UNIVERSIDAD SIMÓN BOLÍVAR | INSTITUTO DE POSGRADOS

Evaluación de marcadores moleculares para el estudio poblacional de *Lecythis minor* Jacq. (Lecythidaceae)

Alfredo Miguel Navas Ramírez
Trabajo de Investigación o Tesis de maestría como requisito para optar el título de magister en Genética

Tutores
PhD. Yani Aranguren Díaz
PhD. Elwi Machado Sierra

El bosque seco tropical es un ecosistema importante para distintos organismos debido a su alta diversidad. Dentro de la amplia diversidad de especies vegetales de este ecosistema se encuentra *Lecythis minor* (olla de mono), perteneciente a la familia Lecythidaceae, y que se distribuye en el norte de Suramérica en Venezuela y Colombia. Esta especie poco estudiada está empezando a ser utilizada en procesos de reforestación debido a su potencial económico, y su capacidad de bioacumular selenio orgánico en sus semillas y del cual se extrae un aceite. Hasta ahora en *L. minor* no se habían realizado estudios genéticos, ni poblacionales y se desconoce su estado de conservación, siendo necesario identificar marcadores moleculares que puedan ser utilizados para el estudio de la diversidad genética de esta especie. En este sentido, el objetivo de este trabajo fue evaluar marcadores moleculares para el estudio poblacional de *L. minor.* El material vegetal fue recolectado de poblaciones naturales de la especie distribuidas en los departamentos de Atlántico, Bolívar, Cesar, Magdalena y Sucre. Posteriormente, se seleccionó un set de 7 marcadores ISSR utilizados en *Berthotellia excelsa* y un grupo de 10 marcadores microsatélites desarrollados para *Lecythis pinosis* y *B. excelsa*, probando la transferibilidad de estos marcadores en *L. minor*, y se continuó identificando los loci polimórficos y las variantes alélicas, analizándose con los programas GenAlex 6.5 y Cervus. El 71 % de los marcadores ISSR probados, generaron bandas claras cuantificables. Por su parte 70 % de los marcadores SSR presentaron transferibilidad en *L. minor*. Los 5 marcadores ISSR amplificados, generaron un total de 31 bandas, de las cuales 25 fueron polimórficas. El número de bandas amplificadas osciló entre 3 y 11 con un promedio de 6,2 bandas. Todos los marcadores ISSR presentaron un alto porcentaje de bandas polimórficas que varió de 66,6% a 100%. El número de alelos efectivos osciló 1,5 y 1,91. El promedio de la diversidad genética de Nei fue de 0,425 y el promedio del índice de información de Shannon fue de 0,526. El contenido de información polimórfica osciló entre 0,218

y 0,416, con un promedio de 0,339. Mientras que los SSR transferidos con éxito registraron un número de alelos que osciló entre 2 a 5 y el número de alelos efectivos varió de 1,280 a 2,909. La heterocigosidad esperada presento un rango entre 0,233 a 0,750. El índice de contenido de información polimórfica presentó un valor medio de 0,447. Los análisis de coordenadas principales PCA, realizados a partir de los marcadores utilizados en el estudio demuestran la capacidad de estos para de detectar variabilidad genética entre los individuos estudiados, demostrando ser marcadores útiles para futuros trabajos. Asimismo, muestran una diferenciación que puede estar influenciada por efectos naturales como geográficos y biológicos, o antrópicos como la fragmentación del bosque seco; sin embargo, es necesario realizar un análisis con un tamaño muestral más grande. Finalmente, se obtuvo un set de 5 marcadores ISSR y 5 marcadores SSR polimórficos en *L. minor*, siendo estos los primeros reportados para la especie, convirtiéndose en una herramienta para futuros estudios en genética poblacional de *L. minor*.

Palabras clave: ISSR, SSR, diversidad genética, variabilidad genética.

