View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Repositorio da Producao Cientifica e Intelectual da Unicamp

UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTIFICA E INTELECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:
https://www.geneticsmr.com/articles/6964

DOI: 10.4238/gmr.15038681

Direitos autorais / Publisher's copyright statement:
©2016 by FUNPECRP. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo
CEP 13083-970 – Campinas SP
Fone: (19) 3521-6493
http://www.repositorio.unicamp.br

Genetics and Molecular Research

Genetic diversity analysis of *Varronia curassavica* Jacq. accessions using ISSR markers

F.A. Brito[1], D.A.C. Nizio[1], A.V.C. Silva[2], L.E.C. Diniz[2], A.R.C. Rabbani[3], M.F. Arrigoni-Blank[1], S.V. Alvares-Carvalho[1], G.M. Figueira[4], I. Montanari Júnior[4] and A.F. Blank[1]

[1]Laboratório de Recursos Genéticos Vegetais e Óleos Essenciais, Departamento de Engenharia Agronômica, Universidade Federal de Sergipe, São Cristóvão, SE, Brasil
[2]Laboratório de Biotecnologia Molecular, Embrapa Tabuleiros Costeiros, Aracaju, SE, Brasil
[3]Instituto Federal da Bahia, Porto Seguro, BA, Brasil
[4]Centro Pluridisciplinar de Pesquisas Químicas Biológicas e Agrícolas, Universidade Estadual de Campinas, Campinas, SP, Brasil

Corresponding author: A.F. Blank
E-mail: afblank@ufs.br

Genet. Mol. Res. 15 (3): gmr.15038681
Received March 31, 2016
Accepted May 25, 2016
Published September 2, 2016
DOI http://dx.doi.org/10.4238/gmr.15038681

Copyright © 2016 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License

ABSTRACT. *Varronia curassavica* Jacq. is a medicinal and aromatic plant from Brazil with significant economic importance. Studies on genetic diversity in active germplasm banks (AGB) are essential for conservation and breeding programs. The aim of this study was to analyze the genetic diversity of *V. curassavica* accessions of the AGB of Medicinal and Aromatic Plants of the Federal University of Sergipe (UFS), using inter-simple sequence repeat molecular markers. Twenty-four primers were tested, and 14 were polymorphic and informative, resulting in 149 bands with 97.98% polymorphism. The UPGMA

dendrogram divided the accessions into Clusters I and II. Jaccard similarity coefficients for pair-wise comparisons of accessions ranged between 0.24 and 0.78. The pairs of accessions VCUR-001/VCUR-503, VCUR-001/VCUR-504, and VCUR-104/VCUR-501 showed relatively low similarity (0.24), and the pair of accessions VCUR-402/VCUR-403 showed medium similarity (0.78). Twenty-eight accessions were divided into three distinct clusters, according to the STRUCTURE analysis. The genetic diversity of *V. curassavica* in the AGB of UFS is low to medium, and it requires expansion. Accession VCUR-802 is the most suitable for selection in breeding program of this species, since it clearly represents all of the diversity present in the AGB.

Key words: *Varronia curassavica*; Conservation; Diversity; ISSR

INTRODUCTION

Varronia curassavica Jacq. (with synonym *Cordia verbenacea* DC.) is popularly known as "erva-baleeira." It is a medicinal and aromatic plant in the Cordiaceae family (previously Boraginaceae) and is native to Central and South America, occurring mainly in Brazil (Barroso et al., 2002; Gasparino and Barros, 2009; Gilbert and Favoreto, 2012). It is a perennial, highly branched shrub that can reach 1.5 to 2.5 m in height, and it is widely used in popular medicine to treat arthritis (Lorenzi and Matos, 2008). The Brazilian National Health Surveillance Agency approved the first herbal medicine produced from an essential oil of this plant in 2004, with the active compound, α-humulene, showing anti-inflammatory activity (Feijó et al., 2014). This shows how important this species is for the pharmaceutical industry. In a study of native populations of *V. curassavica* in the State of Sergipe, the authors reported a high chemical diversity in essential oils, and five chemical groups were detected (Nizio et al., 2015). This finding, together with the medical importance of the species, and the loss of plant genetic resources due to human activities, highlights the importance of conserving the diversity of this species. The partial conservation of medicinal or agricultural plant species can be performed in active germplasm banks (AGB). A representative sample of the genetic variability of the species can be conserved, allowing the identification and selection of accessions of interest that can be used to develop superior cultivars through breeding programs (Blank, 2013).

