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PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS GENÉTICOS VEGETAIS

FABRÍCIO FRANCISCO SANTOS DA SILVA

CARACTERIZAÇÃO DE MATRIZES, TOLERÂNCIA À DESSECAÇÃO DE DUAS LEGUMINOSAS E VARIABILIDADE GENÉTICA de *Anadenanthera* spp. EM FLORESTAS TROPICAIS SECAS

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Tese apresentada ao Programa de Pós-Graduação em Recursos Genéticos Vegetais, da Universidade Estadual de Feira de Santana como requisito parcial para obtenção do título de Doutor em Recursos Genéticos Vegetais.

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Minha filha Flora

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RESUMO

A seleção de plantas matrizes é importante para identificar, localizar e delimitar áreas de coleta de sementes florestais. A prática de coleta de sementes auxilia na melhoria socioeconômica da população local e conservação do ambiente em que as mesmas se encontram. Outra medida que auxilia na conservação é a identificação de espécies tolerantes à dessecação em florestas tropicais sazonalmente secas (SDTF). A tolerância à dessecação (DT) pós-germinativa está diretamente ligada ao sucesso da sobrevivência de plântulas em SDTF. *Cenostigma pyramidale* é endêmica da Caatinga, *Anadenanthera colubrina* e *Anadenanthera peregrina* são árvores de ampla distribuição em SDTF. Ferramentas que auxiliem no estudo de espécies florestais são importantes para conservação dos recursos genéticos. O presente estudo teve como principais objetivos: caracterizar plantas matrizes e avaliar a DT pós-germinativa de *Anadenanthera colubrina* e *Cenostigma pyramidale*, como também avaliar a variabilidade genética do gênero *Anadenanthera* spp. Apresentamos uma lista de plantas matrizes destas duas leguminosas, bem como a localização detalhada de cada matriz. Foram marcadas 60 plantas matrizes, em sete municípios: Uberlândia-MG e Planaltina-DF no Cerrado; Corumbá-MS no Pantanal; Canindé de São Francisco-SE, Lagoa Grande PE, Petrolina-PE, Juazeiro-BA na Caatinga. Para cada planta marcada, os seguintes detalhes foram coletados: dados dendrométricos, coordenadas geográficas e tipo de solo. Para avaliar a DT, plântulas de *A. colubrina* e *C. pyramidale* de diferentes tamanhos foram separadas em quatro categorias de Comprimento Raiz Inicial (IRL) e dessecadas por 24 e 72 h. A sobrevivência das plântulas foi avaliada aos 7 e 14 dias após a reidratação (DAR). Para avaliar quais eram os níveis de variabilidade genética e estrutura de populações no gênero *Anadenanthera*, foi realizada a extração de DNA das folhas. A estimativa de tamanho em pares de bases foi obtida pelo método da mobilidade inversa. O dendrograma UPGMA foi gerado usando o índice de similaridade de Jaccard, com base na distância genética em 39 alelos de nove loci. A análise da variância molecular foi realizada usando a decomposição total entre e dentro de populações de *Anadenanthera* spp. O fluxo gênico (Nm) foi estimado pelo número de migrantes, com base no parâmetro Φ_{ST}. Entre os resultados obtidos, pode-se destacar que a altura de plantas de *A. colubrina* e *C. pyramidale* varia de 4 a 42,5 m e 3 a 10 m, respectivamente. Foram categorizados 10 tipos de solos. *Anadenanthera colubrina* e *C. pyramidale* foram tolerantes à dessecação pós-germinativa. A taxa de sobrevivência das plântulas de *A. colubrina* com IRL entre 7,00 e 10,99 mm que foram secas por 24 horas foi de 70% aos 7 DAR. A taxa de sobrevivência das plântulas de *C. pyramidale* com IRL entre 1,00

e 6,99 mm que foram secas por 72 horas foi de 96% aos 7 DAR. Aos 14 DAR, plântulas de *C. pyramidale* maiores que 6,99 mm quando dessecadas estavam mortas. A sobrevivência de plântulas de *A. colubrina* e *C. pyramidale* à dessecação tem um efeito direto no recrutamento de espécies SDTF, especialmente durante períodos de seca ou anos de seca. No experimento de variabilidade genética de *Anadenanthera* spp. o tamanho dos alelos variou de 175 pb a 794 pb. A média da frequência alélica, Conteúdo de Informação de Polimorfismo (PIC) e heterozigosidade foram de 0,58; 0,52 e 0,45, respectivamente, demonstrando uma alta capacidade de detecção de variabilidade genética. O coeficiente de similaridade variou entre 20 e 80%, com valor cofenético de 0,81. Os dois agrupamentos Bayesianos dividem-se em *A. colubrina* e *A. peregrina*. A variabilidade genética entre as populações é alta, $\Phi_{ST} = 0,217$ ($P < 0,001$), restringindo o Nm para um migrante por geração (0,9). Com o presente estudo concluímos que (1) árvores matrizes marcadas na Caatinga apresentaram menor altura total em relação às árvores do Pantanal e do Cerrado; (2) como estratégia de sobrevivência, algumas mudas de ambas as espécies perdem a raiz primária e emitem raízes adventícias após a dessecação; (3) o uso de marcadores microssatélites possibilita o estudo de genética de populações, bem como auxilia na identificação taxonômica de *Anadenanthera* Speg. Este trabalho pode ser utilizado como referência para futuros estudos de campo de *A. colubrina* e *C. pyramidale* e em excursões de coleta de sementes de *A. colubrina* e *C. pyramidale*.

Palavras-chave: *Anadenanthera colubrina*, *Anadenanthera peregrina*, *Poincianella pyramidalis*, SDTF, reidratação, genética de populações, SSR

ABSTRACT

The selection of mother-plants is important for identifying, locating and delimiting areas for seed collection of forest species. The seed collect improve farmers' quality of life and help conservation environmental. Other step in conservation is identification of these plants could help in the selection of desiccation tolerant species in seasonally dry tropical forests (SDTF). Post-germinative desiccation tolerance (DT) is directly linked to the success of seedling survival of SDTF species. *Cenostigma pyramidale* is endemic to Caatinga, *Anadenanthera colubrina* and *Anadenanthera peregrina* which are widely distributed trees in SDTF. Tools used to study forest trees are important for the conservation of genetic resources. The objective of this study was to characterize mother-trees and to evaluate post-germinative DT of *A. colubrina* and *C. pyramidale*, as well as to evaluate the genetic variability of the *Anadenanthera* spp. This study presents a list of mother-trees of two legumes, as well as their detailed location. Sixty plants were registered in seven municipalities in Brazil: Uberlândia-MG and Planaltina-DF from Cerrado; Corumbá-MS from Pantanal; Canindé de São Francisco-SE, Lagoa Grande-PE, Petrolina-PE and Juazeiro-BA from Caatinga. For each marked plant, the following details were collected: dendrometric data, geographic coordinates and soil type. *Anadenanthera colubrina* and *C. pyramidale* seedlings of different sizes were separated into four Initial Root Length (IRL) categories and dried for 24 and 72 h. The seedling survival was evaluated at 7 and 14 days after rehydration (DAR). DNA was extracted from the leaves. A UPGMA dendrogram was generated using the Jaccard similarity index based on the genetic distance of 39 alleles and nine loci. An analysis of molecular variance was conducted using total decomposition among and within the populations of *Anadenanthera* spp. Gene flow (Nm) was estimated by the number of migrants, based on the parameter Φ_{ST}. The plant height of *A. colubrina* and *C. pyramidale* ranges from 4 to 42.5 m and 3 to 10 m, respectively. Ten types of soils were categorized. *Anadenanthera colubrina* and *C. pyramidale* were tolerant to post-germination desiccation. The survival rate of the *A. colubrina* seedlings with IRL between 7.00 and 10.99 mm that were dried for 24 h was 70% at 7 DAR. The survival rate of the *C. pyramidale* seedlings with IRL between 1.00 and 6.99 mm that were dried for 72 h was 96% at 7 DAR. At 14 DAR, *C. pyramidale* seedlings longer than 6.99 mm when dessicated were dead. The survival of seedlings of *A. colubrina* and *C. pyramidale* to desiccation, has a direct effect on the recruitment of SDTF species, specially during dry spells or drought years. The size of the alleles varied from 175 bp to 794 bp. The averages for allelic frequency, polymorphism information content (PIC) and heterozygosity

were 0,58; 0,52 e 0,45, respectively, demonstrating the high capacity for detecting genetic variability. The coefficient of similarity varied between 20 and 80%, with a cophenetic value of 0,81. The two Bayesian clusters divide *A. colubrina* and *A. peregrina*. The genetic variability among the population is high, Φ_{ST} = 0,217 (P < 0,001), restricting the Nm to one migrant per generation (0,9). With the present study we conclude that (1) The mother-trees marked in the Caatinga ecosystem presented lower total height in relation to the Pantanal and Cerrado ecosystems trees.; (2) As a survival strategy, some seedlings of both species lose the primary root and emit adventitious roots after desiccation; (3) Population genetics can be studied using these markers, which also help in the taxonomic identification of *Anadenanthera* Speg. This work can be used as reference for future field studies of *A. colubrina* and *C. pyramidale* and in seed collection excursions of *A. colubrina* and *C. pyramidale*.

Keywords: *Anadenanthera colubrina*, *Anadenanthera peregrina*, *Poincianella pyramidalis*, SDTF, rehydration, population genetics, SSR

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INTRODUÇÃO GERAL

Plantas matrizes de qualidade são fundamentais para coleta de sementes florestais vigorosas, tendo influência direta na germinação, dormência (quando existente), desenvolvimento e tamanho (massa) das sementes produzidas (Baskin e Baskin, 2019). O uso de características fenotípicas é muito comum no momento da marcação de matrizes, por exemplo, levando em consideração o plantio de mudas na arborização urbana, torna-se vital para o sucesso do plantio observar, nas plantas matrizes, a fisiologia das raízes, se as flores possuem formas e aromas atrativos e que seja de rápido crescimento. Já na produção madeireira, as características almejadas são forma do fuste, volume, etc. (Pinã-Rodrigues *et al.*, 2007). Além disso, o uso de aplicativos para *smartphones* (*e.g.* GPS Essentials, Smart Tools co., Hypsometer) são alternativas de custos reduzidos que auxiliam na caracterização das árvores matrizes em campo (Harfouche *et al.*, 2019), para posterior coleta de sementes.

Dentre as espécies florestais arbóreas que ocorrem na Caatinga e de múltiplos usos podemos destacar as Fabaceae *Anadenanthera colubrina* (Vell.) Brenan e *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis. *Anadenanthera colubrina*, de ampla distribuição, ocorre em floresta estacional semidecídual, floresta ombrófila densa, Cerrado, Caatinga, Pantanal e está distribuída em quase todo o território brasileiro. Normalmente são árvores de médio a grande porte, que geralmente apresentam caducifólia durante os meses de setembro a dezembro (Maia, 2012). Essa espécie é considerada rústica e adaptada a terrenos secos, sendo recomendada para recuperação ambiental, crescendo muito bem em solos degradados. Além disso, também pode ser utilizada na arborização urbana e no paisagismo (Siqueira-Filho *et al.*, 2013). Suas sementes são resistentes aos mais diversos estresses ambientais, como altas temperaturas, estresse salino e déficit hídrico (Dantas *et al.*, 2014; Santos *et al.*, 2016). *Cenostigma pyramidale* é endêmica da Caatinga, muito utilizada na região Nordeste do Brasil devido ao seu uso no reflorestamento, forrageiro, madeireiro e medicinal, podendo chegar aproximadamente 12 metros, seu tronco apresenta cerca de 50 cm de diâmetro, casca cinza-claro, com ritidoma que se desprende em lâminas alongadas e irregulares, com flores amarelas e dispostas em racemos (Maia, 2012). Assim como *A. colubrina*, sementes de *C. pyramidale* apresentam tolerância às diversas condições de

estresses abióticos (Lima *et al.*, 2011; Santos *et al.*, 2016), característica importante para espécies presentes em florestas tropicais sazonalmente secas (SDTF).

A diversidade de formas de vidas em SDTF é influenciada pela heterogeneidade dos ambientes e também pela disponibilidade hídrica (Medina, 1995). Na Caatinga, por exemplo, a precipitação, geralmente, não ultrapassa os 1000 mm ao ano (Sampaio, 1995). Espécies arbóreas encontradas em SDTF apresentam diversas adaptações fisiológicas para tolerar a sazonalidade pluviométrica, como abscisão foliar (Hayden, Greene e Quesada, 2010), presença de raízes primárias tuberosas (Barretto e Ferreira, 2011), folhas coriáceas (Mitchell e Daly, 2015) e tolerância à dessecação pós-germinativa (Martins *et al.*, 2015).