ABSTRACT

The tropical dry forest is an important ecosystem for different organisms due to its high diversity. Within the wide diversity of plant species of this ecosystem is *Lecythis minor* (monkey pot), belonging to the Lecythidaceae family, and which is distributed in the north of South America in Venezuela and Colombia. This little-studied species is beginning to be used in reforestation processes due to its economic potential, and its ability to bioaccumulate organic selenium in its seeds and from which an oil is extracted. Until now, no genetic or population studies have been carried out on *L. minor* and its conservation status is unknown, making it necessary to identify molecular markers that can be used to study the genetic diversity of this species. In this sense, the objective of this work was to evaluate molecular markers for the population study of *L. minor*. The plant material was collected from natural populations of the species distributed in the departments of Atlántico, Bolívar, Cesar, Magdalena and Sucre. Subsequently, a set of 7 ISSR markers used in *Berthotellia excelsa* and a group of 10 microsatellite markers developed for *Lecythis pinosis* and B. excelsa were selected, testing the transferability of these markers in L. minor, and the polymorphic loci and the allelic variants, being analyzed with the GenAlex 6.5 and Cervus programs. 71% of the ISSR markers tested generated clear quantifiable bands. On the other hand, 70% of the SSR markers presented transferability in *L. minor*. The 5 amplified ISSR markers generated a total of 31 bands, of which 25 were polymorphic. The number of amplified bands ranged from 3 to 11 with an average of 6.2 bands. All ISSR markers presented a high percentage of polymorphic bands that ranged from 66.6% to 100%. The number of effective alleles ranged from 1.5 to 1.91. Nei's mean genetic diversity was 0.425 and Shannon's mean information index was 0.526. The polymorphic information content ranged from 0.218 to 0.416,

with an average of 0.339. While the successfully transferred SSRs registered a number of alleles that ranged from 2 to 5 and the number of effective alleles ranged from 1,280 to 2,909. The expected heterozygosity ranged from 0.233 to 0.750. The polymorphic information content index presented a mean value of 0.447. The PCA main coordinate analysis, carried out from the markers used in the study, demonstrate their ability to detect genetic variability among the studied individuals, proving to be useful markers for future work. Likewise, they show a differentiation that can be influenced by natural effects such as geographical and biological, or anthropic such as the fragmentation of the dry forest; however, an analysis with a larger sample size is necessary. Finally, a set of 5 ISSR markers and 5 polymorphic SSR markers were obtained in *L. minor*, these being the first reported for the species, becoming a tool for future studies in population genetics of *L. minor*.

KeyWords: ISSR, SSR, genetic diversity, genetic variability.

REFERENCIAS

1. Pennington, R; Lewis, G; y Ratter J. Neotropical Savannas and Seasonally Dry Forests: Plant Diversity ... - Google Libros [Internet]. [cited 2020 Oct 2]. Available from: https://books.google.es/books?hl=es&lr=&id=AD3MBQAAQBAJ&oi=fnd&pg=PP1&dq=Neotropical+Savannas+and+Seasonally+Dry+Forests:+Plant+Diversity,+Biogeography,+and+Conservation&ots=gBfYyuUI5y&sig=5kXTUVtMjdkrotmUW3U6BJcwFns#v=onepage&q=Neotropical Savannas and S
2. Ballesteros-Correa J, Morelo-García L, Pérez-Torres J. Composición y estructura vegetal de fragmentos de bosque seco tropical en paisajes de ganadería extensiva bajo manejo silvopastoril y convencional en Córdoba, Colombia. Caldasia. 2019;41(1):224–34. DOI: 10.15446/caldasia.v41n1.71320
3. Myers N, Mittermeler RA, Mittermeler CG, Da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000;403:853–8. DOI: 10.1038/35002501
4. Miles L, Newton AC, DeFries RS, Ravilious C, May I, Blyth S, et al. A global overview of the conservation status of tropical dry forests. J Biogeogr. 2006;33(3):491–505. DOI: 10.1111/j.1365-2699.2005.01424.x
5. Pizano, C y Garcia H 2014. EL BOSQUE SECO TROPICAL EN COLOMBIA. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. 2014. 354 p. URL: http://hdl.handle.net/20.500.11761/9333
6. Redford KH, Taber A, Simonetti JA. There Is More to Biodiversity than the Tropical Rain Forests. Conserv Biol [Internet]. 1990 Sep 1 [cited 2020 Oct 2];4(3):328–30. Available from: http://doi.wiley.com/10.1111/j.1523-1739.1990.tb00296.x
7. Aldana-Domínguez J, Montes C, Martínez M, Medina N, Hahn J, Duque M. Biodiversity and Ecosystem Services Knowledge in the Colombian Caribbean. Trop Conserv Sci [Internet]. 2017 Jan 28 [cited 2020 Oct 2];10:194008291771422. Available from: http://journals.sagepub.com/doi/10.1177/1940082917714229
8. Armando Dugand. Observaciones taxonómicas sobre las *Lecythis* del Norte de Colombia. Caldasia. 1947;4(20):411–26.
9. López R, Martín C, Montero González Bogotá I. Manual de identificación de especies forestales en bosques naturales con manejo certificable para las comunidades. 2005. 128 p.
10. Dernovics M, García-Barrera T, Bierla K, Preud'homme H, Lobinski R. Standardless identification of selenocystathionine and its γ-glutamyl derivatives in monkeypot nuts by 3D liquid chromatography with ICP-MS detection followed by nanoHPLC-Q-TOF-MS/MS. Analyst [Internet]. 2007 [cited 2020 Oct 2];132(5):439–49. Available from: https://pubmed.ncbi.nlm.nih.gov/17471390/
11. De La Ossa J, De La Ossa Lacayo A. Densidad poblacional de *Saguinus oedipus* (primates callitrichidae) y disponibilidad de alimento vegetal, Colosó, Sucre - Colombia. Rev UDCA Actual Divulg Científica. 2014;17(2):513–20. DOI: 10.31910/rudca.v17.n2.2014.256