The genetic diversity of a species can be assessed through various types of molecular markers, including inter-simple sequence repeats (ISSRs). These molecular markers use short repeated sequences of DNA to amplify anonymous loci and do not require prior knowledge of the genome. Because they are dominant loci, it is not possible to distinguish heterozygotes from homozygotes; however, multiple loci can be produced from each amplification with polymerase chain reaction (PCR; Goulão and Oliveira, 2001). Several genetic diversity studies on medicinal plants, both in natural populations and in collections of germplasm banks, have been carried out using ISSRs, such as studies on *Capparis spinosa*, *Eucommia ulmoides*, and *Pogostemon cablin* (Liu et al., 2015; Yu et al., 2015; Sandes et al., 2016).

Universidade Federal de Sergipe (UFS) studied the chemical diversity of populations of *V. curassavica* from the State of Sergipe, and genetic resources from those populations are conserved in an AGB that was implemented in 2012. The present study is the first investigation of genetic diversity of this species, using ISSR markers.

Thus, the aim of this study was to analyze the genetic diversity of *V. curassavica* accessions of the AGB of Medicinal and Aromatic Plants of UFS using ISSR molecular markers. This information will then be used to prioritize accessions for conservation and genetic improvement of the species.

MATERIAL AND METHODS

Plant material

After exploitation and collection of plant material from the State of Sergipe in the municipalities of Graccho Cardoso, Tobias Barreto, São Cristóvão, Japaratuba, Tomar do Geru, Itabi, Cedro de São João, and Itabaiana, and with five accessions from seedling exchange with the AGB of Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, collected in the State of São Paulo (Table 1), the *V. curassavica* collection was implemented within the AGB of medicinal and aromatic plants of UFS. The AGB is located at the Research Farm Campus Rural da UFS, in São Cristóvão, State of Sergipe, Brazil (11°00'S; 37°12'W), with 28 accessions of *V. curassavica*.

Fresh and young leaves of each accession from the AGB were collected, wrapped in sterile gauze, and packed in ice at the time of collection, to prevent oxidation. Afterwards, they were frozen at -80°C until lyophilization in a LioTop L101 (Liobras, São Carlos, SP, Brazil). After lyophilization, samples were stored in a desiccator containing silica gel until DNA extraction.

Table 1. Characteristics of 28 accessions of *Varronia curassavica* from the active germplasm bank of medicinal and aromatic plants of Universidade Federal de Sergipe.

Accession code	Source/origin within Brazil (municipality, state)	Georeferenced information
VCUR-001	Donated by Multidisciplinary Center for Chemical, Biological and Agricultural Research, Campinas State University, Campinas, São Paulo	-
VCUR-002	Ubatuba, São Paulo	23°32'18.0"S; 45°03'73.4"W
VCUR-003	Ilha Comprida, São Paulo	25°02'44.0"S; 47°53'17.0"W
VCUR-004	Mongágua, São Paulo	24°08'00.0"S; 46°42'54.0"W
VCUR-005	Ilha Comprida, São Paulo	24°43'08.0"S; 47°30'36.0"W
VCUR-101	Graccho Cardoso, Sergipe	10°14'48.5"S; 37°12'52.8"W
VCUR-102	Graccho Cardoso, Sergipe	10°14'47.6"S; 37°12'52.8"W
VCUR-103	Graccho Cardoso, Sergipe	10°14'47.9"S; 37°12'52.2"W
VCUR-104	Graccho Cardoso, Sergipe	10°14'46.1"S; 37°12'52.8"W
VCUR-105	Graccho Cardoso, Sergipe	10°14'46.4"S; 37°13'26.6"W
VCUR-201	Tobias Barreto, Sergipe	11°03'54.2"S; 38°03'21.1"W
VCUR-202	Tobias Barreto, Sergipe	11°04'10.1"S; 38°04'03.4"W
VCUR-301	São Cristóvão, Sergipe	10°54'26.3"S; 37°11'53.1"W
VCUR-302	São Cristóvão, Sergipe	10°54'59.7"S; 37°11'16.3"W
VCUR-303	São Cristóvão, Sergipe	10°54'48.5"S; 37°11'50.3"W
VCUR-401	Japaratuba, Sergipe	10°38'05.4"S; 36°55'10.5"W
VCUR-402	Japaratuba, Sergipe	10°37'59.9"S; 36°55'16.1"W
VCUR-403	Japaratuba, Sergipe	10°37'39.0"S; 36°55'25.8"W
VCUR-404	Japaratuba, Sergipe	10°37'37.8"S; 36°56'00.0"W
VCUR-501	Tomar do Geru, Sergipe	11°21'12.0"S; 37°50'59.0"W
VCUR-502	Tomar do Geru, Sergipe	11°19'17.1"S; 37°52'02.4"W
VCUR-503	Tomar do Geru, Sergipe	11°19'05.2"S; 37°52'17.5"W
VCUR-504	Tomar do Geru, Sergipe	11°19'01.7"S; 37°52'25.0"W
VCUR-505	Tomar do Geru, Sergipe	11°19'04.0"S; 37°51'51.8"W
VCUR-601	Itabi, Sergipe	10°09'24.9"S; 37°08'27.0"W
VCUR-701	Cedro de São João, Sergipe	10°18'06.9"S; 36°53'27.7"W
VCUR-801	Itabaiana, Sergipe	10°50'27.6"S; 37°12'49.3"W
VCUR-802	Itabaiana, Sergipe	10°48'28.9"S; 37°15'48.6"W