A semente, desde sua formação até a germinação, apresenta estádios de intolerância e tolerância à dessecação (DT). Após a germinação ocorre uma redução da DT, sendo assim, compreender esses mecanismos em sementes ortodoxas servirá de modelo para estudos futuros em sementes recalcitrantes (Farrant e Moore, 2011; Nonogaki, Bassel e Bewley, 2010). Além disso, posteriormente ao desenvolvimento, as sementes ortodoxas se mantêm viáveis após a dessecação, reduzindo a umidade a um baixo teor, podendo chegar a valores em torno de 5%. Sementes recalcitrantes, ao contrário, são dispersas com alto teor de umidade, sendo intolerantes à dessecação (Pammenter e Berjak, 2000). A DT de sementes durante a germinação é importante para que a espécie sobreviva às condições desfavoráveis para o desenvolvimento da plântula (Castro, Bradford e Hilhorst, 2004). A tolerância à dessecação pós-germinativa é a capacidade da plântula sobreviver após secagem (Leprince e Buitink, 2015). Trabalhos sobre tolerância à dessecação pós-germinativa em plântulas são relativamente recentes, sendo importantes para o sucesso do recrutamento de plântulas em ambientes áridos (Martins *et al.*, 2015).

O sucesso do estabelecimento de plântulas é em sua maior parte dependente da qualidade da semente (viabilidade e vigor). A resistência elevada a estresses abióticos é uma característica original de sementes, mas que não é explorada como uma fonte potencial para conferir tolerância a plântulas ou plantas inteiras, assim como não é explorado como um marcador potencial para o estabelecimento e melhoria do plantio como um todo. Nesse sentido, sementes podem estar expostas a estresses severos também durante o desenvolvimento e maturação, incluindo seca e temperaturas elevadas (Bowler e Fluhr, 2000; Pastori e Foyer, 2002).

Em 2001, com parcerias de instituições públicas e privadas, foram criadas as Redes de Sementes, com o objetivo de estruturar todas as informações de produção, armazenamento e comercialização de sementes de espécies florestais (França-Neto, 2009). Programas de restauração em SDTF exigem um grande número de sementes geneticamente diversificadas e adaptadas localmente. As Redes de Sementes, com participação local, melhora a renda da comunidade rural na mesma medida em que auxilia a conservação e restauração da biodiversidade (Schmidt *et al.*, 2018).

O uso de marcadores moleculares é importante como ferramenta em programas de conservação e uso de recursos genéticos, em especial para aquelas espécies que sofrem algum tipo de ameaça (Balbino, Caetano e Almeida, 2018). Quanto a expressão genética, os marcadores podem ser classificados como dominantes e codominantes. Como exemplo de marcadores moleculares dominantes temos o AFLP (*Amplified Fragment Length Polymorphism*), RAPD (*Random Amplified Polymorphic* DNA), ISSR (*Inter Simple Sequence Repeats*); e codominantes as isoenzimas, RFLP (*Restriction Fragment Length Polymorphism*), SSR (*Simple Sequence Repeats*) (Faleiro, 2007). Usando SSR como exemplo, podemos citar algumas vantagens e desvantagens desse marcador. Uma das grandes vantagens dos marcadores codominantes é a diferenciação dos lócus em homozigose e heterozigose. Como limitação, o SSR necessita do desenvolvimento de *primers* específicos para cada espécie, sendo esse processo demorado, trabalhoso de alto custo (Caixeta, Ferrão e Maciel-Zambolim, 2013). Este marcador molecular pode ser usado como uma importante ferramenta na identificação de unidades taxonômicas (Tuler *et al*., 2015), podendo ocorrer ainda a transferibilidade entre espécies do mesmo gênero, como é o caso de *Anadenanthera peregrina* (L.) Speg. e *A. colubrina* (Feres *et al.*, 2012). Até o momento não há registros de *primers* específicos para *C. pyramidale*.

As informações geradas nestes estudos serão de grande importância para o direcionamento de agricultores (pequenos, familiares ou grandes) da região Nordeste, cuja vegetação é historicamente afetada pelos estresses abióticos a serem estudados, para a produção de sementes das espécies e acessos mais indicados em condições adversas, seja para produção com fins lucrativos, seja para recuperação de áreas degradadas e serviços ambientais. Desta forma, tive como objetivos principais caracterizar plantas matrizes e avaliar a tolerância à dessecação de *A. colubrina* e *C. pyramidale*, como também avaliar a variabilidade genética de *A. colubrina* e *A. peregrina*.

REFERÊNCIAS

BALBINO, E.; CAETANO, B.; ALMEIDA, C. Phylogeographic structure of *Spondias tuberosa* Arruda Câmara (Anacardiaceae): seasonally dry tropical forest as a large and continuous refuge. **Tree Genetics & Genomes**, v. 14, p. 1–8, 2018.

BARRETTO, S. S. B.; FERREIRA, R. A. Aspectos morfológicos de frutos, sementes, plântulas e mudas de leguminosae mimosoideae: *Anadenanthera colubrina* (Vellozo) Brenan e *Enterolobium contortisiliquum* (Vellozo) Morong. **Revista Brasileira de Sementes**, v. 33, n. 2, p. 223–232, 2011.

BASKIN, J. M.; BASKIN, C. C. How much influence does the paternal parent have on seed germination? **Seed Science Research**, p. 1-11, 2019.

BOWLER, C.; FLUHR, R. The role of calcium and activated oxygens as signals for controlling cross-tolerance. **Trends in Plant Science**, v. 5, n. 6, p. 241–246, jun. 2000.

CAIXETA, E. T.; FERRÃO, L. F. V.; MACIEL-ZAMBOLIM, E. Marcadores moleculares. *In*: BORÉM, A.; FRITSCHE-NETO, R. (Eds.). . **Biotecnologia aplicada ao melhoramento de plantas**. Visconde do Rio Branco, MG: Suprema, 2013. p. 31–68.

CASTRO, R. D.; BRADFORD, K. J.; HILHORST, H. W. M. Desenvolvimento de sementes e conteúdo de água. *In*: **Germinaçao: Do básico ao aplicado**. [s.l.] Artmed Editora, 2004. p. 51–67.

DANTAS, B. F.; RIBEIRO, R. C.; MATIAS, J. R.; ARAÚJO, G. G. L. Germinative metabolism of Caatinga forest species in biosaline agriculture. **Journal of Seed Science**, v. 36, n. 2, p. 194–203, 2014.

FALEIRO, F. **Marcadores genético-moleculares aplicados a programas de conservação e uso de recursos genéticos**. Planaltina: Embrapa Cerrados, 2007.

FARRANT, J. M.; MOORE, J. P. Programming desiccation-tolerance: From plants to seeds to resurrection plants. **Current Opinion in Plant Biology**, v. 14, n. 3, p. 340–345, 2011.

FERES, J. M.; MONTEIRO, M.; ZUCCHI, M. I.; PINHEIRO, J. B.; MESTRINER, M. A.; ALZATE-MARIN, A. L. Development of microsatellite markers for *Anadenanthera colubrina* (leguminosae), a neotropical tree species. **American Journal of Botany**, v. 99, n. 4, p. 154–156, 2012.

FRANÇA-NETO, J. B. Evolução do conceito de qualidade de sementes. **Informativo Abrates**, v. 19, n. 2, p. 76–80, 2009.

HARFOUCHE, T. B.; CORTE, A. P. D.; RUZA, M.; BEHLING, A. Uso de aplicativos em smartphone para medições de árvores. **BIOFIX Scientific Journal**, v. 4, n. 1, p. 7–15, 2019.

HAYDEN, B.; GREENE, D. F.; QUESADA, M. A field experiment to determine the effect of dry-season precipitation on annual ring formation and leaf phenology in a seasonally dry tropical forest. **Journal of Tropical Ecology**, v. 26, n. 02, p. 237–242, 29 mar. 2010.

LEPRINCE, O.; BUITINK, J. Introduction to desiccation biology: from old borders to new frontiers. **Planta**, v. 242, n. 2, p. 369–378, 2015.

LIMA, C. R. DE; PACHECO, M. V.; BRUNO, R. DE L. A.; FERRARI, C. DOS S.; BRAGA JÚNIOR, J. M.; BEZERRA, A. K. D. Temperaturas e substratos na germinação de sementes de. **Revista Brasileira de Sementes**, v. 33, n. 2, p. 216–222, 2011.

MAIA, G. N. **Caatinga: árvores e arbustos e suas utilidades**. Fortaleza: Printcolor Gráfica e Editora, 2012.

MARTINS, J. R.; EDVALDO, A. A. S.; ALVARENGA, A. A.; RODRIGUES, A. C.; RIBEIRO, D. E.; TOOROP, P. E. Seedling survival of *Handroanthus impetiginosus* (Mart ex DC) Mattos in a semi-arid environment through modified germination speed and post-germination desiccation tolerance. **Brazilian Journal of Biology**, v. 75, n. 4, p. 812–820, 2015.

MEDINA, E. Diversity of life forms of higher plants in neotropical dry forests. *In*: BULLOCK, S. H.; MOONEY, H. A.; MEDINA, E. (Eds.). . **Seasonally dry tropical forests**. Cambridge, UK: Cambridge University Press, 1995. p. 221–242.

MITCHELL, J. D.; DALY, D. C. A revision of Spondias L. (Anacardiaceae) in the Neotropics. **PhytoKeys**, n. 55, p. 1–92, 2015.

NONOGAKI, H.; BASSEL, G. W.; BEWLEY, J. D. Germination-still a mystery. **Plant Science**, v. 179, n. 6, p. 574–581, 2010.

PAMMENTER, N. W.; BERJAK, P. Aspects of recalcitrant seed biology. **Revista Brasileira de Fisiologia Vegetal**, v. 12, p. 56–69, 2000.

PASTORI, G. M.; FOYER, C. H. Common Components, Networks, and Pathways of Cross-

Tolerance to Stress. The Central Role of "Redox" and Abscisic Acid-Mediated Controls. **Plant Physiology**, v. 129, p. 460–468, 2002.

PINÃ-RODRIGUES, F.; FREIRE, J. M.; LELES, P. S. S.; BREIER, T. B. **Parâmetros técnicos para a produção de sementes florestais**. Seropédica: EDUR, 2007.

SAMPAIO, E. V. S. B. Overview of the Brazilian caatinga. *In*: BULLOCK, S. H.; MOONEY, H. A.; MEDINA, E. (Eds.). . **Seasonally dry tropical forests**. Cambridge, UK: Cambridge University Press, 1995. p. 35–63.

SANTOS, C. A. DOS; SILVA, N. V. DA; WALTER, L. S.; SILVA, E. C. A. DA; NOGUEIRA, R. J. M. C. Germinação de duas espécies da caatinga sob déficit hídrico e salinidade. **Pesquisa Florestal Brasileira**, v. 36, n. 87, p. 219–224, 2016.

SCHMIDT, I. B.; URZEDO, D. I. DE; PIÑA-RODRIGUES, F. C. M.; VIEIRA, D. L. M.; REZENDE, G. M. DE; SAMPAIO, A. B.; JUNQUEIRA, R. G. P. Community-based native seed production for restoration in Brazil - the role of science and policy. **Plant Biology**, p. 1–9, 2018.

SIQUEIRA-FILHO, J. A.; MEIADO, M. V.; RABBANI, A. R. C.; SIQUEIRA, A. A.; VIEIRA, D. C. M. **Guia de Campo de Árvores das Caatingas**. Curitiba: Editora Progressiva Ltda, 2013.

TULER, A. C.; CARRIJO, T. T.; NÓIA, L. R.; FERREIRA, A.; PEIXOTO, A. L.; FERREIRA, M. F. S. (2015). SSR markers: a tool for species identification in *Psidium* (Myrtaceae). **Molecular biology reports**, v. 42, n. 11, p. 1501-1513, 2015.

CAPÍTULO 1 - Mapeamento e descrição de árvores matrizes em área de coleta de sementes

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Mapeamento e descrição de árvores matrizes em área de coleta de sementes

RESUMO – A seleção de plantas matrizes é importante para identificar, localizar e delimitar áreas de coleta de sementes florestais. O presente estudo apresenta uma lista de plantas matrizes de *Anadenanthera colubrina* e *Cenostigma pyramidale*, bem como localização detalhada de cada matriz. Para cada planta marcada, os seguintes detalhes foram coletados: dados dendrometricos, coordenadas geográficas e tipo de solo. Foram marcadas 60 plantas matrizes, em sete municípios: Uberlândia-MG e Planaltina-DF no Cerrado; Corumbá-MS no Pantanal; Canindé de São Francisco-SE, Lagoa Grande PE, Petrolina-PE, Juazeiro-BA na Caatinga. Foram categorizados 10 tipos de solos. A altura de plantas de *A. colubrina* e *C. pyramidale* variam de 4,0 a 42,5 m e 3,0 a 10,0 m, respectivamente. As árvores matrizes marcadas na Caatinga apresentaram menor altura total em relação às árvores do Pantanal e do Cerrado. Este trabalho pode ser utilizado como referência para futuros estudos de campo de *A. colubrina* e *C. pyramidale* e em excursões de coleta de sementes de *A. colubrina* e *C. pyramidale*.

