



Biodiversity hotspots for conservation of *Hancornia speciosa* Gomes

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Abstract *Hancornia speciosa* is one target species for genetic diversity ethnobotanical and medicinal applications within the Brazilian Northeast Atlantic Forest biodiversity hotspot. However, information on the genetic variability of populations associated with modeling the potential distribution in the state of Sergipe has not yet been performed. Therefore, the objective of this study was to predict the potential occurrence of *H. speciosa* in areas in which fruits are intensively used in an extractive practice. The maximum entropy method was used to detect the

distribution patterns of *H. speciosa* in variable environments within the referred hotspot. The diversity of four natural populations, situated in areas of extractivism, was determined by ISSR molecular markers. The species appears to occur more densely in the coastal regions of the state of Sergipe. However, data prediction occurrence indicates that the areas of natural presence have been reduced due to anthropic actions.

Keywords Species distribution modeling · Maxent · Mangaba · ISSR, genetic structure

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Introduction

Habitat loss and fragmentation has long been the cause for biodiversity loss and forest ecosystems degradation (Pütz et al. 2011). This biodiversity loss has occurred in areas of the Atlantic Forest, a Brazilian biome that has suffered predatory human actions for centuries, leading to species extinction. Within this biome, Restinga areas are plant phyto-physiognomies under significant maritime influence and exposed to enormous climate and anthropic pressure (Oliveira and Landim 2016).

Restinga areas are scarcely studied given their importance. Moreover, information regarding its biological maintenance dynamic is crucial for conservation strategies since natural areas of high diversity are a refuge for different types of wildlife (Cerqueira 2000).

In the last decades, research has allowed a better understanding of the natural regeneration process of these forests and how management and conservation strategies should be (Almeida 2016). A practical tool to support these strategies is the knowledge of genetic variability, influenced by intrinsic characteristics and the species' environment. Knowledge of genetic diversity within land-use occupation, and climate, contribute to assessing the direct consequences of anthropic actions on the structure of natural populations.

One species impacted by forest fragmentation has been *H. speciosa*, a fruit tree commonly denominated mangaba tree (Fajardo et al. 2018). This species has relevance for the food industry and is part of the List of Native Species of the Brazilian Flora of Current or Potential Economic Value (Pereira et al. 2016; Nunes et al. 2022). *H. speciosa* also has the potential for composition and recovery of restinga areas.

Geno-phenotypic diversity in a natural population of *H. speciosa* and implications for conservation and breeding has been studied. Their fruits, seeds, and seedlings present helpful for use in the species conservation and propagation, as well as in phenotypic variability, attested by heritability, germination, and vigor data. The search of populations with genetic variability is essential for this species, configuring these natural resources as seed trees to provide propagules for the enrichment of natural populations, forest restoration, and genetic breeding (Nunes et al. 2021).

In genetic diversity studies with *H. speciosa*, the heterozygosity variation in populations has been reported in the state of Pernambuco (Jimenez et al. 2015), Rio Grande do Norte (Costa et al. 2015), Mato Grosso (Soares et al. 2017), Sergipe (Silva et al. 2017) and Goiás (Rodrigues et al. 2015). Consequently, preserving this diversity is a determining factor for the conservation and sustainability of fruit production, of which traditional communities, such as Catchers of Mangaba, have part of their income. *H. speciosa* is included in the list of species of Brazilian flora that have current or potential economic value (Nunes et al. 2022). Enabling a sustainable and prosperous future through science and innovation in the bioeconomy at Agriculture and Agri-Food Brazilian perspectives might be a strategy. Therefore, it is considered a priority species for research and development (Coradin et al. 2018), enabling a sustainable and prosperous

future through science and innovation in the bioeconomy at Agriculture and Agri-Food Sectors maybe a strategy. Other intrinsic factors that affect this species' conservation are the recalcitrant character of the seeds and the difficulties of its micropropagation. Thus, the germplasm of *H. speciosa* has been conserved as live plants kept in situ, in permanent preservation areas, legal reserves, conservation units, and in germplasm collections maintained by Federal University of Goiás (Ganga et al. 2009), Embrapa Amapá (Yokomizo, Santos and Freitas 2017), State Agricultural Research Corporation of Paraíba (Souza et al. 2007), and Embrapa Tabuleiros Costeiros in Sergipe (Muniz et al. 2019), that are susceptible to human actions, climate change and natural disasters.