12. López, R., Sarmiento, C., Espitia, L., Barrero, A., Consuegra, C., y Gallego-Castillo B. 100 Plantas del Caribe colombiano. 2016. 241 p.
13. Erickson DL, Hamrick JL, Kochert GD. Ecological determinants of genetic diversity in an expanding population of the shrub *Myrica cerifera*. Mol Ecol. 2004;13:1655–64. DOI: 10.1111/j.1365-294X.2004.02139.x
14. Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, et al. Current trends in microsatellite genotyping. Mol Ecol Resour. 2011;11(4):591–611. DOI: 10.1111/j.1755-0998.2011.03014.x
15. Araya S, Martins AM, Junqueira NT V, Costa AM, Faleiro FG, Ferreira ME. Microsatellite marker development by partial sequencing of the sour passion fruit genome (*Passiflora edulis* Sims). BMC Genomics. 2017:18:549. DOI: 10.1186/s12864-017-3881-5
16. Aranguren-Díaz YC, Varani AM, Michael TP, Miranda VFO. Development of microsatellite markers for the carnivorous plant *Genlisea aurea* (Lentibulariaceae) using genomics data of NGS. Mol Biol Rep. 2018;45(1):57–61. DOI: 10.1007/s11033-017-4140-1
17. Moreira PA, Sousa SA, Oliveira FA, Araújo NH, Fernandes GW, Oliveira DA. Characterization of nine transferred SSR markers in the tropical tree species *Enterolobium contortisiliquum* (Fabaceae). Genet Mol Res. 2012;11(4):3729–34. DOI: 10.4238/2012.August.17.11
18. Cabral JC, Baldoni AB, Tonini H, Azevedo VCR, Giustina LD, Tiago A V., et al. Diversity and genetic structure of the native Brazil nut tree (Bertholletia excelsa bonpl.) population. Genet Mol Res. 2017;16(3):1–8. DOI: 10.4238/gmr16039702
19. Rodrigues AB, Florence CT, Mariano-Neto E, Gaiotto FA. First microsatellite markers for *Lecythis pisonis* (Lecythidaceae), an important resource for Brazilian fauna. Conserv Genet Resour. 2015;7:437–9. DOI: 10.1007/s12686-014-0390-6
20. Hernández G, Konrad H, Krumböck S, Geburek T. Novel nuclear microsatellites in the endangered neotropical timber tree *Lecythis ampla* (Lecythidaceae). Silvae Genet. 2014;63:181–4. DOI: 10.1515/sg-2014-0023
21. Rangel-Acosta JL, Martínez-Hernández NJ. Comparación de los ensamblajes de escarabajos copronecrófagos (Scarabaeidae: Scarabaeinae) entre fragmentos de bosque seco tropical y la matriz adyacente en el departamento del Atlántico-Colombia. Rev Mex Biodivers. 2017;88:389–401. DOI: 10.1016/j.rmb.2017.03.012
22. Institute CB. Biodiversity Hotspots Revisited, Conservation International, 2011 [Internet]. 2011 [cited 2020 Oct 5]. Available from: https://databasin.org/datasets/23fb5da1586141109fa6f8d45de0a260
23. Maass JM, Balvanera P, Castillo A, Daily GC, Mooney HA, Ehrlich P, et al. Ecosystem services of tropical dry forests: Insights from long-term ecological and social research on the Pacific Coast of Mexico. Ecol Soc. 2005;10(1):1–23. DOI: 10.5751/ES-01219-100117
24. Pizano C, González R, González M, Castro F, López R, Rodríguez N, et al. Las plantas de los Bosques Secos Tropicales de Colombia. El Bosque Seco Tropical en Colombia. 2014. 49–93 p.
25. IDEAM, IGAC, IAvH, Invemar IS e I. Ecosistemas continentales, costeros y marinos de Colombia [Internet]. Sistema de Información Ambiental de Colombia.