DNA extraction and ISSR amplification

DNA extraction was carried out using the 2% CTAB method (Doyle and Doyle, 1990), modified by Alzate-Marin et al. (2005). DNA quantification was carried out using a NanoDrop 2000c (Thermo Fisher Scientific, Wilmington, DE, USA), and samples were diluted to a standard concentration. ISSR primers used in this study were produced by Eurofins MWG Operon (Operon Technologies, Louisville, KY, USA), IDT (Integrated DNA Technologies, Coralville, IA, USA), and Invitrogen (Thermo Fisher Scientific, Carlsbad, CA, USA). Twenty-four primers were tested for PCR amplification. Reactions were carried out in a total volume of 20 µL containing 1.0 µL genomic DNA (10 ng/µL), 0.2 µL Taq polymerase from *Thermus aquaticus* recombinant, expressed in *Escherichia coli* (Sigma-Aldrich) (0.05 units/µL), 2 µL 10X buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin) (Sigma-Aldrich, St. Louis, MO, USA), 0.4 µL dNTP (2.5 mM), 1.0 µL primer (25.0 pmol), and 15.4 µL autoclaved ultrapure water.

Amplification was carried out in a ProFlex PCR System thermocycler (Thermo Fisher Scientific, Applied Biosystems, Foster City, CA, USA) programmed with the following protocol: 5 min at 94°C; 45 cycles of 40 s at 94°C, 30 s ranging from 50.4° to 53°C (using the annealing temperature of each primer; Table 2), and 1 min at 72°C; and a final extension for 7 min at 72°C. Amplification products were subjected to electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, visualized under ultraviolet light, and photographed. Molecular weights were estimated using a Ludwig DNA scale of 1 kb for each primer.

Table 2. ISSR primers, their sequence, annealing temperature, and the amplified products used for genetic diversity analysis of *Varronia curassavica*.

Primer name	Sequence (5'-3')	Length (bp)	Annealing temperature (°C)	Total number of bands	Number of polymorphic bands	% polymorphism
ISSR1	CAC ACA CAC ACA GG	100-400	51.5	11	11	100.0
ISSR2	CTC TCT CTC TCT CTC TAC	100-350	51.5	13	13	100.0
ISSR3	CTC TCT CTC TCT CTC TTG	850-2000	51.5	10	10	100.0
ISSR4	CAC ACA CAC ACA AC	250-350	51.5	7	7	100.0
ISSR5	CTC TCT CTC TCT CTC TGC	150-350	51.5	13	13	100.0
ISSR6	CAC ACA CAC ACA AG	50-450	51.5	12	11	91.7
UBC810	GAG AGA GAG AGA GAG AT	200-700	50.4	12	12	100.0
UBC811	GAG AGA GAG AGA GAG AC	100-350	53.0	6	6	100.0
UBC834	AGA GAG AGA GAG AGY T	1000-2000	52.8	11	11	100.0
UBC841	GAG AGA GAG AGA GAG AYC	500-1000	52.0	11	11	100.0
UBC845	CTC TCT CTC TCT CTC TRG	400-2000	51.5	12	12	100.0
UBC855	ACA CAC ACA CAC ACY T	250-1000	53.0	17	17	100.0
UBC857	ACA CAC ACA CAC ACY G	200-1000	53.0	9	7	77.8
UBC858	TGT GTG TGT GTG TGT GRT	450-1000	53.0	5	5	100.00

Data analysis

For the analysis and interpretation of the gel, only the clearly-visible bands were used. Loci were identified as present (1) or absent (0) in each accession, and this binary data matrix was used for the analyses.