Furthermore, despite several studies with the species, there is still no focus on identifying environmental characteristics associated with the potential distribution of *H. speciosa* in Sergipe. Thus, the objective of this study was to predict the potential occurrence of *H. speciosa* and to identify priority areas for the conservation, seed collection for domestication according to the genetic diversity distribution under present and future climatic data.

Material and methods

Distribution modeling

The mangaba trees in areas of extractivism were geo-referenced using GPS coordinates in field in the municipalities of Pirambu municipality in state of Sergipe. (Fig. 1).

Vegetable material (young leaves) was collected in the same areas. For the extraction of DNA were used 2 g of plant material macerated in 2% CTAB buffer [2% CTAB; 100 mM Tris (pH 8.0); 20 mM EDTA (pH 8.0); 1.4 M NaCl and 1% PVP (polyvinylpyrrolidone), 20 µL of β-mercaptoethanol] (Doyle 1991).

The for the amplification reactions occurred in a final volume of 15 µL [1.5 µL of 10 X buffer; 0.6 µL of dNTP; 1.5 µL of MgCl₂ (2.5 mM); 1.8 µL of ISSR primers (2.0 mM); 0.2 µL of Taq DNA polymerase; 2.0 µL of genomic DNA (1: 100) and 7.4 µL of ultrapure water]. The reactions were performed a priori using 14 primers of ISSR (Inter Simple Sequence Repeat); seven were selected due

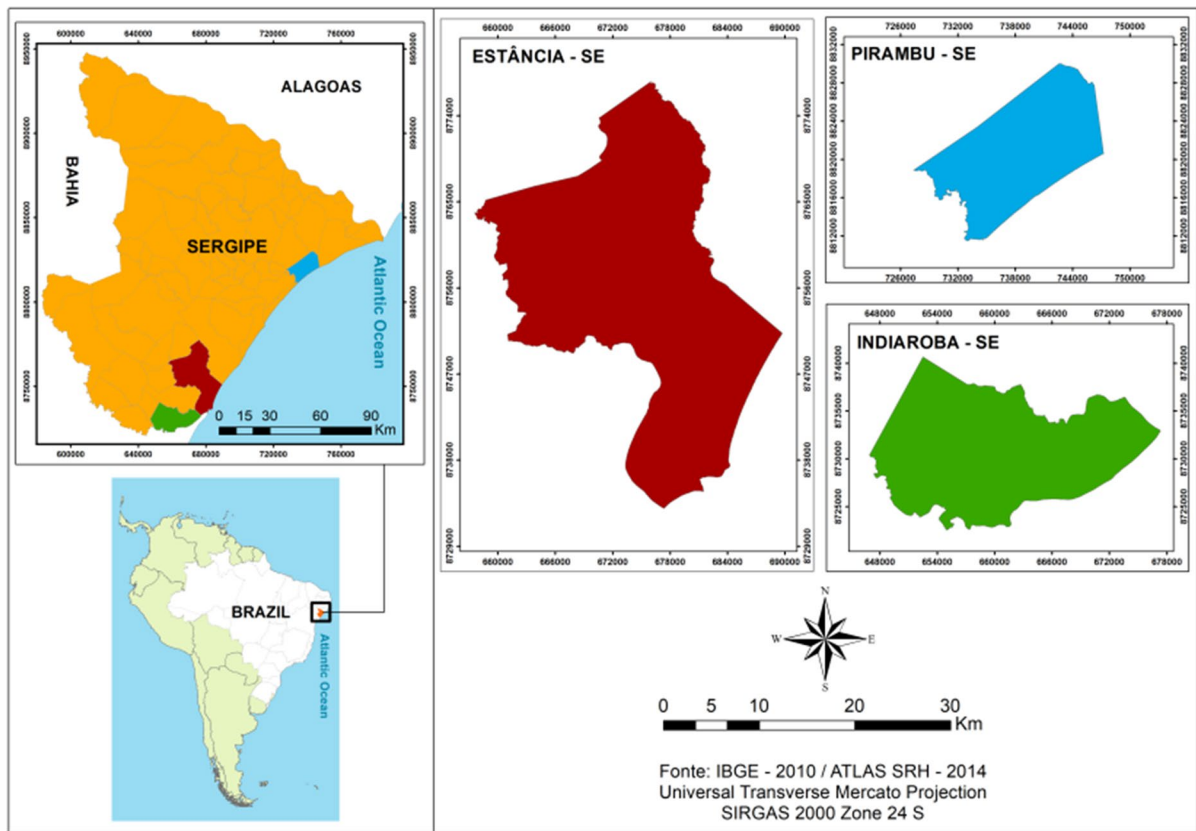


Fig. 1 The geographic location of the municipalities that collected *Hancornia speciosa* Gomes in the state of Sergipe, Brazil

