environmental and earth sciences proceedings

MDPI

Proceeding Paper

Neutral Genetic Diversity of Brazilian Native Flora: Current Approaches and Gaps †

Catarina da Fonseca Lira

Diretoria de Pesquisa, Instituto de Pesquisa Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão, 915/sala 402, Jardim Botânico, Rio de Janeiro 22460-030, RJ, Brazil; catarina@jbrj.gov.br

† Presented at the 4th International Electronic Conference on Forests, 23–25 September 2024; Available online: https://sciforum.net/event/IECF2024.

Abstract: Understanding genetic diversity is crucial for plant adaptation in a changing world. The neutral genetic variation (NGD) is correlated to adaptation capacity, which is crucial for long-term conservation of threatened species. Brazil, a megadiverse nation with habitats encompassing a great variety of ecosystems, harbors a wealth of plant biodiversity, yet studies on NGD remain scarce. This work analyzed published data on NGD in native Brazilian plant populations, identifying 731 papers through a systematic search on the Scopus database. Results indicated microsatellite markers as the most used for population studies, followed by ISSR. The SNP marker is still underutilized, possibly due to its higher costs and labor-intensiveness. Fabaceae, Bromeliaceae, and Arecaceae were the most studied families. Moreover, the two most studied species were *Euterpe edulis* and *Hancornia speciosa*, both economically important species. Notably, trees and herbs dominated the studies with a focus on the Atlantic Forest biome. However, Cerrado and Amazon biomes were also well represented, underscoring the importance of broader investigation across all Brazilian ecosystems. These findings reveal a critical gap in knowledge, where traditional molecular markers are most used and few economically important species are intensively studied. The number of threatened species studied is negligible, and most are not endemic. With looming climate and landscape changes, more comprehensive studies of NGD of threatened flora in Brazil are vital. The lack of genetic diversity information of native species may threaten any conservation efforts in the long term.

Keywords: genetic diversity; native plants; molecular markers; biodiversity; plant conservation

Citation: Lira, C.d.F. Neutral Genetic Diversity of Brazilian Native Flora: Current Approaches and Gaps. *Environ. Earth Sci. Proc.* **2024**, *31*, 7. https://doi.org/10.3390/eesp2024031007

Academic Editor: Giorgos Mallinis

Published: 18 December 2024

1. Introduction

Genetic diversity is crucial for the long-term conservation of a species. The fate of a species and its population depends on its genetic variability and how it is distributed among natural populations [1,2]. Various evolutionary forces, such as migration, mutation, genetic drift, and natural selection, influence the genetic variability of species and their natural populations over time, providing a source of adaptive variation [3]. However, in the short term, there is a lack of information on how the evolutionary process is accelerating and its consequences for many species regarding changes and disequilibrium in speciation and extinction rates [4].

Neutral genetic diversity (NGD) obtained from molecular markers (review in [5,6]) is often used for calculating the genetic diversity of a species. Although there are authors indicating a limited relevance of NGD for conservation genetics [7], it is well accepted that NGD is the source of genetic adaptation and evolvability [8,9]. Moreover, NGD is especially important for gene flow estimates, independently of selective forces [10], restoration, and conservation [11]. However, a huge gap between geneticists, conservationists, and stakeholders hinders advancing on management plans and policy development together with political instruments such as the Convention on Biological Diversity and Strategic

Plan for Biodiversity [12,13]. In Brazil, scientists and decision- and policymakers should align their needs and priorities for improving biodiversity conservation outcomes [14].

Brazil is a megadiverse nation with a wide variety of forests that cover many ecosystems and contain a rich diversity of plant life, equivalent to 19% of the world's flora [15]. The Atlantic and the Amazon Forests are the most well-known hotspots [16,17]. However, Cerrado, Caatinga, Pantanal, and Pampa are other Brazilian ecosystems that are much threatened, with higher conservation gaps, but that receive less attention [18,19]. The information on NGD of Brazilian native species is scarse and dispersed.

The molecular markers have evolved with technological advancements since 2000, such as PCR and NGS (next-generation sequencing). Although generating robust results, NGS methodology has increased costs in developing countries where expenses far exceed available research funding, besides other labor limitations [20]. Moreover, genetic diversity erosion is accelerated even for nonthreatened species, due to habitat loss or modification and climate change [21]. The uncertainties that natural populations, species, and ecosystems now face, such as environmental changes, are critical for plant survival in the long term, and so assessing NGD is pivotal [8].