Similarity coefficients were calculated using the Jaccard index (Jaccard, 1908). NTSYSpc 2.0 (Rohlf, 2001) was used to construct a dendrogram, using the unweighted pair group method with arithmetic mean (UPGMA). The Shannon index (*I*) e o marker index (MI) were calculated using the software GENALEX 6.5 (Peakall and Smouse, 2012).

STRUCTURE v.2.3.3 was used to analyze genetic structure using a Bayesian clustering method (Hubisz et al., 2009). The "admixture" model was used with correlated allele frequencies, and simulations were carried out with a burn-in of 100,000 generations and K values ranging from 2 to 6 clusters. The number of clusters (K) was determined using STRUCTURE HARVESTER (Earl and vonHoldt, 2012).

RESULTS

A high level of polymorphism was found in ISSR markers among *V. curassavica* accessions of the AGB of Medicinal and Aromatic Plants of UFS. The location of the bands can be visualized through the images generated by the photodocumentation of the agarose gels (Figure 1). Of the 24 ISSR primers tested in this study, 14 generated informative bands. There were 149 amplified bands total, ranging from 5 to 17 bands per primer, with a mean of 11 (Table 2). Among them, 146 were polymorphic, which corresponds to 97.98% polymorphism. *I* value indicates genetic diversity and varies from 0 to 1, with values closer to zero having lower diversity (Silva et al., 2015). The *I* value was equal to 0.42, ranging from 0.25 to 0.57 for the loci of the evaluated accessions of *V. curassavica* of the AGB of Medicinal and Aromatic Plants of UFS, which suggests amplitude od medium diversity.

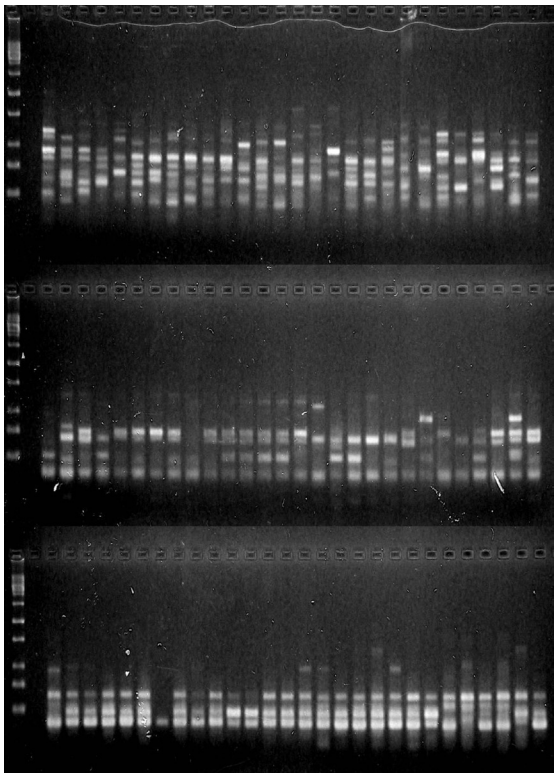

Figure 1. Dendrogram generated by UPGMA analysis of Jaccard similarity indices for 28 accessions of *Varronia curassavica* from the Active Germplasm Bank of Medicinal and Aromatic Plants of Universidade Federal de Sergipe.

Heterozygosity, which is a measure of genetic variability of a population (McManus et al., 2011), was considered low, with a mean of 0.27. The polymorphic information content (PIC), described by Botstein et al. (1980), indicates how much the used marker shows polymorphic information in genetic diversity studies. According to Botstein et al. (1980), PIC values higher than 0.5 are very informative; those between 0.25 and 0.50 are moderately informative; and values lower than 0.25 are considered uninformative. The present study found a mean PIC of 0.27, which is considered moderately informative. On the other hand, the MI, which is a parameter defined as the product of expected heterozygosity and proportion in multiplex, that estimates the overall quality of each molecular marker system (Powell et al., 1996), ranged from 1.22 to 5.48 with a mean of 2.88.

The Jaccard similarity coefficient ranged from 0.24 to 0.78, with a mean of 0.41, which indicates relatively high genetic diversity for many pairs of accessions. Accessions VCUR-402 and VCUR-403, both from Japaratuba/Sergipe, were the most similar genetically, with an index of 0.78. On the other hand, the pairs of accessions VCUR-001 and VCUR-503, VCUR-001 and VCUR-504, and VCUR-104 and VCUR-501 showed the lowest genetic similarity, each with an index of 0.24. There were 15 genotype combinations with medium genetic similarity, i.e., their values ranged between 0.60 and 0.78, and the other combinations presented low genetic similarity, with values below 0.6 (0.24 to 0.59; Table 3).