Table 1 ISSR primers used to estimate the genetic similarity of mangaba trees (*Hancornia speciosa* Gomes). Sequences, annealing temperature (T°C), total number of fragments amplified (NT) and total number of polymorphic fragments (NP)

Primers	Sequences	T °C	NT	NP
GOOFY	(GT) _n -YG	48	12	12
UBC 808	(AG) _n -C	47	15	14
UBC 809	(AG) _n -G	48	10	7
UBC 810	(GA) _n -T	48	11	8
UBC 825	(AC) _n -T	49	14	13
UBC 834	(AG) _n -YT	47	12	10

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

to the high polymorphism and intensity of the generated fragments (Table 1).

Genetic diversity

The amplified fragments were separated in a 1.5% agarose gel in 1.0X TBE buffer and GelRed staining (diluted 1: 1000 H₂O) in horizontal electrophoresis at 100 V for one hour and thirty minutes. Subsequently, the gels were photographed under UV light in a digital transilluminator (Benchtop). Similarities were estimated by the Jaccard coefficient using the NTSYS pc 2.0 program (Rohlf, 2002).

Bayesian model-based methods were used to infer the population structure for the number of genetic groups (K) with the STRUCTURE software version 2.3 (Pritchard et al. 2010).

The occurrence of the species was identified through georeferenced records for the state of Sergipe obtained from herbariums (<http://spling.cria.org.br>) and records of field prospecting, totaling 95 points. Nineteen climatic variables were obtained from the WorldClim database to represent current

and future climate configurations (2050) (Hijmans et al. 2005) with a spatial resolution of 1 km².

The climatic variables were selected using Pearson's correlation coefficient (*r*) using the ENMTools 1.3 software (Warren et al. 2010), and variables with correlation values below ± 0.85 were selected (Remya et al. 2015).

The contributions of each variable were assessed using the jackknife test. Soil and altitude variables were obtained on the National Institute for Space Research website and included in the analysis (INPE <http://www.dpi.inpe.br/Ambdata/index.php>).

The human impact and its influence on the environment and species distribution were evaluated by the Global Human Influence Index (HII) v2 (1995–2004) produced by the Wildlife Conservation Society (WCS) and Columbia University Center for International Earth Science Information Network (CIESIN) (WCS and CIESIN 2005).

The species distribution models (SDM) were developed using the Maxent software version 3.3.3 k (Phillips et al. 2006), which has shown good performance for small data sets (Pearson et al. 2007). The data set was submitted to 10 replications for the model. For each one, a cross-validation bootstrap was performed. The data were divided into two independent sets (75 and 25%). This process was used to calibrate and validate the model (Yang et al. 2013). The parameters in the models were 1–5 convergence threshold with 500 interactions and 10,000 background points.

The estimation of area-under-the-curve (AUC) and the receiver operating characteristic (ROC) were assessed to evaluate the performance of the models. AUC is often used to measure consistency in predictions of the species distribution modeling (SDM) (Pearson et al. 2007). The *p*-value of the binomial probability test was also verified, inferring the significance of the obtained model. The potential future distribution for the year 2050 of *H. speciosa* was evaluated in the Representative Concentration Pathway (RCP 4.5) scenario, developed by three different sources: a—Commonwealth Scientific, Industrial Research Organization and Bureau of Meteorology (ACCESS 1.0); b—Community Climate System Model (CCSM4); and c—Met Office Hadley Center and the National Institute for Space Research (HadGEM2CC). For lack of the Global Human Influence Index (HII) for 2050, the same value for the present time was used.

Results and discussion

The predicted potential distribution of *H. speciosa* was accurately estimated at a high AUC value (0.95) (Table 2) and indicative of high precision models (Swets 1988), an efficient indicator of the adjustment of the model (Manel et al. 2001) for the present and the future predictions.