2007. 276 p. Available from: https://www.siac.gov.co/contenido/contenido.aspx?catID=837&conID=1507
26. Arango N, Armenteras D, Castro M, Gottsmann T, Hernándes OL, Matallana CL, et al. Vacíos de conservación del sistema de parques nacionales naturales de Colombia desde una perspectiva ecorregional. 2003. 62 p.
27. Barrera JI, Contreras SM, Garzón NV, Moreno AC, Montoya SP. Manual para la restauración ecológica de los ecosistemas disturbados del Distrito Capital [Internet]. 2010. 402 p. Available from: http://oab.ambientebogota.gov.co/resultado_busquedas.php?AA_SL_Session=8cf97c692bfb8688eaf05115108c7ab8&x=5473
28. Hernandéz-Jaramillo A, Gonzales-M R, Villegaz F, Martinez S. Bosque seco tropical. Monitoreo comunitario de la biodiversidad. Cuenca río Cañas. 2010. 42 p.
29. Németh A, Dernovics M. Effective selenium detoxification in the seed proteins of a hyperaccumulator plant: The analysis of selenium-containing proteins of monkeypot nut (Lecythis minor) seeds. J Biol Inorg Chem. 2015;20(1):23–33. DOI: 10.1007/s00775-014-1206-6
30. Gladyshev VN, Hatfield DL. Selenocysteine-containing proteins in mammals. J Biomed Sci. 1999;6:151–60. DOI: 10.1007/BF02255899
31. Preedy V. Selenium: Chemistry, Analysis, Function and Effects - Google Libros [Internet]. 2015 [cited 2020 Oct 5]. 666 p. Available from: https://books.google.es/books?hl=es&lr=&id=h3ZwCgAAQBAJ&oi=fnd&pg=PR1&dq=Selenium+Chemistry,+Analysis,+Function+and+Effects.+Food+and+Nutritional+Components+in+Focus&ots=p6WpGGAhtT&sig=KYu2u1MT07pRYpC54rhd8aMMwH4#v=onepage&q=Selenium Chemistry%2C Analysis
32. FAO. Ecosistemas, biodiversidad, recursos genéticos | Objetivos de Desarrollo Sostenible [Internet]. 2015 [cited 2020 Oct 5]. Available from: http://www.fao.org/sustainable-development-goals/overview/fao-and-post-2015/ecosystems-biodiversity-genetics/es/
33. Aguilar R, Ashworth L, Galetto L, Aizen MA. Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. Ecol Lett. 2006;9:968–80. DOI: 10.1111/j.1461-0248.2006.00927.x
34. Crandall KA, Bininda-Emonds ORR, Mace GM, Wayne RK. Considering evolutionary processes in conservation biology. Trends Ecol Evol. 2014;15(7):290–5. DOI: 10.1098/rspb.1999.0918
35. Smith RD, Maltby E. Using the Ecosystem Approach to Implement the Convention on Biological Diversity Key Issues and Case Studies [Internet]. IUCN, Gland, Switzerland and Cambridge, UK; 2003. 118 p. Available from: www.iucn.org/themes.cemwww.iucn.org/bookstore
36. BEER, J; HARVEY, C; IBRAHIM, M; HARMAND, JM; SOMARRIBA, E; JIMENEZ F. Servicios ambientales de los sistemas agroforestales. Agroforestería en las Américas. 2003;10(37–28).
37. Belcher B, Ruíz-Pérez M, Achdiawan R. Global patterns and trends in the use and management of commercial NTFPs: Implications for livelihoods and conservation. World Dev. 2005;33(9):1435–52. DOI: 10.1016/j.worlddev.2004.10.007