Table 3. Jaccard coefficients for *Varronia curassavica* accessions of the active germplasm bank of medicinal and aromatic plants of Universidade Federal de Sergipe.

Accessions	VCUR-001	VCUR-002	VCUR-003	VCUR-004	VCUR-005	VCUR-101	VCUR-102	VCUR-103	VCUR-104	VCUR-105	VCUR-201	VCUR-202	VCUR-301	VCUR-302	VCUR-303	VCUR-401	VCUR-402	VCUR-403	VCUR-404	VCUR-501	VCUR-502	VCUR-503	VCUR-504	VCUR-505	VCUR-601	VCUR-701	VCUR-801	VCUR-802
VCUR-001	-																											
VCUR-002	0.32	-																										
VCUR-003	0.32	0.59	-																									
VCUR-004	0.27	0.61	0.62	-																								
VCUR-005	0.27	0.54	0.53	0.55	-																							
VCUR-101	0.35	0.31	0.37	0.35	0.38	-																						
VCUR-102	0.35	0.31	0.31	0.34	0.34	0.44	-																					
VCUR-103	0.37	0.28	0.33	0.33	0.31	0.45	0.51	-																				
VCUR-104	0.25	0.25	0.29	0.28	0.27	0.35	0.38	0.51	-																			
VCUR-105	0.34	0.29	0.36	0.37	0.36	0.33	0.41	0.49	0.41	-																		
VCUR-201	0.44	0.35	0.34	0.38	0.35	0.44	0.39	0.45	0.38	0.36	-																	
VCUR-202	0.34	0.26	0.30	0.28	0.28	0.40	0.44	0.49	0.38	0.48	0.42	-																
VCUR-301	0.32	0.30	0.28	0.30	0.29	0.40	0.29	0.38	0.30	0.48	0.32	0.37	-															
VCUR-302	0.32	0.42	0.42	0.40	0.43	0.37	0.44	0.45	0.35	0.45	0.35	0.42	0.40	-														
VCUR-303	0.30	0.38	0.40	0.43	0.42	0.37	0.40	0.42	0.28	0.45	0.33	0.36	0.41	0.68	-													
VCUR-401	0.25	0.33	0.32	0.38	0.39	0.35	0.29	0.34	0.25	0.27	0.32	0.26	0.30	0.46	0.51	-												
VCUR-402	0.31	0.43	0.44	0.49	0.43	0.41	0.39	0.44	0.33	0.49	0.38	0.35	0.40	0.59	0.62	0.50	-											
VCUR-403	0.31	0.39	0.39	0.48	0.39	0.36	0.34	0.46	0.37	0.44	0.40	0.33	0.35	0.54	0.55	0.53	0.78	-										
VCUR-404	0.33	0.35	0.35	0.38	0.38	0.34	0.35	0.43	0.34	0.42	0.39	0.33	0.35	0.53	0.58	0.50	0.60	0.62	-									
VCUR-501	0.28	0.40	0.35	0.38	0.41	0.35	0.31	0.31	0.24	0.36	0.30	0.31	0.38	0.42	0.47	0.42	0.46	0.45	0.51	-								
VCUR-502	0.29	0.35	0.38	0.38	0.36	0.42	0.33	0.39	0.35	0.37	0.37	0.30	0.36	0.48	0.51	0.51	0.55	0.57	0.53	0.52	-							
VCUR-503	0.24	0.42	0.40	0.46	0.45	0.40	0.30	0.40	0.32	0.38	0.37	0.27	0.32	0.49	0.53	0.48	0.61	0.59	0.57	0.47	0.56	-						
VCUR-504	0.24	0.41	0.43	0.45	0.42	0.43	0.31	0.35	0.29	0.35	0.38	0.27	0.31	0.43	0.50	0.45	0.47	0.48	0.53	0.43	0.48	0.61	-					
VCUR-505	0.28	0.37	0.40	0.43	0.37	0.39	0.28	0.40	0.29	0.36	0.40	0.30	0.36	0.40	0.46	0.47	0.52	0.55	0.54	0.49	0.60	0.65	0.56	-				
VCUR-601	0.32	0.53	0.46	0.48	0.52	0.40	0.38	0.40	0.33	0.39	0.36	0.33	0.35	0.58	0.51	0.47	0.57	0.52	0.50	0.46	0.52	0.62	0.57	0.55	-			
VCUR-701	0.33	0.50	0.51	0.53	0.41	0.38	0.29	0.35	0.29	0.36	0.40	0.27	0.33	0.48	0.53	0.44	0.63	0.59	0.48	0.42	0.54	0.61	0.53	0.52	0.66	-		
VCUR-801	0.28	0.42	0.44	0.47	0.48	0.36	0.28	0.36	0.30	0.36	0.38	0.30	0.29	0.47	0.50	0.49	0.54	0.55	0.51	0.40	0.43	0.53	0.58	0.52	0.53	0.52	-	
VCUR-802	0.30	0.36	0.38	0.45	0.40	0.34	0.29	0.30	0.30	0.36	0.34	0.33	0.31	0.38	0.41	0.37	0.49	0.48	0.38	0.35	0.44	0.45	0.44	0.41	0.37	0.45	0.48	-