The anthropic activity and soil variables have a high contribution to the potential distribution of the species in Sergipe in the present and future.

Table 2 Percentage of contribution (%) of the variables for the potential distribution models (present and future) of *Hancornia speciosa* Gomes for the state of Sergipe, Brazil

Variables	Present	Future (2050)		
		HadGen	ACCESS	CCSM4
Training AUC	0.9534	0.9332	0.9439	0.9526
Test AUC	0.8755	0.9076	0.8873	0.8852
AUC Standard Deviation	0.0536	0.0418	0.0494	0.0446
Contribution of the variables (%)				
Human Action	53.2817	43.6256	38.7786	43.1574
Altitude	2.5831	3.4797	2.1663	4.8868
Precipitation Seasonality	2.4298	2.4116	21.7901	0.5099
Precipitation in the hottest semester	4.9974	3.124	1.7312	9.3879
Maximum temperature of the hottest month	0.016	0.445	0.0295	1.562
Annual thermal amplitude	0.616	0.877	1.4085	1.0497
Average temperature of the driest semester	0.3651	1.2001	0.9062	0.2443
Soil	35.7108	44.837	33.1895	39.2019

For the present (Fig. 2A) and future (Fig. 2B), the areas of occurrence are in the Agreste and East of Sergipe mesoregions. In the mesoregion Sertão of Sergipe, there are practically no areas suitable for the species.

Considering micro-regions in Sergipe, the nine municipalities comprising the Aracaju metropolitan area (Aracaju, São Cristóvão, Barra dos Coqueiros, Nossa Senhora do Socorro, Laranjeiras, Riachuelo, Maruim, Santo, Amaro das Brotas and Itaporanga D’ajuda) and all of Eastern area of Sergipe (Pirambu, Japarutuba, Capela, Siriri, Carmópolis, Rosário do Catete, General Maynard, Divina Pastora and Santa Rosa de Lima) showed higher probability of occurrence of mangaba with percentages of 69 to 74%. Japoatã, Pacatuba, Nossa Senhora das Dores, Malhador, Estância, Arauá and Itabaianinha are the areas with the highest occurrence.

In these municipalities with a higher probability of occurrence, there is an average rainfall of around 1,300 mm and the presence of Red-Yellow Podzolic soils with low activity clay, Red-Yellow Latosol, Neo soil, and Plano soils (Sergipe, 2016; Santos et al. 2018).

The more intense distribution of the species in coastal areas corroborates with the phytosociological studies in the region (Vieira Neto et al. 2009). The authors were able to estimate the distribution of

H. speciosa in Coastal Tablelands and Coastal Lowlands. The coastal area of the state of Sergipe is characterized by the presence of restinga ecosystems that present edaphoclimatic conditions favorable to the development of the species. However, these areas are under high anthropic impact (Oliveira and Landim 2016). The region of Itaporanga d’Ajuda and Estância has shown intense occupation and land use changes with the expansion of activities with urbanization and real estate exploration on the main beaches.

In addition, in ecological fitting areas of mangaba, there is the cultivation of crops as monocultures such as coconut and sugar cane (Vieira and Rodrigues 2009).

Percentages of 45–64% of the probability of occurrence were observed in the South of Sergipe (Tomar do Geru, Cristinápolis, Umbaúba, Indiaroba, and Santa Luzia do Itanhhy) and in all the municipalities of the Lower course of the São Francisco River. In the Agreste of the Center of Sergipe, there is a medium aptitude for the occurrence of *H. speciosa* (36–45%), mainly in the Serra de Itabaiana, where its presence was cataloged in an ethnobotanical study of non-timber forest products (Lima et al. 2011). In this area, the residents use timber of this species as firewood. This fact corroborates the high rates of human influence on the occurrence of the species and in its present and future distribution. In the regions of the South Center