Termos para indexação: Caatinga, *Anadenanthera colubrina*, *Poincianella pyramidalis*

Mapping and description of mother-trees on seed collection areas

ABSTRACT- The selection of mother-plants is important for identifying, locating and delimiting areas for seed collection of forest species. This study presents a list of mother-trees of *Anadenanthera colubrina* and *Cenostigma pyramidale*, as well as their detailed location. For each marked plant, the following details were collected: dendrometric data, geographic coordinates and soil type. Sixty plants were registered in

seven municipalities in Brazil: Uberlândia-MG and Planaltina-DF from Cerrado; Corumbá-MS from Pantanal; Canindé de São Francisco-SE, Lagoa Grande-PE, Petrolina-PE and Juazeiro-BA from Caatinga. The plant height of *A. colubrina* and *C. pyramidale* ranges from 4 to 42.5 m and 3 to 10 m, respectively. Ten types of soils were categorized. The mother-trees marked in the Caatinga presented lower total height in relation to the Pantanal and Cerrado trees. This work can be used as reference for future field studies of *A. colubrina* and *C. pyramidale* and in seed collection excursions of *A. colubrina* and *C. pyramidale*.

Index terms: Caatinga, *Anadenanthera colubrina*, *Cenostigma pyramidale*

1.1. Introdução

A marcação de plantas matrizes é importante para a coleta de sementes. Informações como fenofase, altura da planta, coordenadas geográficas, herborização de material, descrição dos indivíduos e do local de coleta são fundamentais para o sucesso desta atividade. A frutificação de algumas espécies na Caatinga ocorre durante todo o ano (Meiado et al., 2012), sendo que a época ideal para coleta dos frutos maduros varia de acordo com a espécie e a localização (Matias et al., 2014). Determinadas condições ambientais são favoráveis para uma regularidade na produção dos frutos em certos meses de cada ano. Portanto, a ida prévia ao campo é importante para a obtenção de informações a respeito do fenograma de frutificação de cada espécie (Silva e Dantas, 2012).

Anadenanthera colubrina (Vell.) Brenan e *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis são representantes da família Fabaceae, subfamília

Caesalpinioideae (LPWG, 2017), sendo a primeira distribuída em quase todo território brasileiro e a segunda endêmica da Caatinga (Maia, 2012). Os folículos de *A. colubrina* quando maduros apresentam uma coloração marrom escura brilhante, característica importante para determinar o momento ideal de coleta dos frutos maduros. Essa espécie é indicada para recuperação de áreas degradadas, arborização urbana e paisagismo. Árvore de médio a grande porte, apresenta caducifólia entre os meses de setembro a dezembro. A coleta é feita com o auxílio de podão e lona plástica colocada na base da copa (Maia, 2012; Matias et al., 2014). *Cenostigma pyramidale* é muito utilizada na região Nordeste do Brasil devido ao seu potencial no reflorestamento, forrageiro, madeireiro e medicinal, árvore de médio porte, apresenta flores amarelas e dispostas em racemos (Maia, 2012). Os legumes de *C. pyramidale* apresentam coloração castanho-escuro quando maduros. Por apresentar deiscência explosiva, é recomendado a coleta dos frutos com coloração entre marrom e castanho-claro, sendo feita manualmente antes da dispersão diretamente na árvore ou com o auxílio de podão (Matias et al., 2014).

As necessidades de conhecimento de áreas produtivas pelo pequeno produtor e as políticas públicas relacionadas ao setor produtivo de sementes nativas ainda são poucos exploradas (Freire et al., 2017), sendo imprescindível não só o apoio financeiro, mas também um trabalho de capacitação básica para a coleta, secagem e beneficiamento dessas sementes.

O custo de trabalho para coleta de sementes varia entre países e fornecedores (Schmidt, 2007). A comercialização de sementes nativas poderá auxiliar na melhoria das condições socioeconômicas da população local e conservar a Caatinga. O custo médio do quilo de sementes de *A. colubrina* e *C. pyramidale* fica em torno de R$ 21,66 e R$ 29,16, respectivamente (Espírito Santo et al., 2010). A marcação das plantas matrizes para posterior coleta de frutos é um dos passos iniciais nessa cadeia produtiva.

Dessa forma, o presente estudo apresenta uma lista de plantas matrizes das espécies *A. colubrina* e *C. pyramidale*, bem como localização detalhada de cada matriz incluindo fenofases na época da marcação.

1.2. Material e métodos

Árvores adultas de *A. colubrina* e *C. pyramidale* foram selecionadas para posterior coleta de sementes. Após a seleção, foi feito o georreferenciamento dos indivíduos e a identificação com placas de metal em cada matriz selecionada, bem como a determinação do seu estado fenológico no momento da marcação. Para cada planta marcada, os seguintes dados dendrométricos foram registrados em fichas de campo: altura total da planta, DAP (diâmetro à altura do peito, 1,30 m), DAB (diâmetro à altura de 30 cm do solo), coordenadas geográficas, município de ocorrência e tipo de solo.

Um total de sete municípios foram selecionados para este estudo: Uberlândia-MG (UDI) e Planaltina-DF (BSB) no Cerrado; Corumbá-MS (CMG) no Pantanal; Canindé de São Francisco-SE (CSF), Lagoa Grande-PE (LGP), Petrolina-PE (PNZ), Juazeiro-BA (JUA) na Caatinga (Figura 1). Para caracterização dos tipos de solos foi utilizada a versão *offline* do software "Carolus" (IBGE - Instituto Brasileiro de Geografia e Estatística, 2006; Siqueira et al., 2012).

A medição da altura das árvores matrizes foi realizada com o auxílio do Telêmetro (Smart Tools co.), um aplicativo auxiliar para dispositivo móvel (Santana et al., 2015). Foram consideradas árvores de *A. colubrina* e *C. pyramidale* com DAB e DAP com circunferência a partir de 12 cm (Kurihara et al., 2005). Essa medida foi realizada com o auxílio de trenas diamétricas (cm), permitindo-se ler o perímetro

(circunferência a altura do peito – CAP), em seguida transformando através da relação DAP = CAP/π (Silva e Paula Neto, 1979).

Para o georreferenciamento das matrizes foi utilizado o GPS Essentials, um aplicativo auxiliar para dispositivo móvel. Identificamos uma exsicata de cada local para cada espécie, exceto as matrizes de *A. colubrina* marcadas em Lagoa Grande-PE e *C. pyramidale* em Canindé de São Francisco-SE, pois as plantas estavam em fase vegetativa, e depositamos na coleção do Herbário Trópico Semiárido, na Embrapa Semiárido sob números de vouchers HTSA 6343; 6342; 6341; 6340 para *A. colubrina* e HTSA 7222 para *C. pyramidale*.

1.3. Resultados e discussão

Foram marcadas 60 plantas matrizes, sendo 35 de *A. colubrina* e 25 de *C. pyramidale* entre os anos de 2015 e 2016. Os indivíduos de *Anadenanthera colubrina* estão localizadas nos municípios de Uberlândia-MG e Planaltina-DF (Cerrado); Corumbá-MS (Pantanal); Petrolina-PE e Lagoa Grande-PE (Caatinga). A fenofase predominante durante a marcação foi a frutificação, sendo encontrada apenas 10 indivíduos em fase vegetativa. A altitude onde essas matrizes foram marcadas varia entre 180 e 960 m, a altura de *A. colubrina* varia entre 4,0 e 42,5 m. Todas as matrizes marcadas no Pantanal e Cerrado tinham alturas superiores a 10 m, enquanto que, na Caatinga, apenas quatro plantas ultrapassaram esse patamar. O DAP e DAB médio de todas as populações de *A. colubrina* foi de 42 e 51 cm, respectivamente, variando entre 14,7 e 116,8 cm para o DAP e entre 12,1 e 140,9 cm para o DAB (Tabela 1).

Durante a marcação das matrizes de *C. pyramidale* foi observado as fenofases de floração, frutificação e vegetativa. A altura dessas árvores varia entre 3 e 10 m. O DAP

e DAB médio de *C. pyramidale* foi de 17 e 24 cm, respectivamente, variando entre 7,0 e 38,6 cm para o DAP e entre 9,9 e 48,3 cm para o DAB (Tabela 1).

Na Caatinga, a floração de *A. colubrina* ocorre após o início das primeiras chuvas, seguida da frutificação entre os meses de abril a agosto, sendo que essa fenofase fica concentrada principalmente na estação seca (Barbosa et al., 1989; Griz e Machado, 2001). Após o início do período chuvoso, há o rebrotamento de *C. pyramidale*, geralmente entre os meses de dezembro e março, a floração ocorre entre os meses de janeiro e abril, seguida pela frutificação que vai até agosto (Barbosa et al., 1989; Leite e Machado, 2010; Maia, 2012). Informações sobre crescimento de espécies arbóreas são fundamentais para estruturação de programas de manejo. Em árvores de *A. colubrina* no Pantanal mato-grossense, o tempo médio para atingir 40 cm de diâmetro do caule é de, no mínimo, 55 anos (Mattos e Seitz, 2008). A análise diamétrica é importante para classificar como os indivíduos estão distribuídos no ambiente, por exemplo, indivíduos de menor diâmetro (3.0-5.0 cm) podem ser classificados na categoria de plântulas, informando sobre o sucesso do recrutamento das mesmas (Monteiro et al., 2006).

Foram categorizados seis tipos de solos nos ambientes em que as matrizes de *A. colubrina* foram marcadas: Latossolo vermelho distroférrico e distrófico; Vertissolo ebânico órtico; Planossolo nátrico órtico; Lastossolo vermelho-amarelo eutrófico; Argissolo vermelho-amarelo eutrófico. O solo presente em Canindé do São Francisco-SE é o Planossolo háplico eutrófico e em Juazeiro-BA o Latossolo amarelo distrófico. A altitude onde esses solos foram identificados variam entre 110 e 469 m.

Os Latossolos correspondem a 1/3 da superfície do território brasileiro, variando de fortemente a bem drenados, ou seja, a água é removida rapidamente do perfil, embora existam solos desta categoria que apresentam drenagem moderada ou até mesmo imperfeita, como é o caso dos Latossolos amarelos que tem como principal

característica a coesão (Ker, 1997; Santos et al., 2013). Geralmente esses solos são muito ácidos e apresentam saturação por base de média a alta, principalmente em regiões semiáridas. As matrizes de *C. pyramidale* marcadas em Juazeiro-BA estão presentes em Latossolos amarelos distróficos, solos com saturação por bases baixa. Os Latossolos vermelhos distroférricos e distróficos apresentam uma saturação por base baixa, nestes solos foram marcadas todas as matrizes de *A. colubrina* no Cerrado. Todas as matrizes de *A. colubrina* marcadas em Pernambuco, estavam em Latossolos vermelho-amarelos eutróficos, solos intermediários para Argissolos e Cambissólicos (Santos et al., 2013), com exceção de apenas 5 matrizes que estavam em Argissolo vermelho-amarelo eutrófico.

Os Planossolos compreendem solos minerais imperfeitamente ou mal drenados, geralmente com uma alta concentração de argila. Ocorrem preferencialmente em áreas planas, onde as condições anuais favorecem o acúmulo de água como, por exemplo, no Pantanal. Uma matriz de *A. colubrina* foi marcada em Planossolos nátrico órtico. As matrizes de *C. pyramidale* em Canindé do São Francisco-SE foram marcadas em Planossolos háplicos eutróficos, solos com saturação por bases alta. Apenas uma matriz de *A. colubrina* foi marcada em Vertissolo ebânico órtico (Santos et al., 2013).

As maiores plantas matrizes estavam presentes em Planossolos Vermelhos distróficos e distroférricos. As matrizes marcadas na Caatinga apresentaram menor altura. Possivelmente isso se deve ao menor aporte de chuvas, típico do clima semiárido. Além disso, os Latossolos Vermelho-Amarelo Eutrófico e Amarelo Distrófico, presente em mais da metade das matrizes marcadas na Caatinga (Tabela 1), provavelmente dificultem o crescimento das raízes, seja por adensamento ou compactação.

1.4. Considerações finais

A plantas matrizes marcadas no presente trabalho poderão ser utilizadas para futuras excursões de coleta de sementes de *A. colubrina* e *C. pyramidale*. As plantas matrizes marcadas na Caatinga apresentam uma menor altura total em relação as plantas marcadas no Pantanal e Cerrado.

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REFERÊNCIAS

BARBOSA, D.C.A.; ALVES, J.L.H.; PRAZERES, S.M.; PAIVA, A.M.A. Dados fenológicos de 10 espécies arbóreas de uma área de caatinga (Alagoinha-PE). **Acta Botanica Brasilica**, v. 3, n. 2, p. 109–117, 1989.