The dendrogram was divided into three major clusters (I, II and III) (Figure 2). Cluster I (including 32.15% of the accessions from the AGB) presented Jaccard coefficients below 0.5, whereas similarities for Cluster II (53.57% of the AGB) were between 0.4 and 0.78, and Cluster III (14.28 of the AGB) with similarities between 0.5 and 0.6.

STRUCTURE effectively separated the 28 accessions in three distinct and well-defined clusters (Figure 3).

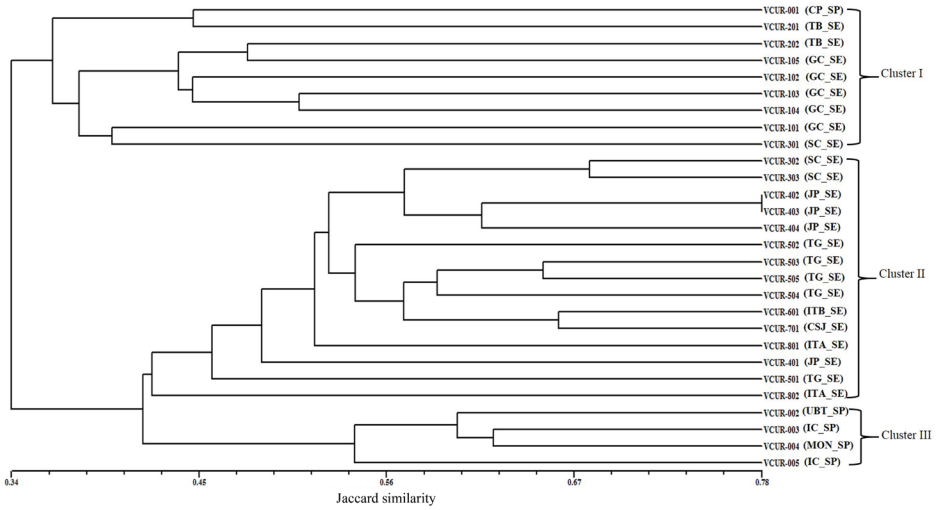

Figure 2. STRUCTURE results for 28 accessions of *Varronia curassavica* with K = 3. Each vertical bar represents one accession, with color indicating cluster membership.

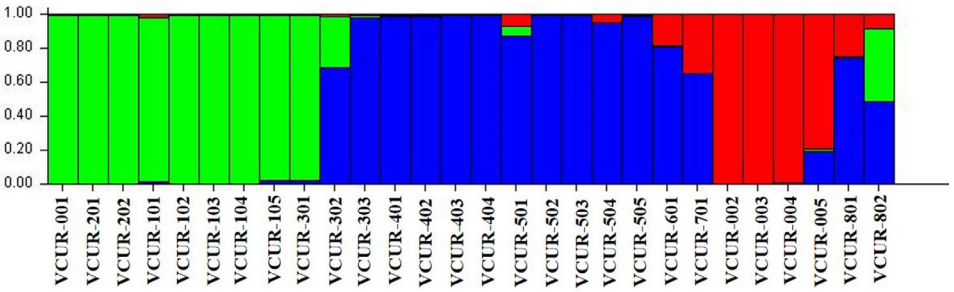

Figure 3. Principal component analysis generated by Statistica based on 149 identified bands.

DISCUSSION

Total genetic diversity of *V. curassavica* accessions is low to medium, as indicated by several diversity statistics. The genetic diversity found in the present study contrasts with the levels of chemical diversity found by Nizio et al. (2015) for five natural populations of *V. curassavica*. They detected high chemical diversity in essential oils and the division into five chemical clusters. The observed chemical diversity in essential oils can be explained in part by genetic factors. Thus, an option for expanding the genetic diversity of the AGB is the addition of some of the plants identified by Nizio et al. (2015) that produced a diversity of oil types. The addition of more ISSR or other molecular markers may identify loci that are associated with the variability seen in essential oil production for different lineages of *V. curassavica*.