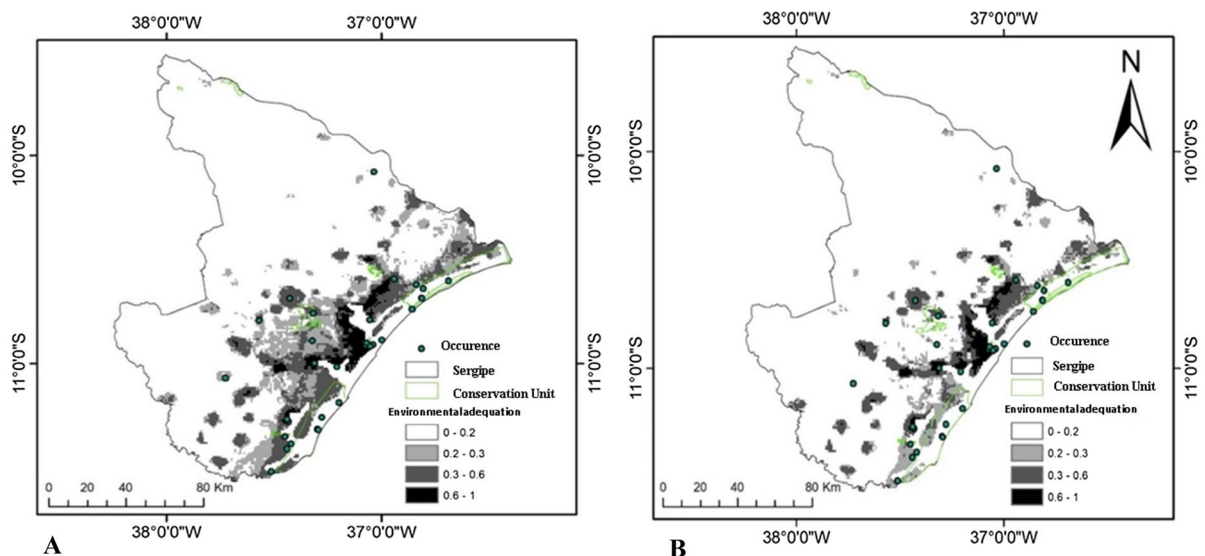


Fig. 2 Potential distribution of *Hancornia speciosa* in the present **A** and future **B** in Sergipe, Brazil

of Sergipe, High and Medium Sertão of Sergipe, there is less probability of occurrence of mangaba (<36%). In these regions, there is a predominance of the Caatinga biome and less rainfall than that required by the species (>750 mm annual) (Soares et al. 2009).

It is also noteworthy the existence of conservation units located on the North and South of the Coast of the state, at the Biological Reserve Santa Isabel, Environmental Protection area (EPA) North of Coast and EPA South of the Coast, involving municipalities of Pirambu, Japoatã, Pacatuba, and Estância. The occurrence justifies the most significant occurrence, which highlights the importance of Conservation Units since there are no protected areas in the central regions of Sergipe until now (Faria et al. 2013). The lack of protected areas can also be one of the causes of reducing the species' occurrence rate in these locations.

For the present scenario, *H. speciosa* is a small part of the Atlantic Forest biome in Sergipe. In the future, there will be a reduction in the geographic distribution of the species, especially in central-south regions, with consequences for the use and exploitation of the species with negative economic influence for communities that depend on the mangaba fruits. In addition, there is a reduction in environments suitable for the survival of *H. speciosa* for 2050.

The progressive reduction of areas of the natural occurrence of *H. speciosa* probably has influenced the distribution of the species' genetic diversity. For populations of Baixa Grande, Riboleirinha, Pontal and São Sebastião, diversity rates ranging from 0.25 to 0.36 (Table 3).

Higher values of Nei (He) and Shannon Index (I) genetic diversity were found in the population of Baixa Grande (He=0.36 and I=0.53). This population also presents higher values of observed alleles (na—1.96) and effective alleles (ne—1.61), showing

low effect of genetic drift (Soares et al. 2017). However, actions that can reduce the number of individuals in the population could contribute to their isolation and genetic drift. It can lead, in the short term, to changes in allele frequencies in the population, resulting in loss of alleles (Soares et al. 2017).

The genetic diversity found are close to those obtained in studies with dominant (ISSR and RAPD) and codominant (SSR) molecular markers, in natural populations of forest species. In studies with populations of *Protium heptaphyllum*, it was observed in the Brazilian Northeast region, a diversity ranging from 0.24 to 0.32 (Freire et al. 2019). Was obtained for *Acrocomia aculeata* the diversity of 0.30–0.42 (Oliveira et al. 2012), amplitude of 0.24–0.27 for *Metrodorea nigra* (Moraes Filho et al. 2015) and 0.36–0.42 for *Myroxylon peruiferum* (Schwarcz et al. 2018).