ESPÍRITO SANTO, F.S; SIQUEIRA FILHO, J.A.; MELO JÚNIOR, J.C.F.; GERVÁSIO, E.S.; OLIVEIRA, A.M.B. Quanto vale as sementes da caatinga? Uma proposta metodológica. **Revista Caatinga**, v. 23, n. 3, p. 137–144, 2010.

FREIRE, J.M.; URZEDO, D.I.; PINA-RODRIGUES, F.C.M. A realidade das sementes nativas no Brasil: Desafios e oportunidades para a produção em larga escala. **Seed News**, p. 24–28, 2017. Disponível em: <https://www.researchgate.net/publication/322581933_A_realidade_das_sementes_nativas_no_Brasil_Desafios_e_oportunidades_para_a_producao_em_larga_escala>.

GRIZ, L.M.S.; MACHADO, I.C.S. Fruiting phenology and seed dispersal syndromes in caatinga, a tropical dry forest in the northeast of Brazil. **Journal of Tropical Ecology**, v. 17, n. 2, p. 303–321, 2001.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Novo Sistema Brasileiro de Classificação de Solos (SBCS)**. Disponível em: <http://mapas.ibge.gov.br/solos/viewer.htm>. Acesso em: 9 maio 2017.

KER, J.C. Latossolos do Brasil: uma revisão. **Revista Geonomos**, v. 5, n. 1, p. 17–40, 1997.

KURIHARA, D.L.; IMAÑA-ENCINAS, J.; ELIAS DE PAULA, J. Levantamento da arborização do campus da universidade de brasilia. **Cerne**, v. 11, n. 2, p. 127–136, 2005.

LEITE, A.V.L.; MACHADO, I.C. Reproductive biology of woody species in Caatinga, a dry forest of northeastern Brazil. **Journal of Arid Environments**, v. 74, n. 11, p. 1374–1380, 2010.

LPWG. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny – The Legume Phylogeny Working Group (LPWG). **Taxon**, v. 66, n. 1, p. 44–77, 2017. Disponível em: <http://www.ingentaconnect.com/content/10.12705/661.3>.

MAIA, G.N. **Caatinga: árvores e arbustos e suas utilidades**. Fortaleza: Printcolor Gráfica e Editora, 2012.

MATIAS, J.R.; OLIVEIRA, G.M.; DANTAS, B.F. Colheita e beneficiamento de algumas espécies da Caatinga. **Informativo Abrates**, v. 24, n. 2, p. 22–26, 2014.

MATTOS, P.P.; SEITZ, R.A. Growth dynamics of *Anadenanthera colubrina* var. *cebil* and *Tabebuia impetiginosa* from Pantanal Mato-grossense, Brazil. **Ciencia Florestal**, v. 18, n. 4, p. 427–434, 2008.

MEIADO, M.V.; SILVA, F.F.S.; BARBOSA, D.C.A.; SIQUEIRA-FILHO, J.A. Diaspores of the Caatinga: a review. *In*: SIQUEIRA-FILHO, J.A. (Org.). **Flora of the Caatingas of the São Francisco River: Natural History and Conservation**. Andrea Jak. Rio de Janeiro: [s.n.], 2012, p. 306–365.

MONTEIRO, J.M.; ALMEIDA, C.F.C.B.R.; ALBUQUERQUE, U.P.; LUCENA, R.F.P.; FLORENTINO, A.T.N.; OLIVEIRA, R.L.C. Use and traditional management of *Anadenanthera colubrina* (Vell.) Brenan in the semi-arid region of northeastern Brazil. **Journal of Ethnobiology and Ethnomedicine**, v. 2, n. 6, p. 1–7, 2006.

SANTANA, O.A.; SANTOS, N.K.B.; SILVA, M.M; MORAIS, R.L.; ENCINAS, J.I. Árvores potenciais a danos urbanos: manejo através da tecnologia, educação e mobilização social. **Revista tecnologia e sociedade**, v. 11, n. 23, p. 71–88, 2015.

SANTOS, H.G.; JACOMINE, P.K.T.; ANJOS, L.H.C.; OLIVEIRA, V.A.; LUMBRERAS, J.F.; COELHO, M.R., ... OLIVEIRA, J.B. **Sistema brasileiro de classificação de solos**. 3. ed. Brasília: Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), 2013.

SCHMIDT, L. **Tropical Forest Seed**. Springer S. Heidelberg: Springer-Verlag Berlin

Heidelberg, 2007.

SILVA, F.F.S.; DANTAS, B.F. Coleta e beneficiamento de sementes da caatinga. **Informativo ABRATES**, v. 22, n. 3, p. 16–19, 2012. Disponível em: <http://www.alice.cnptia.embrapa.br/alice/handle/doc/944649>.

SILVA, J.A.A.; PAULA-NETO, F. **Princípios básicos de dendrometria**. Recife: Universidade Federal Rural de Pernambuco, Departamento de Ciência Florestal, 1979. Disponível em: <http://esalqlastrop.com.br/img/aulas/Apostila - Dentrometria.pdf>.

SIQUEIRA, A.A.; SANTOS FILHO, J.V.; SIQUEIRA FILHO, J.A. Carolus : A new system for handling electronic botanical collections. **Rodriguésia**, v. 63, n. 3, p. 715–732, 2012.

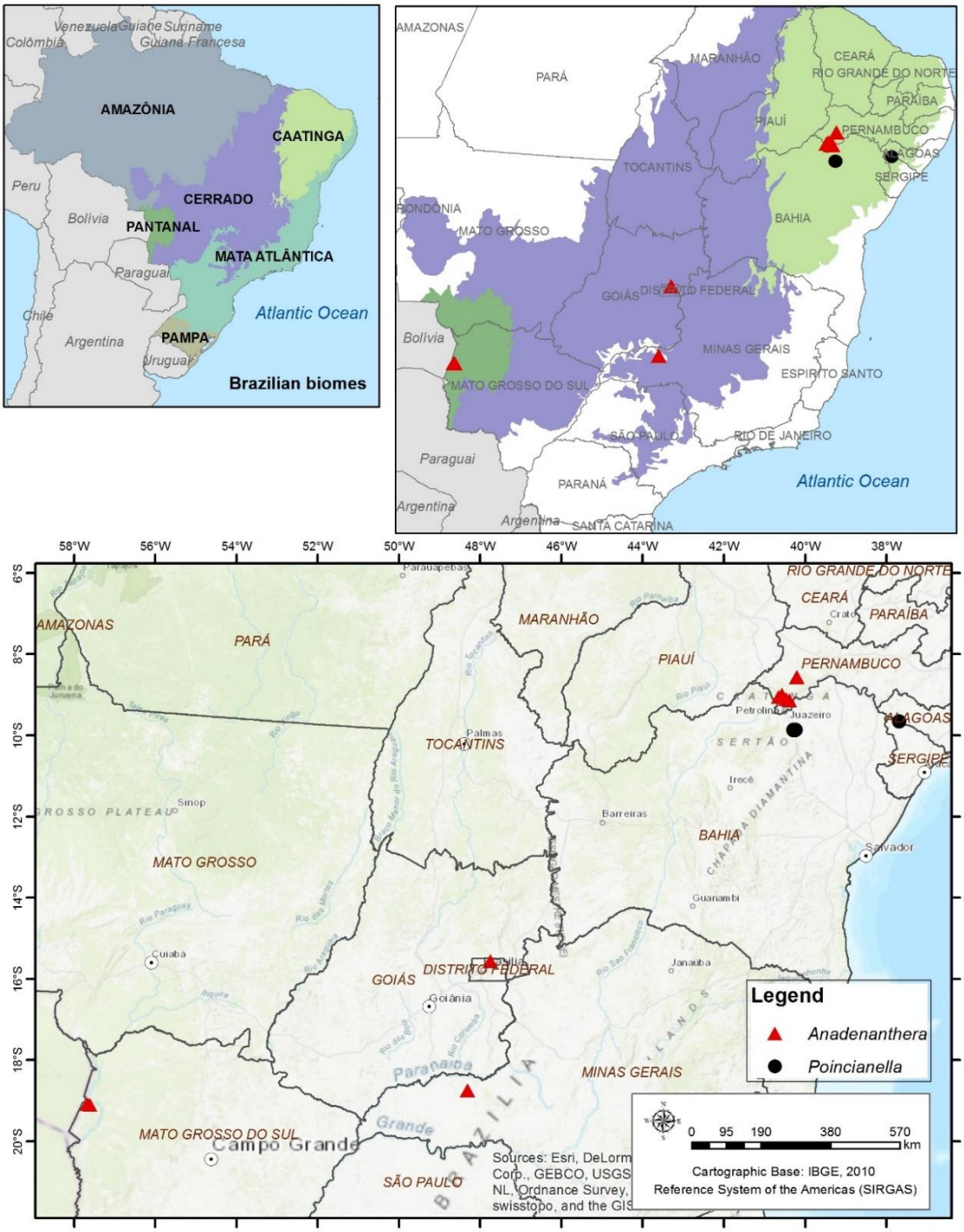

Figura 1. Localização das populações de *Anadenanthera colubrina* (Vell.) Brenan e *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis em diferentes ecossistemas brasileiros.

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285 Tabela 1. Localização e características de matrizes de *Anadenanthera colubrina* (Vell.)
286 Brenan e *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis.

Matriz	**F****	**A (m)**	**DAP**	**DAB**	**Longitude**	**Latitude**	**Altitude (m)**	**Município**	**Solo**
					Anadenanthera colubrina				
1*	Fr	31	69,6	81,8	783041	7924521	689	UDI	LVdf
2	Vg	12,3	44,3	61,1	783065	7924503	684	UDI	LVdf
3	Vg	24	74,0	77,0	783070	7924513	683	UDI	LVdf
4	Vg	15	61,9	76,1	783104	7924495	684	UDI	LVdf
5	Vg	10,7	46,0	43,0	783114	7924480	689	UDI	LVdf
8	Vg	12,3	49,2	60,0	783080	7924420	709	UDI	LVdf
9	Fr	42,5	116,8	140,9	783810	7924377	693	UDI	LVdf
21	Fr	18	38,5	44,5	205437	8277724	950	BSB	LVd
23	Vg	20,3	22,8	30,2	205468	8277754	950	BSB	LVd
24	Fr	13,6	47,9	72,1	205423	8277493	940	BSB	LVd
25	Vg	15,8	35,7	44,7	205350	8277442	960	BSB	LVd
26	Vg	23,6	59,4	67,4	205385	8277338	948	BSB	LVd
27	Fr	13,2	63,7	78,9	435420	7888237	180	CMG	VEo
30	Fr	17	47,5	44,8	429354	7890509	180	CMG	SNo
33	Fr	8,6	29,0	46,9	338274	8993224	401	PNZ	LVAe
34	Fr	5	31,1	39,8	338249	8993204	389	PNZ	LVAe
36	Fr	8	22,9	31,6	338207	8993238	400	PNZ	LVAe
37	Fr	6,5	27,6	40,1	338216	8993204	401	PNZ	LVAe
38	Fr	6	21,7	24,2	338188	8993178	400	PNZ	LVAe
40	Vg	5	24,2	31,2	338193	8993188	399	PNZ	LVAe
54	Fri	13	49,4	51,4	327545	9005694	379	PNZ	LVAe
55	Fri	11	43,9	46,6	327511	9005668	370	PNZ	LVAe
110	Fri	7	71,9	86,9	347526	8989876	377	PNZ	PVAe

Matriz	F**	A (m)	DAP	DAB	Longitude	Latitude	Altitude (m)	Município	Solo
111	Fri	7	23,7	27,2	347551	8989794	376	PNZ	PVAe
112	Fri	8	14,7	23,0	347540	8989798	380	PNZ	PVAe
114	Fri	5	14,6	12,4	347582	8989794	381	PNZ	PVAe
115	Fri	6	32,2	40,6	347583	8989790	384	PNZ	PVAe
131	Fr	6	27,1	32,5	318366	8999252	420	PNZ	LVAe
132	Fr	7	28,7	43,2	318291	8999238	418	PNZ	LVAe
133	Fr	4	26,9	12,1	318280	8999270	419	PNZ	LVAe
71	Fri	8	23,2	35,8	366941	9052800	409	LGP	LVAe
76	Vg	13	43,7	48,2	366968	9052792	415	LGP	LVAe
84	Fri	12	61,2	109,9	367421	9053004	407	LGP	LVAe
88	Fri	6	31,8	38,2	367579	9053648	408	LGP	LVAe
90	Fri	6	43,4	50,3	367592	9053558	399	LGP	LVAe
Cenostigma pyramidale									
13	Vg	7,2	16,5	25,5	644765	8932440	110	CSF	SXe
14	Vg	7,6	9,9	11,8	644589	8932100	119	CSF	SXe
16	Vg	8,5	19,8	22,2	644216	8931606	192	CSF	SXe
60	Fl	9	38,6	45,7	359825	8908774	469	JUA	LAd
61*	Fl	10	25,9	48,3	359856	8908712	462	JUA	LAd
62	Fr	6	25,1	35,4	359729	8908754	464	JUA	LAd
63	Fr	5	14,7	23,2	359707	8908730	461	JUA	LAd
64	Fl	5	13,1	22,2	359751	8908514	464	JUA	LAd
65	Fl	6	14,3	16,6	364403	8908762	457	JUA	LAd
66	Fl	5	11,7	16,6	364405	8908910	456	JUA	LAd
79	Fl	3	20,7	22,6	364532	8908866	452	JUA	LAd
119	Vg	5,5	8,6	42,3	364469	8908828	452	JUA	LAd
120	Vg	5,5	21,1	24,5	364357	8908630	450	JUA	LAd
121	Fl	4	23,4	25,6	364328	8908634	454	JUA	LAd
122	Fl	4	16,7	24,9	364554	8908828	452	JUA	LAd