Therefore, a survey on papers published since 2000 using NGD information for Brazilian native plant species was carried out. The aim was to evaluate the molecular markers mostly used in Brazil for population studies, the species and taxonomic families with NGD information available, and the importance of the studies for improving Brazilian flora conservation outcomes. This work provides valuable information for future research on genetic diversity conservation for Brazilian flora species.

2. Methods

The literature review was based on the Scopus database. The search was limited for the period of 2001 to 2024 and the terms used were as follows:

((ALL("genetic diversity") AND ALL(plant) AND ALL(Brazil))) AND (marker)

The search result presented 10,518 papers. These were filtered for (1) English or Portuguese papers, (2) Brazilian native species and natural populations, and (3) population studies. Also, works based on phylogenetic analysis, species delimitation, crop species, and germplasm collections were excluded from this analysis. As a result, 731 papers were registered for this review (Supplemental Table S1).

Each plant species studied in these papers was registered separately, totaling 935 observations (Supplemental Table S1). Using the Flora and Funga Database [22], the correct species name, endemism, Red List classification, life form, substrate, biome, habitat, and distribution were registered. Missing information was registered as NA. The names of 86 species were updated to the correct and accepted name. For the molecular marker analysis, we had a total of 760 observations.

Graphics were created in RStudio using the packages dplyr [23] and ggplot2 [24].

3. Results and Discussion

The filtering excluded more than 90% of papers obtained from Scopus search, showing that most papers about genetic diversity in Brazil using markers are not populational diversity studies with Brazilian native species. Of the 731 papers registered, 760 observations were obtained for molecular markers, and 935 for the species, due to works using more than one marker and/or analyzing more than one species.

The most used molecular marker was microsatellite or Simple Sequence Repeats (SSRs; N = 403), followed by Inter-Simple Sequence Repeat (ISSRs; N = 126), Random Amplification of Polymorphic DNA (RAPD; N = 75), Allozyme (N = 42), Single Nucleotide Polymorphisms (SNPs; N = 42), Amplified Fragment Length Polymorphism (AFLP; N = 38), and Isozyme (N = 20). SSR, ISSR, and SNP are markers frequently used in recent years, while other markers, such as RAPD, Allozyme, AFLP, and Isozyme, were mostly used before 2020 (Figure 1). The SNP marker appeared in a 2014 paper and had a crescent tendency, as expected due to its robustness and information refinement, revolutionizing

NGD assessment projects [5]. The SSR markers, also known as microsatellites, have appeared since 2001 frequently and are the most used molecular marker for studying Brazilian flora's NGD (Figure 1). Limited access to SNP due to elevated costs and frail funding in developing countries [20] may have favored the SSR use. It is considered a better cost–benefit marker, even though SSR markers are less informative than SNP, especially for genotyping collections and populations [25].