38. Naciones unidas. Objetivos y metas de desarrollo sostenible – Desarrollo Sostenible [Internet]. [cited 2020 Oct 7]. Available from: https://www.un.org/sustainabledevelopment/es/objetivos-de-desarrollo-sostenible/
39. CONPES. Política de Bosques. 1996;38.
40. Planeación. El plan nacional de desarrollo 2018-2022: “Pacto por Colombia, pacto por la equidad.” 2018. 212 p. DOI: 10.19053/01203053.v38.n68.2019.9924
41. Kageyama PY, Gandara FB, Souza LMI De. Conseqüências genéticas da fragmentação sobre populações de espécies arbóreas. Série Técnica IPEF. 1998;12(32):65–70.
42. McKay JK, Christian CE, Harrison S, Rice KJ. “How local is local?” - A review of practical and conceptual issues in the genetics of restoration. Restor Ecol. 2005;13(3):432–40. DOI: 10.1111/j.1526-100X.2005.00058.x
43. Allendorf FW, Hohenlohe PA, Luikart G. Genomics and the future of conservation genetics. Nat Rev Genet. 2010;11(10):697–709. DOI: 10.1038/nrg2844
44. Jiménez P, Collada Collada C. Técnicas para la evaluación de la diversidad genética y su uso en los programas de conservación. Investig Agrar Sist y Recur For. 2000;9(4):237–48.
45. Reed DH, Frankham R. Correlation between fitness and genetic diversity. Conserv Biol. 2003;17(1):230–7. DOI: 10.1046/j.1523-1739.2003.01236.x
46. Schaberg PG, DeHayes DH, Hawley GJ, Nijensohn SE. Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. For Ecol Manage. 2008;256(5):855–62. DOI: 10.1016/j.foreco.2008.06.038
47. Magdalena River Nuts. La Nuez-Natural Selium: Lecyrhisminor- Monkey Pot [Internet]. [cited 2020 Oct 7]. Available from: http://www.magdalenarivernuts.com/lanuez.html
48. Sánchez-Azofeifa GA, Quesada M, Rodríguez JP, Nassar JM, Stoner KE, Castillo A, et al. Research priorities for neotropical dry forests. Biotropica. 2005;37(4):477–85. DOI: 10.1111/j.1744-7429.2005.00066.x
49. Mooney HA, Bullock SH, Medina E. Introduction Seasonally dry tropical forests. 1995. 1–8 p.
50. BANCO DE OCCIDENTE. Bosque Seco Tropical - Colombia - Libros de la Colección Ecológica del Banco de Occidente [Internet]. Editorial I/M. On line. 2006 [cited 2020 Oct 7]. Available from: https://www.imeditores.com/banocc/seco/cap1.htm
51. Hernández-Camacho J, Heliodoro Sánchez Páez. Biomas terrestres de Colombia-LA DIVERSIDAD BIOLÓGICA DE IBEROAMERICA. Geriatrika. 1992. 153–172 p. DOI: 10.2307/40117169
52. Espejo N, Morales N. Variación de la diversidad taxonómica y funcional de la avifauna en un bosque seco tropical (bs-T) en diferentes estados de sucesión en el sur del Valle del Magdalena, Huila, Colombia. Caldasia. 2019;41(1):108–23. DOI: 10.15446/caldasia.v41n1.71272
53. Rojas A. Verificación de coberturas de bosque seco tropical en el nodo norandino (Santander y Norte de Santander). Bogotá: Instituto de Investigación Biológica Alexander von Humboldt. 2017.