For *H. speciosa*, the diversity values varied of 0.11–0.31 considering dominant markers. In Pernambuco there was a variation of 0.197–0.229 (Jimenez et al. 2015), in Rio Grande do Norte of 0.18 (Costa et al. 2015) and in Mato Grosso of 0.27 (Soares et al. 2016). In Sergipe, populations with heterozygosity (He) ranged 0.2 to 0.31 (Silva et al. 2017) and 0.11 to 0.26 (Costa et al. 2011). In this previous study, the number of observed alleles (na) ranged of 0.55 to 1.3, while the number of effective alleles (ne) varied of 1.00 to 1.47, with Shannon's index (I) of 0 to 0.38 (Costa et al. 2011) (Fig. 3).

This population is not isolated, and gene flow occurs. This fact is contributed by the lower values of genetic differentiation (Fst) of individuals in this population and the populations of Pontal (0.161), Riboleirinha (0.172), and São Sebastião (0.148). Individuals from Pontal and São Sebastião showed higher rates of genetic differentiation (Fst) (0.355). The same

Table 3 Number of observed alleles (na), number of effective alleles (ne), Nei genetic diversity (H), Shannon index (I), number of polymorphic loci (nlp), and percentage of polymor-

phism (% p) in the natural population of *Hancornia speciosa* Gomes in the state of Sergipe, Brazil

Population	na	ne	H	I	nlp	%p
Baixa Grande (Pirambu)	1.96	1.61	0.36	0.53	71	96
Pontal (Estância)	1.77	1.52	0.29	0.43	57	77
Riboleirinha (Indiaroba)	1.77	1.48	0.28	0.41	57	77
São Sebastião (Pirambu)	1.81	1.42	0.25	0.38	60	81
Average Value	1.83	1.516	0.29	0.44	62	83

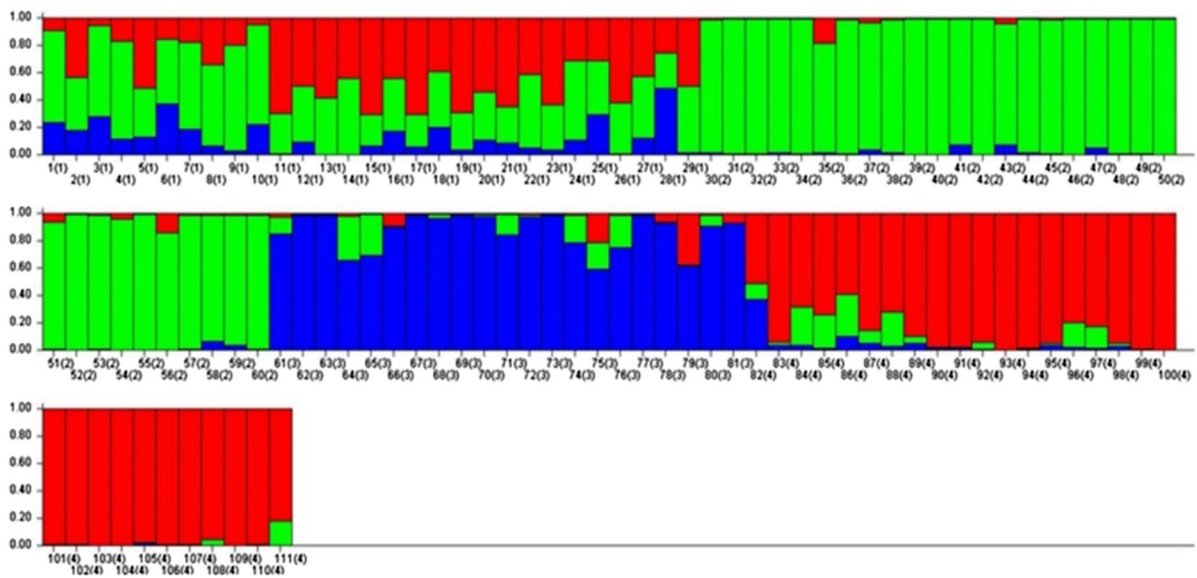


Fig. 3 Genetic structure in natural populations of *Hancornia speciosa* Gomes. Different colors in the same bar indicate the percentage of the genome shared with each group ($k=3$)

is verified for Pontal and Riboleirinha (0.262); and Riboleirinha and São Sebastião (0.206). Studies on the genetic diversity of *H. speciosa* in natural populations in the state of Sergipe (Table 4) estimated variations in H_e of 0.06–0.57. The populations of Abaís and Barra dos Coqueiros presented the highest values. However, these may be due to the type of molecular marker used (codominant). The low H_e values in Santa Luzia and Japarutuba are probably associated with the low number of individuals studied (2 and 5). A worrying factor is that the other populations also had low values of diversity.