Matriz	F**	A (m)	DAP	DAB	Longitude	Latitude	Altitude (m)	Município	Solo
144	Fr	4	21,9	26,3	348764	8921480	457	JUA	VCo
145	Fr	3	13	24,5	348861	8921626	452	JUA	VCo
146	Fr	3,5	10,5	12,2	345619	8935114	422	JUA	VCo
147	Fr	4	12,3	9,9	345595	8935174	420	JUA	VCo
148	Fr	4	7,2	9,9	345683	8935124	419	JUA	VCo
149	Fr	4	13,9	14,8	326665	8926304	413	JUA	CXve
150	Fr	6	19,2	30,6	326632	8926392	430	JUA	CXve
151	Fr	6	27,7	26,6	326458	8922958	423	JUA	CXve
152	Fr	3,5	7	16,8	326460	8922928	421	JUA	CXve
153	Fr	6	15,1	30,9	326482	8923054	418	JUA	CXve

*Material depositado na coleção do Herbário do Trópico Semiárido (HTSA), sob números de vouchers HTSA 6343; 6342; 6341; 6340 para *A. colubrina* e HTSA 7222 para *C. pyramidale*.

** F= Fenologia; Fl=Floração; Fr=Frutificação; Fri=Frutificação (imaturo); Vg=Vegetativa; A= Altura total; DAP (diâmetro à altura do peito); DAB (diâmetro à altura da base); UDI=Uberlândia-MG; BSB=Planaltina-DF; CMG=Corumbá-MS; PNZ=Petrolina-PE; LGP=Lagoa Grande-PE; CSF=Canindé do São Francisco-SE; JUA=Juazeiro-BA; LVdf=Latossolo vermelho distroférrico; LVd=Latossolo vermelho distrófico; LVAe=Latossolo vermelho-amarelo eutrófico; LAd=Latossolo amarelo distrófico; VEo=Vertissolo ebânico órtico; VCo=Vertissolo Crômico Órtico; SNo=Planossolo nátrico órtico; SXe=Planossolo háplico eutrófico; PVAe=Argissolo vermelho-amarelo eutrófico; CXve=Cambissolo Háplico Ta Eutrófico.

CAPÍTULO 2 - Plântulas de espécies adaptadas à floresta seca retomam o crescimento após dessecação parcial. *Seedlings of dry forest adapted species resume growth after nearly total desiccation*.

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Abstract: Desiccation tolerance (DT) in germinated seeds is directly linked to the success of seedling survival of seasonally dry tropical forests (SDTF) species. The objective of this study was to evaluate whether the seeds of *Anadenanthera colubrina* and *Cenostigma pyramidale* present post-germinative DT and until what stage of seedling development the tolerance persists. *Anadenanthera colubrina* and *C. pyramidale* seedlings of different sizes were separated into four Initial Root Length (IRL) categories and dried for 24 and 72 h. The seedling survival was evaluated at 7 and 14 days after rehydration (DAR). *Anadenanthera colubrina* and *C. pyramidale* were tolerant to post-germination desiccation. The survival rate of the *A. colubrina* seedlings with IRL between 7.00 and 10.99 mm that were dried for 24 h was 70% at 7 DAR. The survival rate of the *C. pyramidale* seedlings with IRL between 1.00 and 6.99 mm that were dried for 72 h was 96% at 7 DAR. At 14 DAR, *C. pyramidale* seedlings longer than 6.99 mm when desiccated were dead. As a survival strategy, some seedlings of both species lose the primary root and emit adventitious roots after desiccation. The survival of seedlings of *A. colubrina* and *C. pyramidale* to desiccation has a direct effect on the recruitment of SDTF species, specially during dry spells or drought years.

Keywords: Fabaceae; *Poincianella pyramidalis*; recruitment; rehydration; semi-arid; desiccation tolerance.

Key Message: Recently germinated seeds of the dry forest specialist trees, *Anadenanthera colubrina* and *Cenostigma pyramidale*, are tolerant to desiccation, losing the primary root and emitting adventitious roots as a survival strategy.

2.1. Introduction

Desiccation tolerance (DT) is observed in several organisms that are able to survive extreme water loss (Dekkers et al. 2015), such as algae (Carniel et al. 2016), bryophytes (Stark 2017), pteridophytes (Pampurova and Van Dijck 2014), and angiosperms (Giarola et al. 2017). DT also occurs in pollen grains (Marks et al. 2014) and seeds during development (Dekkers et al. 2015; Leprince et al. 2016). At the beginning of development, seeds are intolerant to desiccation, due to cell expansion and intense metabolic activity until maturation. At the end of development, metabolism is reduced and DT of orthodox seeds starts (Nonogaki et al. 2010). Mature orthodox seeds are able to tolerate desiccation at low water content (WC), around 5%, and maintain their viability, while recalcitrant seeds fail to germinate when desiccated beyond critical WC (Pammenter and Berjak 2000; Mayrinck et al. 2019). The post-germinative DT of seedlings of these orthodox seed species acts as a model for understanding the mechanisms of stress tolerance and how to improve development (e.g. germination and growth of seedlings) of recalcitrant seeds and non-DT species to environmental drought conditions (Masetto et al. 2015; Barak and Farrant 2016).

According to most post-germinative behavior models, following germination DT is lost; however, in seedlings of some species, DT may be re-induced and followed by the resumption of growth after subsequent rehydration to their initial WC before desiccation (Lyall et al. 2014). Most of the DT tolerant seedling are tree species, such as *Sesbania virgata* (Cav.) (Pers.), *Bauhinia forficata* Link and *Senna multijuga* (Rich.) Irwin et Barn., dryland specialist legumes, which have the ability to tolerate desiccation only during initial development, immediately after root protrusion (Masetto et al. 2015; Rodrigues et al. 2015; Ribeiro et al. 2016). In addition, literature points out Velloziaceae (*Xerophyta viscosa* Baker) and Bignoniaceae (*Handroanthus impetiginosus* [Mart ex DC.]) dryland species, which also

present same response (Lyall et al. 2014; Martins et al. 2015). To enhance survival of young seedlings, these can be primed in polyethylene glycol solution or treated with growth regulators (*i.e.*, abscisic acid) prior to desiccation (Vieira et al. 2010; Masetto et al. 2015; Rodrigues et al. 2015).

In environments with seasonal droughts, a set of physiological strategies are necessary to guarantee the conservation and evolution of local biodiversity (de Lima et al. 2012; Méndez-Alonzo et al. 2013). The legumes present several water-saving mechanisms, such as deciduous leaves, different densities of the wood and variations of water potential of leaves (de Lima et al. 2012; Reyes-García et al. 2012). *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis is a Fabaceae endemic to *Caatinga*, and *Anadenanthera colubrina* (Vell.) Brenan and is a dryland specialist species, native to *Caatinga* with a wide distribution in different ecosystems, mainly in seasonally dry tropical forests (SDTF) of South America (Albuquerque et al. 2007). The Pleistocene Arc Theory explains the distribution of legumes in non-contiguous fragments of SDTF in South America, which comprises the Brazilian ecosystems *Caatinga*, *Cerrado*, *Pantanal*; the *Chacos* in Argentina and Paraguay and dry inter-Andean valleys in Peru and Ecuador. *Anadenanthera colubrina* presents strong evidence of a more continuous distribution that was interrupted by the climate change after Pleistoscene (Prado and Gibbs 1993; Mogni et al. 2015).

SDTF are environments characterized by long periods of a well defined dry season (Locosselli et al. 2016). In the tropics, more than 50% of the SDTF area is found in South America, with the *Caatinga* having the largest contiguous territorial extension, with more than 800,000 km² (Miles et al. 2006; Silva and Souza 2018). This SDTF, located in Northeastern Brazil, has a rich vegetal biodiversity (Queiroz et al. 2017) and a dry period of often more than four months with low volume and poorly distributed rainfall (Santiago et al. 2017). Understanding the ecological mechanisms operating in this environment is important

to assist in priority conservation actions, especially in high biodiversity SDTF areas and those with risk of anthropic activity (Miles et al. 2006). In SDTF, non-dormant Fabaceae seeds germinate up to twice as fast as other taxa and are highly synchronized with their environment. This adaptation provides a competitive advantage in low rainfall environments, where small rainfall events are fundamental for maintaining a viable plant population and germination from the soil seed bank (Vargas et al. 2015; Silva et al. 2017).

To understand the set of mechanisms favoring the post-germinative DT existence in SDTF species, it is necessary to evaluate, in addition to seedling survival, the moisture variation during desiccation and rehydration and the resumption of post-desiccation growth. Most post-germinative DT studies do not evaluate seedlings with primary roots length longer than 5 mm before desiccation, neither do they study different time length of desiccation to understand until which stage of seedlings development DT persists (Masetto et al. 2015; Rodrigues et al. 2015; Ribeiro et al. 2016). In the present study, we tested three hypotheses: (1) SDTF specialists trees, show seedling DT after germination. (2) Root length prior to desiccation will influence the survival of seedlings. (3) Desiccation time length will affect DT.

2.2. Materials and methods

Fruit harvesting and seed processing

Seeds of *A. colubrina* and *C. pyramidale* were harvested in July 2016 at different sites of *Caatinga* SDTF with some anthropogenic disturbance. The vegetation of both sites is classified as Steppic Savannah, with a hot and dry climate classified as BSh, semi-arid where the average temperature is more than 18 °C and less than 700 mm of annual precipitation (SEI 1998; Alvares et al. 2013).

Follicles of *A. colubrina* were harvested from six parent trees, in Lagoa Grande, Pernambuco, Brazil (8°34′01.00″S, 40°12′32.00″O and 409 m asl), with a predominance of

Red-Yellow Eutrophic Latosol soil (IBGE 2012). Legumes of *C. pyramidale* were harvested from 17 trees in Juazeiro, Bahia, Brazil (9°52′09.00″S, 40°16′42.00″W, 469 m asl), in a Dystrophic Yellow Latosol area (IBGE 2015). We identified an exsiccate for each species and deposited it in the collection of the Semi-Arid Tropic Herbarium under voucher numbers HTSA 6340 for *A. colubrina* and HTSA 7222 for *C. pyramidale*.

Fruits of *A. colubrina* and *C. pyramidale* were shade-dried, under a canvas to allow all fruits to open completely. Seeds were separated from fruit remains, branches, and seeds of other species and stored for 6 months in cloth bags in a cold and dry chamber (±10 °C/45% RH) until the beginning of the experiment. Fresh *A. colubrina* and *C. pyramidale* seeds showed 89% and 96% germination and WC was 8% and 8.1%; whilst after six months of storage, prior to DT trials, seed germination was 85% and 90%, respectively, with similar WC.

Seed germination and seedlings categorization

Seeds were sowed on germination paper moistened with distilled water 2.5 times the dry paper weight and incubated at 25 °C for *A. colubrina* and 30 °C for *C. pyramidale* and photoperiod of 12/12 h (white light with photon flux density of 30 W/m^2) light/dark for 24 hours. After this period, radicle protrusion was observed and measured using a digital caliper (0.001 mm accuracy). The seedlings were separated into four Initial Root Length (IRL) categories: 1.00–2.99 mm, 3.00–4.99 mm, 5.00–6.99 mm, and 7.00–10.99 mm (Fig. 1).

