev. Bras. Bioci. 5(1):011-1013.
Silva AB, Silva T, Franco ES, Rabelo SS, Lima ER, Mota RA, Câmara CAG, Pontes-Filho NT, Lima-Filho JV (2010a). Antibacterial activity, chemical composition, and cytotoxicity of leaf's essential oil from Brazilian pepper tree (*Schinus terebinthifolius*, Raddi.). Braz. J. Microbiol. 41(1):158-163. http://dx.doi.org/10.1590/S1517-83822010000100023, PMid:24031476 PMCid:PMC3768621.
Silva M, Pessotti BM S, Zanini S F, Colnago GL, Nunes LC, Rodrigues MRA, Ferreira, L (2010b). Uso de óleo de aroeira-vermelha sobre o desempenho e a morfometria intestinal de frangos de corte. Ciênc. Rural. 40(10):2151-2156. http://dx.doi.org/10.1590/S0103-84782010005000160
Singh AK, Singh J, Gupta KC, Brophy JJ (1998). Essential oil of leaves and inflorescence of *Schinus terebinthifolius*: An exotic plant of India. J. Essent. Oil Res. 10(6):697-699. http://dx.doi.org/10.1080/10412905.1998.9701013
Sneath PH, Sokal RR (1973). Numerical Taxonomy: the principles and practice of numerical classification. W. H. Freeman, San Francisco.
Statsoft (2001). Statistica for Windows: computer program manual. Versão 6.0. Tulsa.
Telles MPC, Silva SP, Ramos JR, Soares TN, Melo DB, Resende LV, Batista EC, Vasconcellos BF (2010). Estrutura genética em populações naturais de *Tibouchina papyrus* (pau-papel) em áreas de campo rupestre no cerrado. Rev Bras Botânica. 33(2):291-300. http://dx.doi.org/10.1590/S0100-84042010000200010
Van den Dool H, Kratz PDJ (1963). A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. J. Chromatogr. 11: 463–71. http://dx.doi.org/10.1016/S0021-9673(01)80947-X
Wu YG, Guo QS, He JC, Lin YF, Luo LJ, Liu GD (2010). Genetic diversity analysis among and within populations of Pogostemon cablin from China with ISSR and SRAP markers. Biochem. Syst. Ecol. 38:63-72. http://dx.doi.org/10.1016/j.bse.2009.12.006
Yamamoto PY, Colombo CA, Azevedo Filho JA, Lourenção AL, Marques MOM, Morais GDS, Chiorato AF, Martins ALM, Siqueira WJ (2008). Performance of ginger grass (*Lippia alba*) for traits related to the production of essential oil. Sci. Agric. 65(5):481-489. http://dx.doi.org/10.1590/S0103-90162008000500006
Yeh FC, Yang R, Boyle T (1999). POPGENE version 1.32: Microsoft Window-based freeware for population genetics analysis. University of Alberta, Edmonton, Canada.
Yu H, Yang Z, Liu R (2011). Genetic diversity and relationship of endangered plant *Magnolia officinalis* (Magnoliaceae) assessed with ISSR polymorphisms. Biochem. System. Ecol. 39:71-78. http://dx.doi.org/10.1016/j.bse.2010.12.003