The overlap of these data of genetic diversity (H_e) and the distribution of occurrence of the mangaba trees in the state (Fig. 4) shows the necessity for immediate actions for the conservation of the species, since of the 17 studied populations, only the population of Abaís has H_e above of 0.57; and 10 populations (58%) are in a critical condition of diversity.

There is a small number of natural populations with high genetic diversity in Sergipe for *H. speciosa*. Even with ex-situ conservation, diversity sampling was not successful, and, in some studies, the low genetic diversity of germplasm was reported in the active germplasm bank in Sergipe. Furthermore, there is a solid genetic relationship between individuals from the states of Paraíba, Sergipe, and Pará. (Costa

et al. 2011). These populations were probably connected in the remote past, but fragmentation occurred with the occupation and use for other purposes (Álvares-Carvalho et al. 2015).

Indeed, the areas of the natural occurrence of *H. speciosa* have been suffering an increased reduction due to the advancement of agriculture, real estate speculation, and tourism. In the lack of legal instruments to control the use and suppression of mangaba trees—such as conservation units—the natural habitats state’s fragmentation continues to occur. In addition, there is an intense extractivism of fruits without a management plan. In the long term and over the generations, without management for species and their fruits, which are total harvested, negatively affect the development of regenerants as seedlings banks in the soil.

Human occupation and its activities result transform natural ecosystems’ structure, distribution, and functioning (Saunders et al. 1991). Because of this exploration, *H. speciosa* has become one of the fruit species most endangered by genetic erosion in Brazil (Moura et al. 2011).

These factors corroborate the necessity to implement conservation practices in-situ and ex-situ. The population of Baixa Grande and São Sebastião (Pirambu), Riboleirinha (Estância), and Pontal

Table 4 Genetic diversity of *Hancornia speciosa* Gomes in natural populations in the state of Sergipe, Brazil

Population	Coordinates	He	Molecular marker	Author
Baixa Grande	–10.6579 –36.7668	0.40	ISSR	–
Abaís	–11.305 –37.2883	0.57	SSR	Amorim et al. 2015
Barra dos Coqueiros	–10.8194 –36.9478	0.49		
Itaporanga	–11.1417 –37.1883	0.09	ISSR	Costa et al. 2015
Pacatuba	–10.4931 –36.545	0.06		
Indiaroba	–11.4403 –37.4083	0.08		
Estância	–11.3056 –37.295	0.11		
Barro dos Coqueiros	–10.9058 –37.0217	0.10		
Pirambu	–10.6708 –36.7908	0.09		
Japaratuba	–10.6144 –36.8694	0.07		
Brejo Grande	–10.4625 –36.6372	0.10		
Neópolis	–10.3719 –36.6561	0.10		
Santa Luzia	–11.3236 –37.4033	0.03		
Baixa grande	–10.622 –36.8232	0.36	ISSR	–
Pontal	–11.4894 –37.4139	0.29		
São Sebastião	–10.6161 –36.7501	0.25		
Riboleirinha	–11.3643 –37.324	0.28		

(Itaporanga) present significant indices of diversity and can figure as priority areas for conservation in the state. We can also help define conservation strategies, such as genetic enrichment in areas of low diversity and selection of matrices trees for seed collection aiming seedling production, in addition to increasing plantations in conservation units and legal reserves.

The lack of information on the genetic and biological potential of the species that compose the restinga areas could culminate with local biodiversity at risk. Despite these environments having a significant floristic richness, with about 1590 species of plants (Oliveira and Landim 2016), the lack of management, monitoring, and public policies that efficiently protect

the vegetation jeopardize the future of species like *H. speciosa*, that is the tree of high importance in the bioeconomic context of the state of Sergipe.

Conclusion

From the variables provided, it was possible to generate models of the potential present and future occurrence of *H. speciosa* in the state of Sergipe.