54. Prance GT, Kartawinata EK. Lecythidaceae. Vol. 21. Flora Malesiana; 2013. 1–118 p.
55. Calderon E, Galeano G, García N. Libro rojo de plantas Fanerógamas de Colombia Volumen 1: Chrysobalanaceae, Dichapetalaceae y Lecythidaceae. Instituto Alexander von Humboldt; 2002. 218 p.
56. Rimieri P. La diversidad genética y la variabilidad genética: Dos conceptos diferentes asociados al germoplasma y al mejoramiento genético vegetal*. J Basic Appl Genet. 2017;XXVIII(2):7–13.
57. Agarwal M, Shrivastava N, Padh H. Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep. 2008;27(4):617–31. DOI: 10.1007/s00299-008-0507-z
58. Schloterrer C. The evolution of molecular markers: Just a matter of fashion? Nat Rev Genet. 2004;5(1):63–9. https://doi.org/10.1038/nrg1249
59. Garrido-Cardenas JA, Mesa-Valle C, Manzano-Agugliaro F. Trends in plant research using molecular markers. Planta [Internet]. 2018;247(3):543–57. Available from: https://doi.org/10.1007/s00425-017-2829-y
60. Zane L, Bargelloni L, Patarnello T. Strategies for microsatellite isolation: A review. Mol Ecol. 2002;11(1):1–16. Doi: 10.1046/j.0962-1083.2001.01418.x
61. Ellegren H. Microsatellites: Simple sequences with complex evolution. Nat Rev Genet. 2004;5(6):435–45. DOI: 10.1038/nrg1348
62. Annaliese S. Mason. SSR Genotiping. In Plant Genotiping. Jacqueline Batley, editor. Vol. 1245, Methods in Molecular Biology. 2015. 77–89 p. DOI: 10.1007/978-1-4939-1966-6_7
63. Pastelín Solano MC, Salinas Ruíz J, González Arnao MT, Castañeda Castro O, Galindo Tovar ME, Bello Bello JJ. Evaluation of in vitro shoot multiplication and ISSR marker based assessment of somaclonal variants at different subcultures of vanilla (Vanilla planifolia Jacks). Physiol Mol Biol Plants. 2019;25(2):561–7. DOI: 10.1007/s12298-019-00645-9
64. Kumar V, Yadav HK. Assessment of genetic diversity in Lepidium sativum L. using inter simple sequence repeat (ISSR) marker. Physiol Mol Biol Plants [Internet]. 2019;25(2):399–406. Available from: https://doi.org/10.1007/s12298-018-0622-4
65. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20(2):176–83. DOI: 10.1006/geno.1994.1151
66. Verma KS, ul Haq S, Kachhwaha S, Kothari SL. RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. 3 Biotech. 2017;7(5):288. DOI: 10.1007/s13205-017-0918-z
67. Amom T, Tikendra L, Rahaman H, Potshangbam A, Nongdam P. Evaluation of genetic relationship between 15 bamboo species of North-East India based on ISSR marker analysis. Mol Biol Res Commun. 2018;7(1):7–15. DOI: 10.22099/mbrc.2018.28378.1303
68. Purayil FT, Robert GA, Gothandam KM, Kurup SS, Subramaniam S, Cheruth AJ. Genetic variability in selected date palm (*Phoenix dactylifera* L.) cultivars of United Arab Emirates using ISSR and DAMD markers. 3 Biotech [Internet]. 2018;8(2):109. Available from: https://doi.org/10.1007/s13205-018-1108-3

69. Buckley DP, O'Malley DM, Apsit V, Prance GT, Bawa KS. Genetics of Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.: Lecythidaceae) - 1. Genetic variation in natural populations. Theor Appl Genet. 1988;76(6):923–8. DOI: 10.1007/BF00273682

70. O'Malley DM, Buckley DP, Prance GT, Bawa KS. Genetics of Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.: Lecythidaceae) - 2. Mating system. Theor Appl Genet. 1988;76(6):929–32. DOI: 10.1007/BF00273683

71. REIS, A.M.M., BRAGA, A.C., LEMES, M.R., GRIBEL, R. and COLLEVATTI R. Development and characterization of microsatellite markers for the Brazil nut tree *Bertholletia excelsa* Humb. y Bonpl. (Lecythidaceae). Mol Ecol Resour. 2009;9:920–3. DOI: 10.1111/j.1755-0998.2008.02479.x

72. Guidugli MC, De Campos T, De Sousa ACB, Feres JM, Sebbenn AM, Mestriner MA, et al. Development and characterization of 15 microsatellite loci for *Cariniana estrellensis* and transferability to *Cariniana legalis*, two endangered tropical tree species. Conserv Genet. 2009;10:1001–4. DOI: 10.1007/s10592-008-9672-4

73. Guidugli MC, Accoroni KAG, Mestriner MA, Contel EPB, Martinez CA, Alzate-Marin AL. Genetic characterization of 12 heterologous microsatellite markers for the giant tropical tree *Cariniana legalis* (Lecythidaceae). Genet Mol Biol. 2010;33(1):131–4. DOI: 10.1590/S1415-47572010000100022

74. Tambarussi EV, Sebbenn AM, Moreno MA, Ferraz EM, Kageyama PY, Vencovsky R. Microsatellite Markers for *Cariniana legalis* (Lecythidaceae) and Their Transferability to *C. estrellensis* . Appl Plant Sci. 2013;1(6):1200493. DOI: 10.3732/apps.1200493

75. Tambarussi E V., Boshier D, Vencovsky R, Freitas MLM, Sebbenn AM. Paternity analysis reveals significant isolation and near neighbor pollen dispersal in small *Cariniana legalis* Mart. Kuntze populations in the Brazilian Atlantic Forest. Ecol Evol. 2015;5(23):5588–600. DOI: 10.1002/ece3.1816