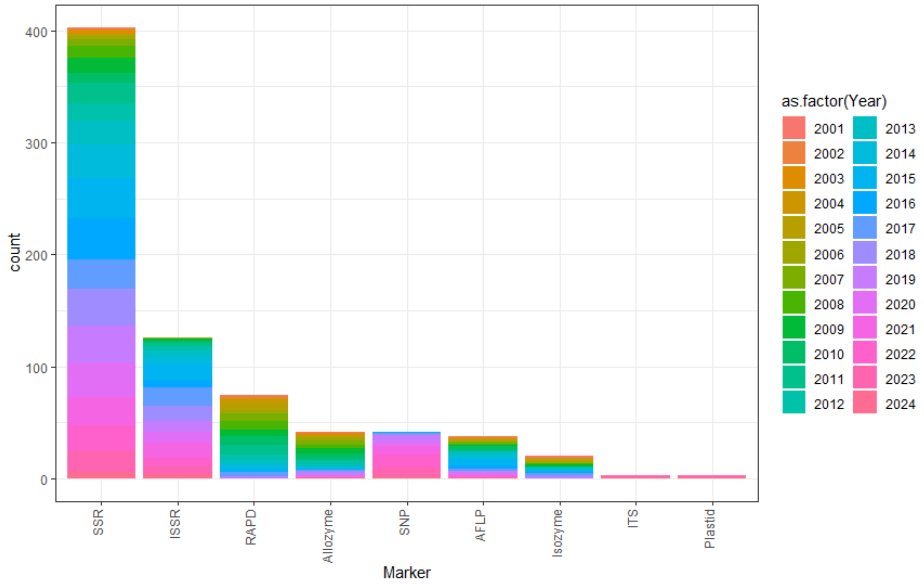

Figure 1. The number of publications with the usage of each molecular marker (with more than two occurrences in the survey) for the Brazilian native plant population analysis since 2001. Colors correspond to the year of the publication. Blue to pink colors are the most recent works (2013–2024) and orange to green colors are older publications (2001–2012).

Interestingly, the ISSR marker, which is a dominant marker, has overcome the usage of RAPD, AFLP, Allozyme, and Isozyme throughout the years (Figure 1). The low-cost and low-labor work demanded by this marker may have popularized it in Brazil for plant genetic studies. Although it is less informative than SSR or SNP, it overcomes technical problems found in RAPD and AFLP markers, such as lack of reproductibility and high labor consumption, being established as a good marker for population studies [11,26].

Most studied families were Fabaceae (N = 131), Bromeliaceae (N = 91), and Arecaceae (N = 82) (Figure 2). The families with the highest number of threatened species are Asteraceae and Bromeliaceae [27], which were the sixth and the second most studied families. Additionaly, the most studied genera were *Euterpe* (N = 29; Arecaceae), *Petunia* (N = 26; Solanaceae), and *Eugenia* (N = 24; Myrtaceae). The two most studied species were *Euterpe edulis* (N = 23) and *Hancornia speciosa* (N = 17), followed by *Dipteryx alata*, *Eugenia dysenterica*, and *Eugenia uniflora* (N = 10 each). *Euterpe edulis* and *H. speciosa* are both economically important species. The first was heavily explored for palm heart production [28] and suffers the impacts of habitat loss in the Atlantic Forest [29]. Recent work shows a strong genetic structure as a result of restricted gene flow caused by habitat loss, with the loss of private alelles and increasing inbreeding [30]. *Hancornia speciosa* produces an edible fruit called "Mangaba". It is seen as a species with incredible economic potential and it is suffering genetic diversity erosion due to exploitation and habitat loss [31,32]. However, studies also show natural populations of *H. speciosa* with high levels of genetic diversity that can be used for genetic rescue and sustainable plant breeding [33,34].

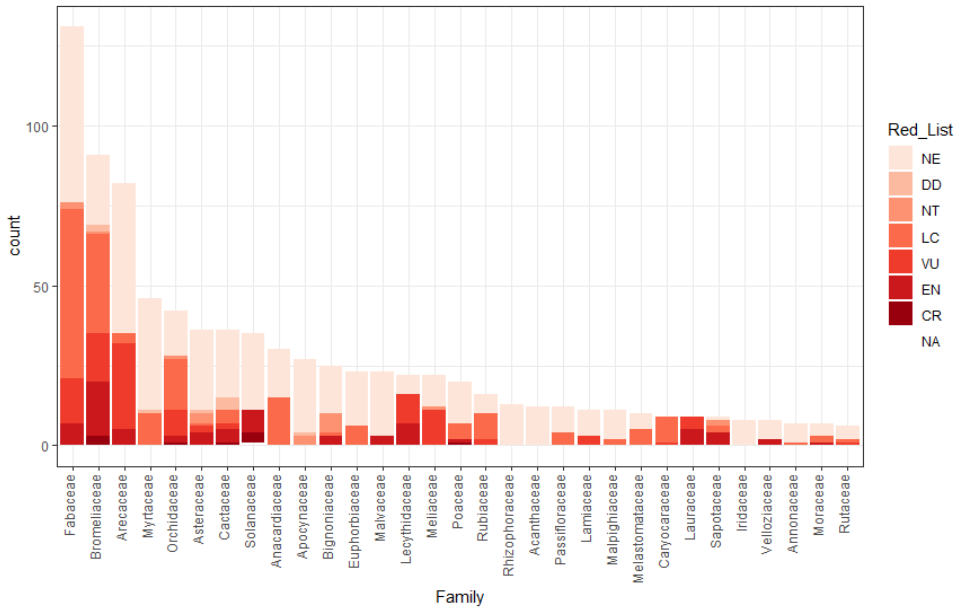

Figure 2. Number of publications by taxonomic families of plants species studied in Brazil. Graphic showing families with more than 5 occurrences. Most publications studied Fabaceae, Bromeliaceae, and Arecaceae species. Colors are correspondent to Red List classification of the studied species, showing a great number of studies with species not yet evaluated for threat risk. NE—not evaluated; DD—data deficient; NT—not threatened; LC—least concern; VU—vulnerable; EN—endangered; CR—critically endangered; NA—not available.