According to the dendrogram (Figure 1), there is a trend of clustering according to the geographic origin of the accessions, except for accessions from São Cristóvão/SE and São Paulo/SP, which are each present in both Clusters I and II. Subcluster 1 of Cluster I has only

accessions VCUR-001 and VCUR-201 from São Paulo/SP and Tobias Barreto/SE. Subcluster 1 of Cluster II includes all of the samples from Graccho Cardoso/SE, together with VCUR-202 from Tobias Barreto/SE. With 15 accessions from several geographical locations, subcluster 3 from Cluster II is the largest subcluster. These accessions have average and low pair-wise genetic similarity. Finally, o Cluster 4 of Cluster II consists only of additional accessions from São Paulo/SP, which have relatively high similarity within the cluster but low similarity to other accessions from São Paulo/SP in Cluster I.

The three clusters resolved by STRUCTURE are consistent with those inferred in the dendrogram. Using the same type of molecular marker, Liu et al. (2015) observed similar results, the formation of three groups in *C. spinosa*. Accession VCUR-802 from Itabaiana/SE shows some affinity to each of the three groups in the STRUCTURE plot, suggesting admixture. Thus, utmost care is necessary in order to avoid losing this accession, since it may be most important for future studies on genetic improvement. Ten patchouli accessions from the AGB of medicinal and aromatic plants of UFS were efficiently identified as two distinct genetic clusters by STRUCTURE (Sandes et al., 2016), highlighting the importance of such methods in studies with AGBs of medicinal and aromatic plants.

There are no reports of studies on plants of the Cordiaceae family using ISSR markers. However, ISSR analyses of *Echium vulgare* L. (Boraginaceae) found lower polymorphism (93.15%), an average of 6-17 bands per primer, a mean PIC of 0.373 (similar to that found here), and an MI of 3.1 (Dresler et al., 2015). Zhao et al. (2014) observed high genetic diversity when evaluating germplasm of 20 plants of *Lilium* (Liliaceae), with an average of 17-22 amplified bands per primer, but a lower *I* (0.35). Lopez et al. (2015) evaluated genetic diversity in *Omphalodes littoralis* subsp *gallaecica* (Boraginaceae) with AFLP markers, which are also dominant, and observed heterozygosity of 0.356 and moderate genetic diversity.

Knowledge of levels of genetic diversity helps in the proper handling of an AGB, which is meant to be a sample of the genetic variation of a species (Ramalho et al., 2012). In a breeding program, this knowledge on the established accessions is of fundamental importance. The use of techniques such as assays of DNA markers can provide accurate estimates of this diversity for a portion of the genome without the potential problems of environmental influence on gene expression. According to Celestino et al. (2015), molecular tools are essential to characterize the genetic diversity of aromatic and medicinal plants. Although agronomic and chemical characteristics may distinguish different groups, expression of traits such as essential oils can be altered by environmental factors.

The genetic diversity of the collection of *V. curassavica* at UFS is low to medium. Its expansion is required, including more accessions from other locations/regions. Accession VCUR-802 is the most suitable for conservation of this species, since it represents important diversity present in the AGB. This is the first study on the genetic diversity of a collection of *V. curassavica* plants using ISSR markers, and the results are important for the conservation of this species, indicating the need to expand the diversity of the germplasm bank and producing useful information for breeding programs that may use the studied accessions.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by CNPq, FAPITEC/SE, CAPES, and FINEP.

REFERENCES

Alzate-Marin AL, Guidugli MC, Soriani HH and Mestriner MA (2005). Otimização de um método econômico e rápido de extração de DNA para quatro espécies de árvores tropicais. In: Anais do 51° Congresso Brasileiro de Genética, Águas de Lindóia.

Barroso ICE, Oliveira F, Branco LHZ, Kato ETM, et al. (2002). O gênero *Cordia* L: botânica, química e farmacologia. *Rev. Lecta* 20: 15-34.

Blank AF (2013). Transformação de recursos genéticos de plantas aromáticas nativas em riqueza: o potencial do alecrim-de-tabuleiro (*Lippia gracilis*). *Hortic. Bras.* 31: 512. http://dx.doi.org/10.1590/S0102-05362013000300029

Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Celestino RS, Zucchi MI, Pinheiro JB, Campos JB, et al. (2015). Molecular and chemical characterization of vetiver, *Chrysopogon zizanioides* (L.) Roberty, germplasm. *Genet. Mol. Res.* 14: 9452-9468. http://dx.doi.org/10.4238/2015.August.14.9