76. Sujii PS, Inglis PW, Ciampi AY, Solferini VN, Azevedo VCR. Isolation and characterization of microsatellite markers for *Bertholletia excelsa* (Lecythidaceae) population genetic analysis. Genet Mol Res. 2013;12(4):5278–82. DOI: 10.4238/2013.November.7.2

77. Sujii PS, Martins K, Wadt LH de O, Azevedo VCR, Solferini VN. Genetic structure of *Bertholletia excelsa* populations from the Amazon at different spatial scales. Conserv Genet. 2015;16:955–64. DOI: 10.1007/s10592-015-0714-4

78. Xie H, Yuan Y, Fang X, Liu Y, Yang C, Jin J, et al. Development of EST-SSR Markers in *Barringtonia racemosa* (Lecythidaceae) and Cross-Amplification in Related Species. Appl Plant Sci. 2015;3(12):1500080. DOI: 10.3732/apps.1500080

79. Martos F, Lebreton G, Rivière E, Humeau L, Chevallier M-H. Microsatellites in the Tree *Foetidia mauritiana* (Lecythidaceae) and Utility in Other *Foetidia* Taxa from the Mascarene Islands. Appl Plant Sci. 2016;4(8):1600034. DOI: 10.3732/apps.1600034

80. Cuénin N, Flores O, Rivière E, Lebreton G, Reynaud B, Martos F. Great genetic diversity but high selfing rates and short-distance gene flow characterize populations of a tree (*Foetidia*; Lecythidaceae) in the fragmented tropical dry forest

of the mascarene Islands. J Hered. 2019;110(3):287–99. DOI: 10.1093/jhered/esy069
81. Doyle, JJ; Doyle J. Isolation of plant DNA from fresh tissue. Vol. 12, Focus. 1990. p. 13–5.
82. Ramalho AB, Rossi AAB, Dardengo JFE, Zortéa KÉM, Tiago AV, Martins KC. Diversidade genética entre genótipos de *Bertholletia Excelsa* por meio de marcadores moleculares issr. Floresta. 2016;46(2):207–14. DOI: 10.5380/rf.v46i2.41970
83. Peakall R, Smouse PE. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006;6:288–95. DOI: 10.1111/j.1471-8286.2005.01155.x
84. Peakall R, Smouse PE. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 2012;28(19):2537–9. DOI: 10.1093/bioinformatics/bts460
85. Wang J sheng, He J hu, Chen H rui, Chen Y yuan, Qiao F. Genetic Diversity in Various Accessions of Pineapple [*Ananas comosus* (L.) Merr.] Using ISSR and SSR Markers. Biochem Genet. 2017;55(5–6):347–66. DOI: 10.1007/s10528-017-9803-z
86. Kalinowski ST, Taper ML, Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol. 2007;16(5):1099–106. DOI: 10.1111/j.1365-294X.2007.03089.x
87. Yeh, F. C., Yang, R. C., & Boyle T. POPGENE version 1.32: Microsoft Windows–based freeware for population genetic analysis, quick user guide. enter Int For Res Univ Alberta, Edmonton, Alberta, Canada. 1999;1(29).
88. Raymond M. & Rousset F. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered. 1995;86:248–9.
89. Rousset F. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour. 2008;8:103–6.
90. Nudin NFH, Ali AM, Ngah N, Mazlan NZ, Mat N, Ghani MNA, et al. ISSR marker-assisted genetic diversity analysis of *Dioscorea hispida* and selection of the best variety for sustainable production. Comptes Rendus - Biol. 2017;340(8):359–66. https://doi.org/10.1016/j.crvi.2017.08.003
91. Monfared MA, Samsampour D, Sharifi-Sirchi GR, Sadeghi F. Assessment of genetic diversity in *Salvadora persica* L. based on inter simple sequence repeat (ISSR) genetic marker. J Genet Eng Biotechnol. 2018;16(2):661–7. DOI: 10.1016/j.jgeb.2018.04.005
92. Bocanegra-González KT, Thomas E, Guillemin M-L, Alcázar Caicedo C, Moscoso Higuita LG, Gonzalez MA, et al. Diversidad y estructura genética de cuatro especies arbóreas clave del Bosque Seco Tropical en Colombia. Caldasia. 2019;41(1):78–91. DOI: https://doi.org/10.15446/caldasia.v41n1.71327
93. Carpejani AA, Pena GF, Vieira FS, Tiago PV, Rossi AAB. Genetic Diversity of *Bertholletia excelsa* Bonpl.: A Native Species of the Amazon Rainforest. Floresta e Ambient. 2020;27(4). https://doi.org/10.1590/2179-8087-floram-2018-0180.
94. Da Silva AVC, Muniz EN, Almeida CS, Da Vitória MF, Da Silva Ledo A, De Vasconcelos Melo MF, et al. Genetic diversity and sex identification in *genipa*