In the Red List classification of the studied plant species, 524 entries (56%) were observed, classified as NE or not evaluated, followed by least concern (LC; N = 195), vulnerable, (VU; N = 100), endangered (EN; N = 78), not threatened (NT; N = 19), data-deficient (DD; N = 9), and critically endangered (CR; N = 9) species. All taxonomic families registered contained species with NE status (Figure 2), showing an important limitation of the Brazilian plant science. Although huge advancements and national centers were created for this porpose, such as the CNCFlora ("Centro Nacional de Conservação da Flora"—National Center for Flora Conservation), only 15.5% of the known flora in Brazil, with 48% of them threatened at some level, were evaluated by 2018 [27].

Brazilian scientists are concentrating efforts on trees (N = 445) and herbs (N = 231), followed by shrubs/subshrubs (N = 107), palm trees (N = 82), succulents (N = 32), and lianas (N = 26) (Table 1). Only five bamboo species were studied in four papers. The genetic studies are not focusing on threatened native species since the vast majority of them are herbs [27]. Most of the studied species were not endemic from Brazil (59%), and mostly inhabited exclusively the Atlantic Forest (18%), Cerrado (10.9%), Amazon (7.9%), and Caatinga (2.6%). Also, many species were widely distributed (73.2%), inhabiting different biomes (Table 1).

The high flora biodiversity in Brazil results in limiting resources and instruments for evaluating NGD in natural populations. The results we see are a tendency of using low-cost molecular markers, and studying economically important species even if they are not critically endangered. However, genetic conservation cannot be overlooked anymore. It must be taken into account in every management and conservation plan. ISSR and SSR markers are broadly used with increasing usage of SNP more recently, despite its elevated cost and labor.

Scientists efforts are limited to a few taxonomic families and species, so new incentives should be launched for expanding the number of studied species, especially the most threatened by climate change and habitat loss. One solution is the incentive for more integrative and interdisciplinary projects, with elevated funding for genetic conservation studies and not only genome sequencing. A second solution is for scientists to expand

their curiosity and knowledge for studying new unexplored species, since many molecular markers can be used without previous sequence knowledge, such as ISSR and SNP (i.e., GBS [35,36] and MIGseq [37]).

Table 1. The umber of publications corresponding to plant life habit, species endemism, and biome of occurrence. All data were obtained from Brazilian Flora and Funga Database [22] based on each studied species in the survey.

Data Obtained From Brazilian Flora and Funga Database						
Life habit	**Tree**	**Herb**	**Shrubs/subshrubs**	**Palm tree**	**Succulent**	**Liana**
	445	231	107	82	32	26
Endemism	**Endemic**		**Not-endemic**		**Data Deficient**	
	370		552		13	
Biome	**Atlantic Forest**	**Cerrado**	**Amazon**	**Caatinga**	**Pampa**	**>2 biomes**
	169	102	74	24	16	536

4. Conclusions

This work showed critical gaps in the knowledge of Brazilian plant genetic diversity. Researchers still underutilize the marker SNP. Much effort is directed toward a few species, such as *Euterpe edulis* Mart. (heart of palm tree) and *Hancornia speciosa* Gomes (mangaba tree), both economically important and broadly found in Brazil. Research efforts should focus on endangered and endemic species, which are understudied. For this accomplishment, funding is crucial. The NGD information is essential for safeguarding Brazil's irreplaceable plant biodiversity, menaced by climate and landscape changes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/eesp2024031007/s1.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article/Supplemental Table S1.

Acknowledgments: The author thank the organizers and reviewers of the 4th International Electronic Conference on Forests (IECF 2024).