Doyle JJ and Doyle JL (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Dresler S, Tyrka M, Szeliga M, Ciura J, et al. (2015). Increased genetic diversity in the populations of *Echium vulgare* L. colonising Zn-Pb waste heaps. *Biochem. Syst. Ecol.* 60: 28-36. http://dx.doi.org/10.1016/j.bse.2015.03.003

Earl DA and vonHoldt BM (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4: 359-361. http://dx.doi.org/10.1007/s12686-011-9548-7

Feijó EVRS, Oliveira RA and Costa LCB (2014). Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency. *Rev. Bras. Farmacogn.* 24: 516-523. http://dx.doi.org/10.1016/j.bjp.2014.10.005

Gasparino EC and Barros MAVC (2009). Palinotaxonomia das espécies de Cordiaceae (Boraginales) ocorrentes no Estado de São Paulo. *Rev. Brasil. Bot.* 32: 33-55.

Gilbert B and Favoreto R (2012). *Cordia verbenacea* DC-Boraginaceae. *Rev. Fitos* 7: 17-25.

Goulão L and Oliveira CM (2001). Molecular characterization of cultivars of apple (*Malus* x *domestica* Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica* 122: 81-89. http://dx.doi.org/10.1023/A:1012691814643

Hubisz MJ, Falush D, Stephens M and Pritchard JK (2009). Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* 9: 1322-1332. http://dx.doi.org/10.1111/j.1755-0998.2009.02591.x

Jaccard P (1908). Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.* 44: 223-270.

Liu C, Xue GP, Cheng B, Wang X, et al. (2015). Genetic diversity analysis of *Capparis spinosa* L. populations by using ISSR markers. *Genet. Mol. Res.* 14: 16476-16483. http://dx.doi.org/10.4238/2015.December.9.19

Lopez L, Retuerto R, Roiloa S, Santis X, et al. (2015). A multi-faceted approach for assessing evolutionary significant conservation units in the endangered *Omphalodes littoralis* subsp. *gallaecica* (Boraginaceae). *Perspect. Plant Ecol. Evol. Syst.* 17: 54-65. http://dx.doi.org/10.1016/j.ppees.2014.09.003

Lorenzi H and Matos FJA (2008). Plantas medicinais no Brasil: nativas e exóticas. 2nd edn. Editora Instituto Plantarum, Nova Odessa.

McManus C, Paiva S, Corrêa PS, Seixas L, et al. (2011). Estatísticas para descrever genética de populações. Informação Genético-Sanitária da Pecuária Brasileira (INCT). Available at [http://inctpecuaria.com.br/images/informacoes-tecnicas/serie_tecnica_genetica_populacoes.pdf]. Accessed February 30, 2016.

Nizio DAC, Brito FA, Sampaio TS, Melo JO, et al. (2015). Chemical diversity of native populations of *Varronia curassavica* Jacq. and antifungal activity against *Lasiodoplodia theobromae*. *Ind. Crops Prod.* 76: 437-448. http://dx.doi.org/10.1016/j.indcrop.2015.07.026

Peakall R and Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28: 2537-2539. http://dx.doi.org/10.1093/bioinformatics/bts460

Powell W, Morgante R, Andre C, Hanafey M, et al. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* 2: 225-238. http://dx.doi.org/10.1007/BF00564200

Ramalho MAP, Santos JB, Pinto CABP, Souza EA, et al. (2012). Genética na Agropecuária. 5th edn. Editora UFLA, Lavras.

Rohlf FJ (2001). NTSYSpc: numerical taxonomy system, Version 2.0. Exeter Publishing, Setauket.

Sandes SS, Zucchi MI, Pinheiro JB, Bajay MM, et al. (2016). Molecular characterization of patchouli (*Pogostemon* spp) germplasm. *Genet. Mol. Res.* 15: 2-12. http://dx.doi.org/10.4238/gmr.15017458

Silva AVC, Muniz EN, Almeida CS, Vitória MF, et al. (2015). Genetic diversity and sex identification in *Genipa americana* L. *Trop. Subtrop. Agroecosys.* 18: 81-86.

Yu J, Wang Y, Ru M, Peng L, et al. (2015). Genetic diversity in intraspecific hybrid populations of *Eucommia ulmoides* Oliver evaluated from ISSR and SRAP molecular marker analysis. *Genet. Mol. Res.* 14: 7417-7425. http://dx.doi.org/10.4238/2015.July.3.17

Zhao L, Liu H, Cai G and Xia M (2014). Assessment of the genetic diversity and genetic relationships of *Lilium* in China using ISSR marker. *Biochem. Syst. Ecol.* 55: 184-189. http://dx.doi.org/10.1016/j.bse.2014.03.024