americana L. Trop Subtrop Agroecosystems. 2015;18(1):81–6. https://www.redalyc.org/articulo.oa?id=93938025010
95. Feitosa-Alcantara RB, Silva AVC, Blank AF, Almeida CS, Alvares-Carvalho S V., Arrigoni-Blank MF. Analysis of genetic diversity of *Hyptis pectinata* (L.) Poit. plants using ISSR markers. Genet Mol Res. 2017;16(3). DOI: 10.4238/gmr16039603
96. Nagy S, Poczai P, Cernák I, Gorji AM, Hegedűs G, Taller J. PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet. 2012;50(9–10):670–2. DOI: 10.1007/s10528-012-9509-1
97. Aranguren Y, Briceño A, Fermin G. Assessment of the variability of Venezuelan guava landraces by microsatellites. Acta Hortic. 2010;849:147–54. DOI: 10.17660/ActaHortic.2010.849.16
98. Chirinos-Aria MC, Jiménez JE. Transferencia de algunos marcadores moleculares microsatélites de la familia Fabaceae en tarwi (*Lupinus mutabilis* Sweet). Sci Agropecu. 2015;6(1):51–8. http://dx.doi.org/10.17268/sci.agropecu.2015.01.05
99. Gimenes MA, Hoshino AA, Barbosa AVG, Palmieri DA, Lopes CR. Characterization and transferability of microsatellite markers of the cultivated peanut (*Arachis hypogaea*). BMC Plant Biol. 2007;7(9):1–13. DOI:10.1186/1471-2229-7-9
100. Vieira FS, Rossi AA, Pena GF, Tiago A V., Zortéa KÉM, Schmitt KFM, et al. Genetic diversity of brazil-nut populations naturally occurring in the municipality of alta floresta, mt, brazil. Genet Mol Res. 2019;18(2):gmr18174. DOI: 10.4238/gmr18174
101. Mori, S. A., and Prance GT. Lecythidaceae. Part II. The zygomorphic-flowered New World genera (*Couroupita, Corythophora, Bertholletia, Couratari, Eschweilera, & Lecythis*). Flora Neotrop Monogr. 1990;21:1– 376.
102. Huang YY, Mori SA, Prance GT. A phylogeny of *Cariniana* (Lecythidaceae) based on morphological and anatomical data. Brittonia. 2008;60(1):69–81. https://doi.org/10.1007/s12228-008-9014-3
103. Mori SA, Tsou CH, Wu CC, Cronholm B, Anderberg AA. Evolution of Lecythidaceae with an emphasis on the circumscription of neotropical genera: Information from combined ndhF and trnL-F sequence data. Am J Bot. 2007;94(3):289–301. DOI: 10.3732/ajb.94.3.289
104. Botstein D, White RL, Skolnick M, Davis RW. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. Am J Hum Gen. 1980;32:314–31.
105. Ellegren H, Galtier N. Determinants of genetic diversity. Nat Rev Genet. 2016;17(7):422–33. https://doi.org/10.1038/nrg.2016.58
106. Fernández-Mazuecos M, Jiménez-Mejías P, Rotllan-Puig X, Vargas P. Narrow endemics to Mediterranean islands: Moderate genetic diversity but narrow climatic niche of the ancient, critically endangered Naufraga (Apiaceae). Perspect Plant Ecol Evol Syst. 2014;16(4):190–202. DOI: 10.1016/j.ppees.2014.05.003
107. Melito S, La Bella S, Martinelli F, Cammalleri I, Tuttolomondo T, Leto C, et al. Morphological, chemical, and genetic diversity of wild myrtle (*Myrtus communis* L.) populations in sicily. Turkish J Agric For. 2016;40(2):249–61. DOI: 10.3906/tar-1505-118

108. Jackson GC, Salas JB. Insect Visitors of *Lecythis elliptica*. J Agric Univ Puerto Rico. 1961;49(1):133–41. https://doi.org/10.46429/jaupr.v49i1.13014
109. Kramer AT, Ison JL, Ashley M V., Howe HF. The paradox of forest fragmentation genetics. Conserv Biol. 2008;22(4):878–85. DOI: 10.1111/j.1523-1739.2008.00944.x