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Siomos, M.F. Shaped by the environment—Adaptation in plants. *FEBS J.* **2009**, *276*, 4705–4714. [CrossRef]
2. Frankham, R. Challenges and opportunities of genetic approaches to biological conservation. *Biol. Conserv.* **2010**, *143*, 1919–1927. [CrossRef]
3. Charlesworth, D.; Barton, N.H.; Charlesworth, B. The sources of adaptive variation. *Proc. R. Soc. B Biol. Sci.* **2017**, *284*, 20162864. [CrossRef]
4. Thomas, C.D. Rapid acceleration of plant speciation during the Anthropocene. *Trends Ecol. Evol.* **2015**, *30*, 448–455. [CrossRef]
5. Grover, A.; Sharma, P.C. Development and use of molecular markers: Past and present. *Crit. Rev. Biotechnol.* **2016**, *369*, 290–302. [CrossRef]
6. Amom, T.; Nongdam, P. The Use of Molecular Marker Methods in Plants: A Review. *Int. J. Curr. Res. Rev.* **2017**, *9*, 1–7. [CrossRef]
7. Teixeira, J.C.; Huber, C.D. The inflated significance of neutral genetic diversity in conservation genetics. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2015096118. [CrossRef]
8. Jump, A.S.; Marchant, R.; Peñuelas, J. Environmental change and the option value of genetic diversity. *Trends Plant Sci.* **2009**, *14*, 51–58. [CrossRef]
9. García-Dorado, A.; Caballero, A. Neutral genetic diversity as a useful tool for conservation biology. *Conserv. Genet.* **2021**, *22*, 541–545. [CrossRef]
10. Holderegger, R.; Kamm, U.; Gugerli, F. Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landsc. Ecol.* **2006**, *21*, 797–807. [CrossRef]