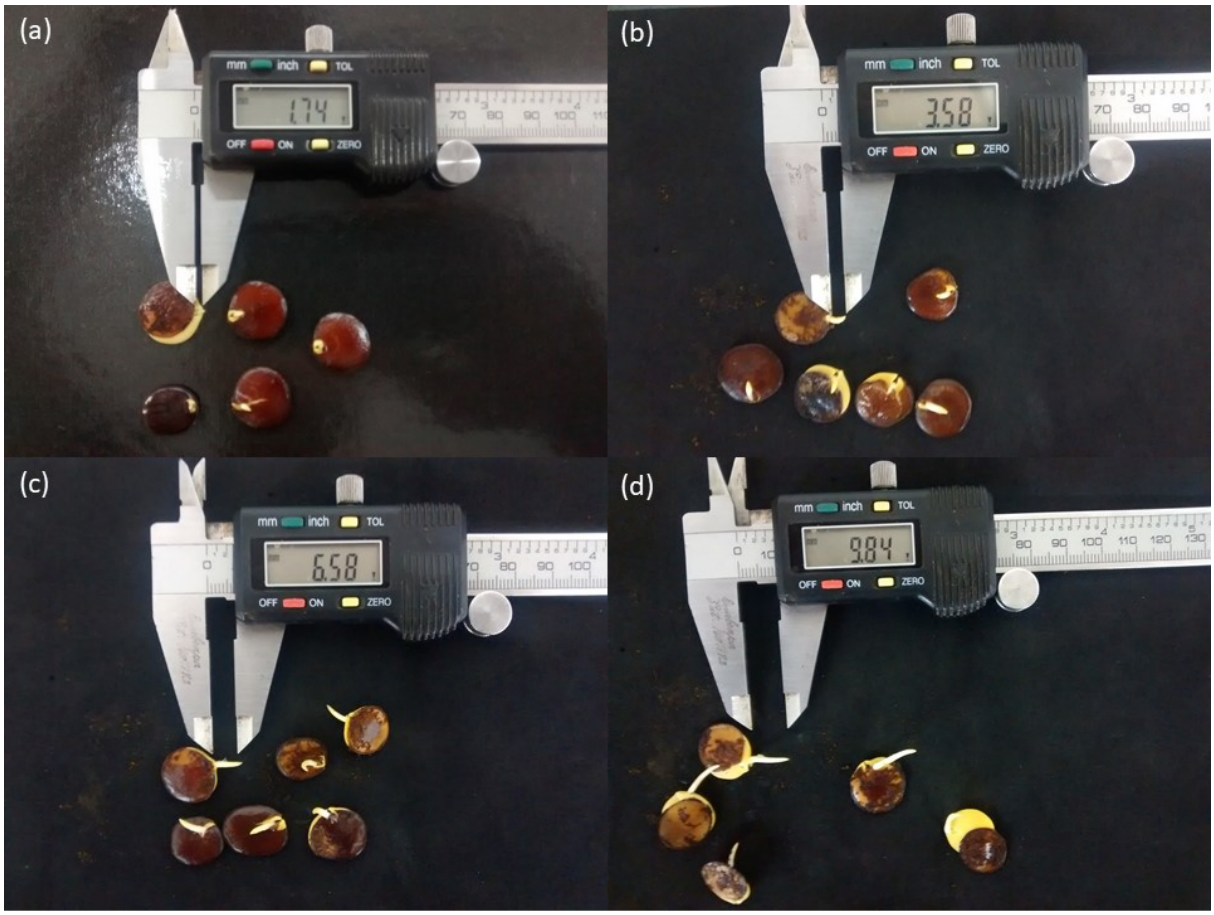


Fig. 1 The seedlings of different initial root length (IRL) categories, 1.00–2.99 mm (a), 3.00–4.99 mm (b), 5.00–6.99 mm (c), and 7.00–10.99 mm (d) before desiccation of *Anadenanthera colubrina* (Vell.) Brenan

Seedlings desiccation and rehydration

Seedlings (5 replicates of 10 seeds) from each IRL category were desiccated for 24 and 72 hours. Desiccation was performed by transferring seedlings to aluminium screens placed in germination boxes (11 × 3.5 cm) with 100 g of silica gel blue (4–8 mm) and incubated at 25 °C for *A. colubrina* and 30 °C for *C. pyramidale*, photoperiod of 12/12 h light/dark (Brazil 2013).

After 24 and 72 hours desiccation, seedlings were rehydrated and allowed to grow by transferring to germination paper moistened with distilled water and incubated at same temperature and photoperiod conditions as previously described, for 7 and 14 days.

WC before and after desiccation

WC of seed lots was determined gravimetrically by oven-drying two samples of 25 seeds (approximately 3 g) of each species at 105 ± 3 °C for 24 h (Brazil 2009). The results were expressed as a mean percentage (fresh weight basis) and this result was considered the initial WC of each individual dry seed.

Each dry seed was individually weighed prior to germination. Seedlings of each category were individually weighed after radicle protrusion, after 24 or 72h of desiccation, and after 7 or 14 days of subsequent rehydration. To evaluate changes in WC, we used the following equation adapted from Hong and Ellis (1996):

$$WC2 = 100 - \frac{M1(\,100 - WC1)}{M2} \qquad (1)$$

where:

WC2 = seedling water content after each stage of hydration, desiccation and rehydration;

WC1= quiescent seed water content;

M1= = quiescent seed mass;

M2= seedling mass, after each stage of hydration, desiccation and rehydration.

177 **Evaluation of seedling DT and development**

178 Desiccation tolerance (DT) percentage was evaluated by desiccated and rehydrated
179 seedlings which presented root development at 7 and 14 days after rehydration (DAR). At the
180 end of the experiment, at 14 DAR, final root and shoot length (FRL and SL, respectively) of
181 desiccated and rehydrated seedlings were individually measured using a digital caliper (0.001
182 mm accuracy).

183 **Statistical analysis**

184 Data were submitted to analysis of variance, with Sisvar statistical program (Ferreira
185 2014) and differences among treatments were explored by Tukey test at 0.05 probability
186 level.

187

188 2.3. Results

189

190 Prior to the DT experiments, seeds of *A. colubrina* and *C. pyramidale* had, respectively,
191 8% and 8.1% WC. First seeds germinated of *A. colubrina* and *C. pyramidale* occurred after
192 17h of hydration, reaching around 70 and 60% WC, respectively. Seedlings of *A. colubrina*
193 with shorter primary roots lost more water during desiccation than those with longer roots.
194 For example, the seedlings with IRL 1.00-2.99 mm reached 33 and 6.7% WC after 24 h and
195 72 h of desiccation respectively, while the 7.00-10.99 mm IRL seedlings reached 43 and 32%
196 WC (Fig. 2a, b). After 24 h of desiccation, all seedling IRL categories of *C. pyramidale*
197 showed approximately 5% WC (Fig. 2c). Water loss was steeper after 72 h of desiccation
198 reaching 2.7% WC, in seedlings with IRL between 3.00 and 10.99 mm (Fig. 2d). After
199 rehydration, WC remained around 70% in *A. colubrina* and 80% in *C. pyramidale* for both
200 rehydration periods, 7 and 14 days (Fig. 2).

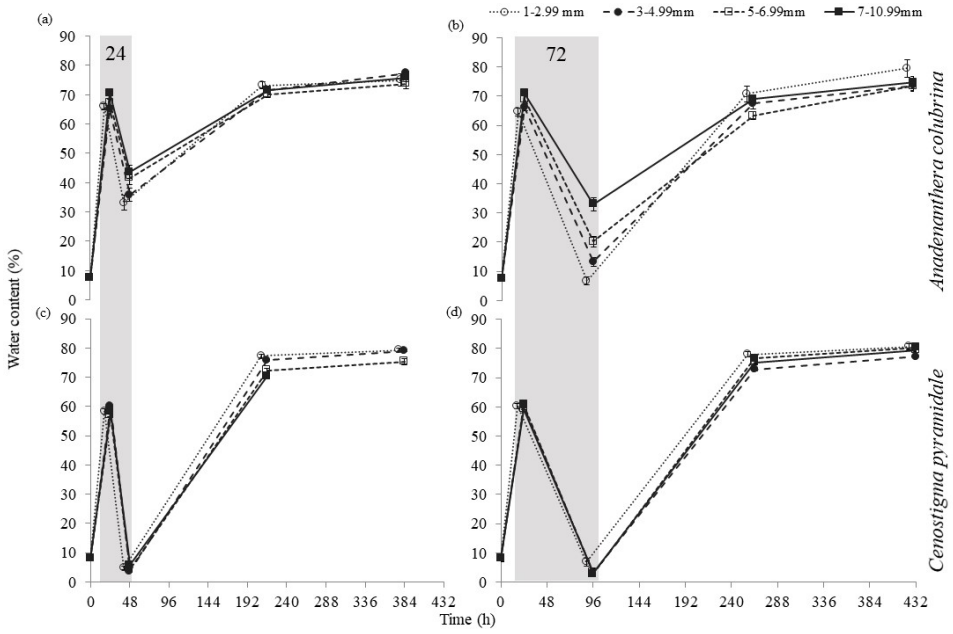


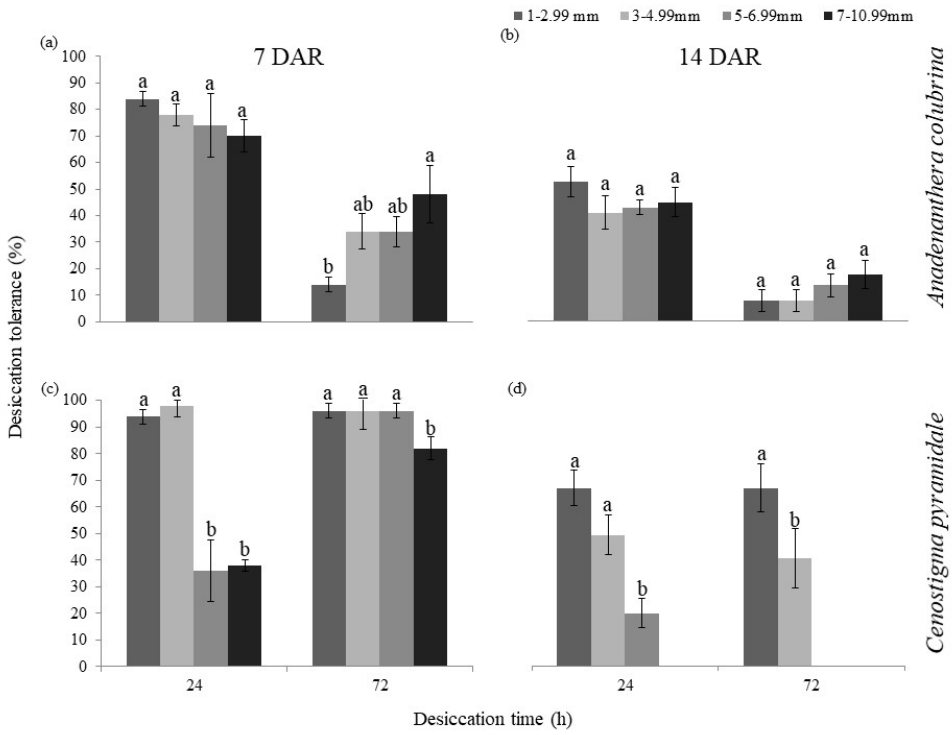
Fig. 2 Water content of quiescent seeds and seedlings of different initial root length (IRL) categories (1.00–2.99 mm, 3.00–4.99 mm, 5.00–6.99 mm, and 7.00–10.99 mm) before and after desiccation and rehydration of *Anadenanthera colubrina* (Vell.) Brenan (a and b) and *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis (c and d) seedlings at 7 and 14 days after rehydration. The grey range is the desiccation period per 24 h (a and c) and 72 h (b and d) in silica gel. Values represent mean and ± standard deviation of 50 replicates

The seedlings of both species showed evidence of seedling survival resuming growth after nearly total desiccation (Fig. 3). Nevertheless, root length of all seedlings of *A. colubrina* and *C. pyramidale* was reduced due to desiccation. As a survival strategy, some seedlings of both species lost the primary root and developed adventitious roots (Figs. 4 and 5).

The DT of *A. colubrina* seedlings ranged from 84 to 70%, desiccated for 24 hours at 7 DAR. Desiccated *A. colubrina* seedlings of all IRL categories survived after both desiccation time lengths, maintaining growth at 7 and 14 DAR. In spite of that, DT was reduced as the rehydration period advanced (Fig. 3a, b). Up to 54% of small seedlings, with IRL up to 2.99 mm, desiccated for 24 hours had survived at 14 DAR (Fig. 3b). On the other hand, when

desiccated for 72 h they survived only 14 and 8 % DT at 7 and 14 DAR, respectively (Fig. 3a, b).

Although survival *of C. pyramidale* seedlings at 7 DAR was total for seedlings with small IRL between 1.00 and 4.99 mm, desiccated for 24 h, larger seedlings, with IRL between 5.00 and 10.99 mm, fail to resume growth after desiccation, indicating, less plasticity regarding DT, when compared to *A. colubrina* seedlings. *Cenostigma pyramidale* seedlings with IRL between 1.00 and 4.99 mm desiccated for 24 hours, as well as, all categories desiccated for 72 hours, obtained DT above 80% at 7 DAR, while seedlings with IRL between 5.00 and 10.99 mm, desiccated for 24 h, presented a DT of less than 40% at 7 DAR (Fig. 3c). As the IRL increased, a reduction in DT at 14 DAR was observed. At 14 DAR, seedlings did not tolerate desiccation for 24 h when IRL was 7-10.99mm length nor for 72 h when IRL was above 5 mm length (Fig. 3d).