The populations of Baixa Grande, São Sebastião, Pontal and Riboleirinha are hotspots for *H. speciosa* in Sergipe.

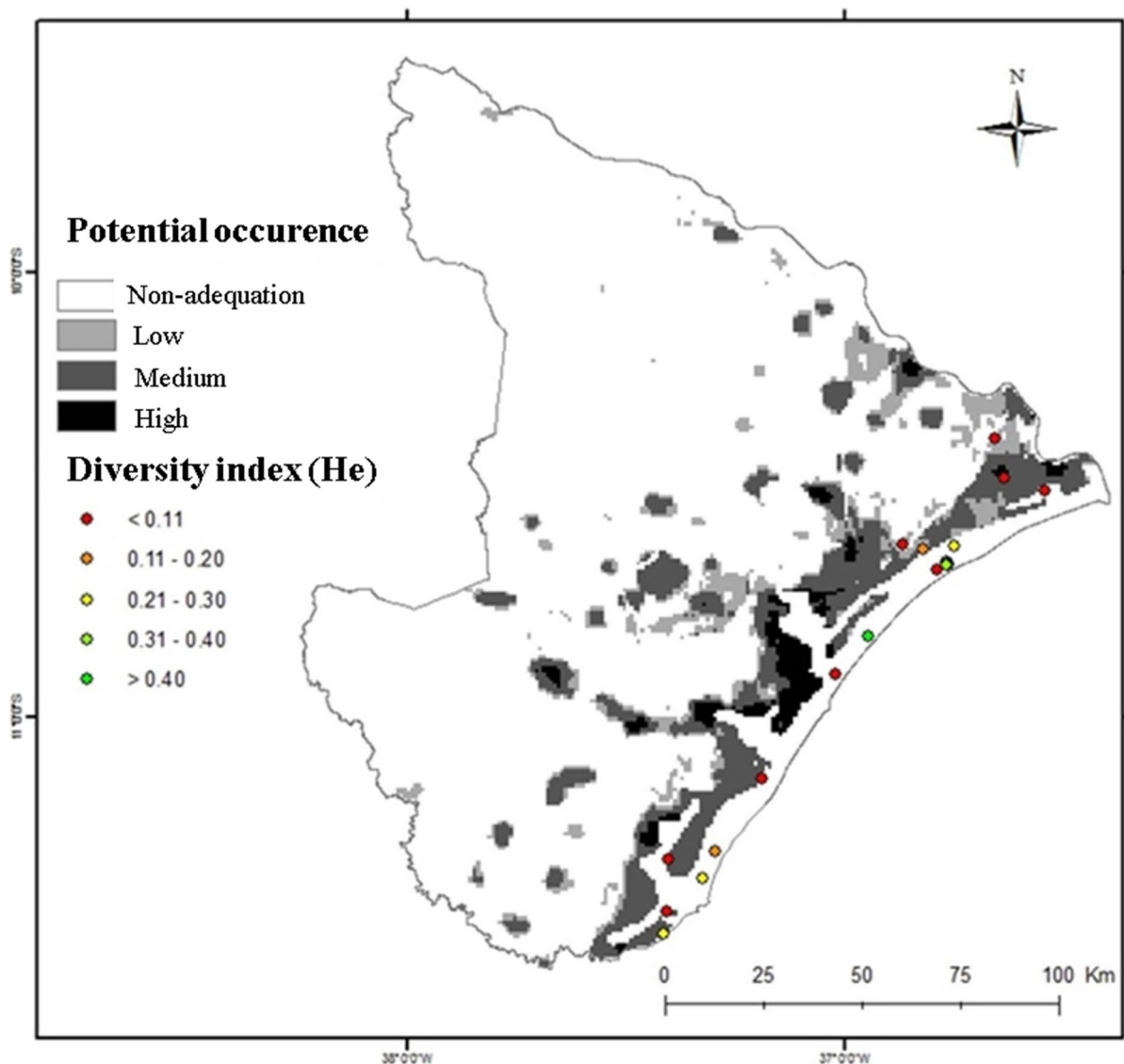


Fig. 4 Potential distribution and genetic diversity of *Hancornia speciosa* Gomes in natural populations in the state of Sergipe, Brazil

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Declarations

Conflict of interest All the authors of this manuscript declare that they have no conflict of interest.

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