11. Granado, R.; Neta, L.C.P.; Nunes-Freitas, A.F.; Voloch, C.M.; Lira, C.F. Assessing genetic diversity after mangrove restoration in Brazil: Why is it so important? *Diversity* **2018**, *10*, 27. [CrossRef]
12. Hoban, S.M.; Hauffe, H.C.; Pérez-Espona, S.; Arntzen, J.W.; Bertorelle, G.; Bryja, J.; Frith, K.; Gaggiotti, O.E.; Galbusera, P.; Godoy, J.A.; et al. Bringing genetic diversity to the forefront of conservation policy and management. *Conserv. Genet. Resour.* **2013**, *5*, 593–598. [CrossRef]
13. Laikre, L. Genetic diversity is overlooked in international conservation policy implementation. *Conserv. Genet.* **2010**, *11*, 349–354. [CrossRef]
14. Karam-Gemael, M.; Loyola, R.; Penha, J.; Izzo, T. Poor alignment of priorities between scientists and policymakers highlights the need for evidence-informed conservation in Brazil. *Perspect. Ecol. Conserv.* **2018**, *16*, 125–132. [CrossRef]
15. Giulietti, A.M.; Harley, R.M.; de Queiroz, L.P.; Wanderley, M.D.G.L.; van den Berg, C. Biodiversity and conservation of plants in Brazil. *Conserv. Biol.* **2005**, *19*, 632–639. [CrossRef]
16. Carnaval, A.C.; Hickerson, M.J.; Haddad, C.F.B.; Rodrigues, M.T.; Moritz, C. Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot. *Science* **2009**, *323*, 785–789. [CrossRef] [PubMed]
17. Peres, C.A.; Campos-Silva, J.; Ritter, C.D. Environmental policy at a critical junction in the Brazilian Amazon. *Trends Ecol. Evol.* **2023**, *38*, 113–116. [CrossRef] [PubMed]
18. Fonseca, C.R.; Venticinque, E.M. Biodiversity conservation gaps in Brazil: A role for systematic conservation planning. *Perspect. Ecol. Conserv.* **2018**, *16*, 61–67. [CrossRef]
19. Colli, G.R.; Vieira, C.R.; Dianese, J.C. Biodiversity and conservation of the Cerrado: Recent advances and old challenges. *Biodivers. Conserv.* **2020**, *29*, 1465–1475. [CrossRef]
20. Helmy, M.; Awad, M.; Mosa, K.A. Limited resources of genome sequencing in developing countries: Challenges and solutions. *Appl. Transl. Genom.* **2016**, *9*, 15–19. [CrossRef]
21. Exposito-Alonso, M.; Booker, T.R.; Czech, L.; Gillespie, L.; Hateley, S.; Kyriazis, C.C.; Lang, P.L.M.; Leventhal, L.; Nogues-Bravo, D.; Pagowski, V.; et al. Genetic diversity loss in the Anthropocene. *Science* **2022**, *377*, 1431–1435. [CrossRef]
22. Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. Available online: http://floradobrasil.jbrj.gov.br/ (accessed on 5 November 2024).
23. Wickham, H.; François, R.; Henry, L.; Müller, K.; Vaughan, D. Dplyr: A Grammar of Data Manipulation. R Package Version.1.4. Available online: https://CRAN.R-project.org/package=dplyr (accessed on 5 July 2024).
24. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016.
25. Testolin, R.; Messina, R.; Cipriani, G.; de Mori, G. SSR-based DNA fingerprinting of fruit crops. *Crop Sci.* **2023**, *63*, 390–459. [CrossRef]
26. Grativol, C.; Lira-Medeiros, C.F.; Hemerly, A.S.; Ferreira, P.C.G. High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L. *accessions. Mol. Biol. Rep.* **2011**, *38*, 4245–4256. [CrossRef] [PubMed]
27. Martins, E.; Martinelli, G.; Loyola, R. Brazilian efforts towards achieving a comprehensive extinction risk assessment for its known flora. *Rodriguesia* **2018**, *69*, 1529–1537. [CrossRef]
28. dos Reis, M.S.; Fantini, A.C.; Nodari, R.O.; Reis, A.; Guerra, M.P.; Mantovani, A. Management and Conservation of Natural Populations in Atlantic Rain Forest: The Case Study of Palm Heart (*Euterpe edulis* Martius). *Biotropica* **2000**, *32*, 894–902. [CrossRef]
29. Santos, A.S.; Cazetta, E.; Morante Filho, J.C.; Baumgarten, J.; Faria, D.; Gaiotto, F.A. Lessons from a palm: Genetic diversity and structure in anthropogenic landscapes from Atlantic Forest, Brazil. *Conserv. Genet.* **2015**, *16*, 1295–1302. [CrossRef]
30. Soares, L.A.S.S.; Cazetta, E.; Santos, L.R.; França, D.S.; Gaiotto, F.A. Anthropogenic Disturbances Eroding the Genetic Diversity of a Threatened Palm Tree: A Multiscale Approach. *Front. Genet.* **2019**, *10*, 1090. [CrossRef]
31. Soares, A.N.R.; Vitória, M.F.; Nascimento, A.L.S.; Ledo, A.S.; Rabbani, A.R.C.; Silva, A.V.C. Genetic diversity in natural populations of mangaba in Sergipe, the largest producer State in Brazil. *Genet. Mol. Res.* **2016**, *15*, gmr.15038624. [CrossRef]
32. Fajardo, C.G.; da Costa, D.F.; das Chagas, K.P.T.; Vieira, F.A. Genetic diversity in natural populations of Hancornia speciosa gomes: Implications for conservation of genetic resources. *Cienc. E Agrotecnologia* **2018**, *42*, 623–630. [CrossRef]
33. Nunes, V.V.; Silva-Mann, R.; Souza, J.L.; Calazans, C.C. Geno-phenotypic diversity in a natural population of Hancornia speciosa Gomes: Implications for conservation and improvement. *Genet. Resour. Crop Evol.* **2021**, *68*, 2869–2882. [CrossRef]
34. Maia, A.K.S.; Terto, J.; Oliveira, I.F.; Nascimento, W.F.; Almeida, C.; Silva, E.F. Genetic diversity and structure of Hancornia speciosa Gomes populations characterized by microsatellites markers. *Crop Breed. Appl. Biotechnol.* **2022**, *22*, e38012228. [CrossRef]
35. Elshire, R.J.; Glaubitz, J.C.; Sun, Q.; Poland, J.A.; Kawamoto, K.; Buckler, E.S.; Mitchell, S.E. A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. *PLoS ONE* **2011**, *6*, e19379. [CrossRef]
36. Deschamps, S.; Llaca, V.; May, G.D. Genotyping-by-Sequencing in Plants. *Biology* **2012**, *1*, 460–483. [CrossRef]
37. Suyama, Y.; Matsuki, Y. MIG-seq: An effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Sci. Rep.* **2015**, *5*, 16963. [CrossRef] [PubMed]