232 **Fig. 3** Desiccation tolerance (%) of *Anadenanthera colubrina* (Vell.) Brenan (a and b) and
233 *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis (c and d) seedlings with different
234 Initial Root Length (IRL) categories (1.00–2.99 mm, 3.00–4.99 mm, 5.00–6.99 mm, and
235 7.00–10.99 mm), desiccation time (24 h and 72 h), and rehydration after 7 (a and c) and 14
236 days (b and d). Values represent mean and ± standard deviation of five replicates. Different
237 lowercase letters indicate significant differences ($P \leq 0.05$)
238

239

240 **Fig. 4** *Anadenanthera colubrina* (Vell.) Brenan seedlings after 24 h of desiccation at 14 days
241 after rehydration. In the left seedling, four adventitious roots; in the right seedling, two
242 adventitious roots. The arrow indicates the necrotic primary roots (IRL= 7.00–10.99 mm)

Fig. 5 *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewis seedlings after 72 h of desiccation. In the left seedling, three adventitious roots, and in the right seedling, only adventitious root formation. In the central seedling, the arrow indicates the necrotic primary roots (IRL=1.00–2.99 mm) at 14 days after rehydration

Smaller roots before desiccation, showed larger growth and longer seedlings lengths after rehydration for both species (Fig. 6). Seedlings of *A. colubrina*, with IRL between 1.00 and 2.99 mm, grew in total approximately 90 mm (FRL+SL), regardless of the desiccation time, in addition, achieving the larger root lengths, up to 44 mm and 40 mm in 24 and 72 hours desiccated seedlings, respectively. Seedlings with IRL between 3.00 and 10.99 mm had their growth compromised when desiccated for a longer period (Fig. 6a, b). Although seedlings of 3.00 to 10.99 mm IRL present DT (Fig. 3a, b), the FRL values of *A. colubrina* in these categories were lower than SL values (Fig. 6a, b).

Seedlings of *C. pyramidale* with IRL between 1.00 and 2.99 mm presented the larger lengths after rehydration (FRL + SL). When the desiccation time was increased, the final development of *C. pyramidale* seedlings with IRL up to 2.99 mm was reduced. For this

260 species there was no significant difference between FRL within each desiccation period (Fig.
261 6c, d).

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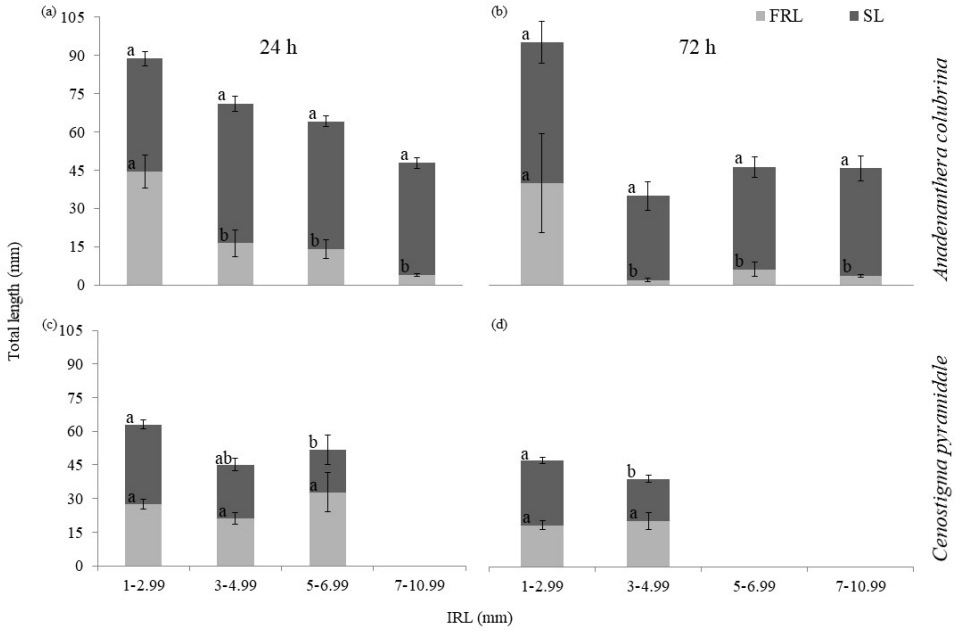
263



264

265 **Fig. 6** Seedling final root and shoot length (FRL and SL, respectively) of *Anadenanthera*
266 *colubrina* (Vell.) Brenan (a and b) and *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P.
267 Lewis (c and d) with different Initial Root Length (IRL) categories (1.00–2.99 mm, 3.00–4.99
268 mm, 5.00–6.99 mm, and 7.00–10.99 mm), desiccation time for 24 (a and c) and 72 hours (b
269 and d), and rehydration after 14 days. Values represent mean and ± standard deviation of five
270 replicates. Different lowercase letters indicate significant differences ($P \leq 0.05$)

271

272 2.4. Discussion

273

274 Forest species seedlings DT is directly linked to the success of its survival in an
275 environment where water is scarce and post-germinative DT may help the recruitment of

these species. Previous studies have shown that there is a minor lapse after germination, in which the seedlings would be tolerant to desiccation up to a certain IRL (Martins et al. 2015; Costa et al. 2016; Silva et al. 2017). For example, only 30% of seedlings of *H. impetiginosus* grown in a dry environment, with IRL above 3.5 mm tolerated desiccation (Martins et al. 2015). After desiccation, hypocotyls of tolerant seedlings remained viable and supported the development of adventitious roots in substitution of the primary root, maintaining seedling establishment (Costa et al. 2016). Our work reveals an important ecological adaptation mechanism for SDTF species, in which seedlings with larger IRL, even if desiccated for a longer period, had approximately 40% survival (Fig. 3a, b).

Evaluating the post-desiccation growth recovery may help to understand the survival mechanisms of SDTF species. Non-stressed *A. colubrina* and *C. pyramidale* seedlings reach 140 and 190 mm in length, respectively, after 14 days germination (Lima et al. 2012; Bispo et al. 2017) and desiccation survived seedling reaching at 14 DAR around 90 mm and 60 mm, respectively (Fig. 6). This and also the reduction in the number of DT seedlings from 7 DAR to 14 DAR (Fig. 3) are likely to be due to the same two reasons: (1) a degradation of the seed reserves in cotyledons (Silva and Dantas 2016), as seeds imbibition as germination progresses, and (2) the seedlings used the remaining reserves to form adventitious roots, depleting resources to continue their initial development (Bewley et al. 2013).

The desiccation by silica gel quickly reduces the WC of seedlings (Fig. 2), damaging at a cellular level, mainly the root system (Pereira et al. 2014; Rodrigues et al. 2015). The loss of cellular water in organisms non-desiccation tolerant, results in an irreversible aggregation of enzymes and other proteins leading to the disintegration of organelles. On the other hand, the ability to accumulate reducing sugars, LEA proteins, antioxidant enzymes can improve DT by preventing protein aggregation (Alpert 2006).

300 *Cenostigma pyramidale* seedlings with lower IRL were more tolerant to desiccation
301 than those with higher IRL (Fig. 3c, d), probably due to the regulation and time of
302 accumulation of protection macromolecules during and after germination (Nonogaki et al.
303 2010; Gaff and Oliver 2013), or even since seed maturation (Leprince et al. 2016). This might
304 also have led to an accumulation of protection macromolecules in more developed seedlings
305 of *A. colubrina*, evidenced by survival of all IRL seedlings (Fig 3a, b).

306 The results obtained for *Cenostigma pyramidale* confirm the indication in literature that
307 seedlings with longer IRL prior to desiccation, showed an increase in the percentage of dead
308 cells after desiccation and reduction of the nuclear size of the root meristematic region, than
309 those with shorter IRL (Martins et al. 2015; Masetto et al. 2015). However, we observed that
310 DT was maintained even in the *A. colubrina* seedlings with IRL longer than 10 mm prior to
311 desiccation (Fig. 3b). DT of initially emerged seedlings and adventitious root emission are
312 part of mechanisms of drought tolerance, supporting a reduction in the mortality of the
313 seedlings found in arid regions (Martins et al. 2015). A change the intensity at which these
314 mechanisms occur may have contributed to the widespread geographic distribution of *A.*
315 *colubrina* in distinct ecosystems with seasonal variations (Mogni et al. 2015).

316 Soon after desiccation, a slight reduction in the root size of *A. colubrina* and *C.*
317 *pyramidale* was observed, due to cell retraction during desiccation (Figs. 4 and 5). As the
318 environment becomes dryer, the number of species or accessions of a same species with DT
319 in seeds and seedlings increases (Wyse and Dickie 2017). Seedlings from *A. colubrina* seeds
320 produced in a semi-deciduous seasonal forest *Cerradão* at southeastern Brazil (1460 mm
321 annual precipitation), lost DT after germination (Castro et al. 2017). In our study, the same
322 species developed in *Caatinga* (550 mm annual precipitation) produced DT seedlings, even
323 with roots as long as 10 mm before desiccation (Fig. 3). Similar response may occur in other
324 widely distributed species. For example, in warm and dry climates, seedlings of *Copaifera*

325 *langsdorffii* tend to be more tolerant to desiccation than those collected from a cold and
326 humid environment (Pereira et al. 2017). In addition, *H. impetiginosus* seedlings of different
327 environments and precipitation patterns have a high plasticity compared to DT, contributing
328 to the distribution of the species in SDTF (Martins et al. 2015). Thus, similarly to *C.*
329 *langsdorffii* and *H. impetiginosus*, the plasticity of *A. colubrina* against DT probably explain
330 its wide distribution across the South American (Albuquerque et al. 2007; Mogni et al. 2015).
331 Thus, in the SDTF, small rainfall events are fundamental for maintaining a viable
332 population and completing the life cycle in seed banks (Sala and Lauenroth 1982). Less than
333 10mm rain events can result in only partial seed hydration. However, several of small rain
334 events in narrow time gaps (no more than 10 days apart) may help to improve seed
335 germination of legume seeds, due to seed hydration memory without compromising the
336 survival of the seedlings (Aragão et al. 2002; Lima and Meiado 2017; Silva et al. 2017). The
337 sparse and low rainfall events (300 to 800 mm $year^{-1}$) do not inhibit the germination of *C.*
338 *pyramidale* from soil seed bank; however, the survival of the seedlings is compromised after
339 40 day with no rainfall (Prado 2005; Silva et al. 2017). The rainfall seasonality and
340 stochasticity, as well as the water deficit, high temperatures, light intensity, and evaporation
341 rates occurring in the *Caatinga* SDTF contribute in making this ecosystem one of the most
342 susceptible areas to climate change (Trovão et al. 2007; IPCC 2013). The most pessimistic
343 climate change scenarios predicted rainfall volume in the *Caatinga* will reduce approximately
344 30%, with a narrower rainy season along with high temperatures (Oliveira et al. 2019) and
345 ultimately the desertification of this environment (Angelotti et al. 2009). Plant species, which
346 are not tolerant to intermittent desiccation in some point of their life cycle, may not survive in
347 their actual occuring sites and at climate change may shift their occurrence distribution in
348 other drylands or become extinct (Mogni et al. 2015).

The studied SDTF species have several mechanisms of drought tolerance. Since young plants, *A. colubrina* develop a tuberous primary root (Barretto and Ferreira 2011), which acts as a water storage mechanism. In contrast, *C. pyramidale* sheds its leaves during the dry season and rapidly sprouts and blooms after the first rains (Figueiredo et al. 2012). Our work showed that seedlings of *A. colubrina* and *C. pyramidale* survive, even when desiccated for up to 72 h. This strategy is important for seedling survival during the dry season in semi-arid environments (Gutterman and Gozlan 1998), as in SDTF. Since the soils of the *Caatinga* takes c. 7 days to dry again after a 27 mm rain (Santos et al. 2011; Moura et al. 2015), desiccation tolerance allows the establishment of these seedlings in the *Caatinga* environment even up to 10 days of no rainfall events. Post-germinative DT of *A. colubrina* and *C. pyramidale* cannot be taken alone in explaining SDTF distribution, considering that these species present several phenological and physiological mechanisms that might benefit their development in drylands (de Lima et al. 2012). The survival of seedlings of *A. colubrina* and *C. pyramidale* to desiccation, thus, may have a direct effect on the recruitment of SDTF species, especially during dry spells or drought years.

2.5. Author contribution statement

FFSS, BFD and CRP conceived and designed the experiments. FFSS, GMO and MNA performed the experiments. FFSS, CES, and BFD wrote the paper. All authors have read and approved the final manuscript.

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2.7. Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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REFERENCES

Albuquerque UP, de Medeiros PM, de Almeida ALS, et al (2007) Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. J Ethnopharmacol 114:325–354. doi: 10.1016/j.jep.2007.08.017

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? J Exp Biol 209:1575–1584. doi: 10.1242/jeb.02179

Alvares CA, Stape JL, Sentelhas PC, et al (2013) Köppen's climate classification map for Brazil. Meteorol Zeitschrift 22:711–728. doi: 10.1127/0941-2948/2013/0507

Angelotti F, Sá IB, Menezes EA, Pellegrino GQ (2009) Mudanças climáticas e desertificação no Semiárido brasileiro. Embrapa Semiárido, Petrolina

Aragão CA, Dantas BF, Alves E, Corrêa MR (2002) Sementes de Feijão Submetidas a Ciclos e Períodos de Hidratação-Secagem. Sci Agric 59:87–92. doi: 10.1590/S0103-90162002000100013

Barak S, Farrant JM (2016) Extremophyte adaptations to salt and water deficit stress. Funct Plant Biol 43:v–x. doi: 10.1071/FPv43n7_FO

Barretto SSB, Ferreira RA (2011) Aspectos morfológicos de frutos, sementes, plântulas e mudas de leguminosae mimosoideae: *Anadenanthera colubrina* (Vellozo) Brenan e *Enterolobium contortisiliquum* (Vellozo) Morong. Rev Bras Sementes 33:223–232. doi: 10.1590/S0101-31222011000200004

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Mobilization of Stored Reserves. In: Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (eds) Seeds: Physiology of Development, Germination and Dormancy, 3rd edn. pp 183–246

Bispo JS, da Costa DCC, Gomes SEV, et al (2017) Size and vigor of *Anadenanthera colubrina* (Vell.) Brenan seeds harvested in Caatinga areas. J Seed Sci 39:363–373. doi: 10.1590/2317-1545v39n4173727

Brazil (2009) Regras para análise de sementes. Mapa/ACS, Brasília

418 Brazil (2013) Instruções para Análise de Sementes de Espécies Florestais. MAPA/DAS,
419 Brasília.

420 Carniel FC, Gerdol M, Montagner A, et al (2016) New features of desiccation tolerance in the
421 lichen photobiont *Trebouxia gelatinosa* are revealed by a transcriptomic approach. Plant
422 Mol Biol 91:319–339. doi: 10.1007/s11103-016-0468-5

423 Castro L, Guimarães C, Faria J (2017) Physiological, cellular and molecular aspects of the
424 desiccation tolerance in *Anadenanthera colubrina* seeds during germination. Brazilian J
425 Biol Eletronic:1–7. doi: http://dx.doi.org/10.1590/1519-6984.00616

426 Costa M-CD, Faria JMR, José AC, et al (2016) Desiccation tolerance and longevity of
427 germinated *Sesbania virgata* (Cav.) Pers. seeds. J Seed Sci 38:50–56. doi: 10.1590/2317-
428 1545v38n1155510

429 de Lima ALA, de Sá Barretto Sampaio EV, de Castro CC, et al (2012) Do the phenology and
430 functional stem attributes of woody species allow for the identification of functional
431 groups in the semiarid region of Brazil? Trees - Struct Funct 26:1605–1616. doi:
432 10.1007/s00468-012-0735-2

433 Dekkers BJW, Costa MCD, Maia J, et al (2015) Acquisition and loss of desiccation tolerance
434 in seeds: from experimental model to biological relevance. Planta 241:563–577. doi:
435 10.1007/s00425-014-2240-x

436 Ferreira DF (2014) Sisvar: a guide for its bootstrap procedures in multiple comparisons. Cienc
437 e Agrotecnologia 38:109–112. doi: 10.1590/S1413-70542014000200001

438 Figueiredo J, Araújo J, Pereira O, et al (2012) Revegetation of degraded Caatinga sites. J Trop
439 For Sci 24:332–343

440 Gaff DF, Oliver M (2013) The evolution of desiccation tolerance in angiosperm plants: A rare
441 yet common phenomenon. Funct Plant Biol 40:315–328. doi: 10.1071/FP12321

442 Giarola V, Hou Q, Bartels D (2017) Angiosperm Plant Desiccation Tolerance: Hints from
443 Transcriptomics and Genome Sequencing. Trends Plant Sci 22:705–717. doi:
444 10.1016/j.tplants.2017.05.007

445 Gutterman Y, Gozlan S (1998) Amounts of winter or summer rain triggering germination and
446 'the point of no return' of seedling desiccation tolerance, of some Hordeum spontaneum
447 local ecotypes in Israel. Plant Soil 204:223–234. doi: 10.1023/A:1004314017674

448 Hong TD, Ellis RH (1996) A protocol to determine seed storage behaviour. International
449 Plant Genetic Resources Institute, Rome

450 IBGE (2012) Manual Técnico da Vegetação Brasileira. Editora IBGE - Instituto Brasileiro de
451 Geografia e Estatística, Rio de Janeiro

452 IBGE (2015) Manual Técnico de Pedologia.
453 https://ww2.ibge.gov.br/home/geociencias/recursosnaturais/sistematizacao/manual_pedo
454 logia.shtm. Accessed 10 Oct 2017

455 IPCC (2013) Climate Change 2013: The physical science basis. Working Group I
456 Contribution to the Intergovernmental Panel on Climate Change Fifth Assessment
457 Report. Cambridge University Press, Cambridge, UK

458 Leprince O, Pellizzaro A, Berriri S, Buitink J (2016) Late seed maturation: Drying without
459 dying. J Exp Bot 68:827–841. doi: 10.1093/jxb/erw363

460 Lima AT, Meiado M V (2017) Discontinuous hydration alters seed germination under stress
461 of two populations of cactus that occur in different ecosystems in Northeast Brazil. Seed

462 Sci Res 1–11. doi: 10.1017/S0960258517000241

463 Lima CR de, Bruno R de LA, Silva KRG da RG da, et al (2012) Physiological maturity of
464 fruits and seeds of *Poincianella pyramidalis* (Tul.) L.P. Queiroz. Rev Bras Sementes
465 34:231–240. doi: 10.1590/S0101-31222012000200007

466 Locosselli GM, Schöngart J, Ceccantini G (2016) Climate/growth relations and
467 teleconnections for a Hymenaea courbaril (Leguminosae) population inhabiting the dry
468 forest on karst. Trees - Struct Funct 30:1127–1136. doi: 10.1007/s00468-015-1351-8

469 Lyall R, Ingle RA, Illing N (2014) The window of desiccation tolerance shown by early-stage
470 germinating seedlings remains open in the resurrection plant, *Xerophyta viscosa*. PLoS
471 One 9:. doi: 10.1371/journal.pone.0093093

472 Marks TR, Seaton PT, Pritchard HW (2014) Desiccation tolerance, longevity and seed-siring
473 ability of entomophilous pollen from UK native orchid species. Ann Bot 114:561–569.
474 doi: 10.1093/aob/mcu139

475 Martins JR, Edvaldo AAS, Alvarenga AA, et al (2015) Seedling survival of *Handroanthus*
476 *impetiginosus* (Mart ex DC) Mattos in a semi-arid environment through modified
477 germination speed and post-germination desiccation tolerance. Brazilian J Biol 75:812–
478 820. doi: 10.1590/1519-6984.23413

479 Masetto TE, Faria JMR, Fraiz ACR (2015) Loss and re-establishment of desiccation tolerance
480 in the germinated seeds of *Sesbania virgata* (Cav.) (Pers.). Acta Sci Agron 37:313–320.
481 doi: 10.4025/actasciagron.v37i3.19373

482 Mayrinck RC, Vilela LC, Pereira TM, et al (2019) Seed desiccation tolerance / sensitivity of
483 tree species from Brazilian biodiversity hotspots : considerations for conservation. Trees
484 0:1–9. doi: 10.1007/s00468-019-01815-8

485 Méndez-Alonzo R, Pineda-García F, Paz H, et al (2013) Leaf phenology is associated with
486 soil water availability and xylem traits in a tropical dry forest. Trees - Struct Funct
487 27:745–754. doi: 10.1007/s00468-012-0829-x

488 Miles L, Newton AC, DeFries RS, et al (2006) A global overview of the conservation status
489 of tropical dry forests. J Biogeogr 33:491–505. doi: 10.1111/j.1365-2699.2005.01424.x

490 Mogni VY, Oakley LJ, Prado DE (2015) The distribution of woody legumes in neotropical
491 dry forests: The pleistocene arc theory 20 years on. Edinburgh J Bot 72:35–60. doi:
492 10.1017/S0960428614000298

493 Moura MSB de, Souza LSB de, Randow C von, Silva TGF da (2015) Perfil vertical de CO_2
494 na Caatinga preservada: resultados preliminares. In: UFLA (ed) XIX Congresso
495 Brasileiro de Agrometeorologia. Lavras, MG, Brazil, pp 307–316

496 Nonogaki H, Bassel GW, Bewley JD (2010) Germination-still a mystery. Plant Sci 179:574–
497 581. doi: 10.1016/j.plantsci.2010.02.010

498 Oliveira GM de, Silva FFS da, Araujo M do N, et al (2019) Environmental stress, future
499 climate, and germination of *Myracrodruon urundeuva* seeds. J Seed Sci 41:

500 Pammenter NW, Berjak P (2000) Aspects of recalcitrant seed biology. Rev Bras Fisiol Veg
501 12:56–69

502 Pampurova S, Van Dijck P (2014) The desiccation tolerant secrets of *Selaginella*
503 *lepidophylla*: What we have learned so far? Plant Physiol Biochem 80:285–290. doi:
504 10.1016/j.plaphy.2014.04.015

505 Pereira WVS, Faria JMR, José AC, et al (2017) Is the loss of desiccation tolerance in
506 orthodox seeds affected by provenance? South African J Bot 112:296–302. doi:

10.1016/j.sajb.2017.06.008

Pereira WVS, Faria JMR, Tonetti OAO, Silva EAA (2014) Loss of desiccation tolerance in *Copaifera langsdorffii* Desf. seeds during germination. Brazilian J Biol 74:501–508. doi: 10.1590/1519-6984.19712

Prado DE (2005) As Caatingas da América do Sul. In: Leal IR, Tabarelli M, Silva JMC da (eds) Ecologia e conservação da Caatinga, 2nd edn. Recife, pp 3–74

Prado DE, Gibbs PE (1993) Patterns of Species Distributions in the Dry Seasonal Forests of South America. Missouri Bot Gard 80:902–927

Queiroz LP, Cardoso D, Fernandes MF, F. MM (2017) Diversity and Evolution of Flowering Plants of the Caatinga Domain. In: J.M.C. S, I.R. L, M. T (eds) Caatinga, Springer. Cham, pp 23–63

Reyes-García C, Andrade JL, Simá JL, et al (2012) Sapwood to heartwood ratio affects whole-tree water use in dry forest legume and non-legume trees. Trees - Struct Funct 26:1317–1330. doi: 10.1007/s00468-012-0708-5

Ribeiro DE, De Alvarenga AA, Martins JR, et al (2016) Germinação e reindução da tolerância à dessecação em sementes de *Senna multijuga* (Rich.) irwin et barn. Cienc Florest 26:1133–1140. doi: 10.5902/1980509825031

Rodrigues AC, de Alvarenga AA, Ribeiro DE, et al (2015) Reindução da tolerância à dessecação em sementes de *Bauhinia forficata* link (Fabaceae). Cerne 21:579–586. doi: 10.1590/01047760201521041377

Sala OE, Lauenroth WK (1982) Small Rainfall Events: An Ecological Role in Semiarid Regions. Oecologia 53:301–304. doi: doi.org/10.1007/BF00389004

529 Santiago EJP, Oliveira GM de, Leitão M de MVBR, et al (2017) Expected rainfall at different
530 levels of probability in the region of Juazeiro-BA. J Environ Anal Prog 2:457–464

531 Santos TEM dos, Montenegro AAA, Silva DD (2011) Umidade do solo no semiárido
532 pernambucano usando-se reflectometria no domínio do tempo (TDR). Rev Bras Eng
533 Agrícola e Ambient 15:670–679. doi: 10.1590/S1415-43662011000700004

534 SEI (1998) Tipologia climática: köppen, escala 1:2.000.000. SEI- Superintendência de
535 Estudos Econômicos e Sociais da Bahia, Salvador

536 Silva AC, Souza AF (2018) Aridity drives plant biogeographical sub regions in the Caatinga,
537 the largest tropical dry forest and woodland block in South America. PLoS One 13:1–22.
538 doi: 10.1371/journal.pone.0196130

539 Silva FFS, Dantas BF (2016) Quantification of storage proteins during seed imbibition of
540 native species from the brazilian Caatinga vegetation. Semina 37:1733–1744. doi:
541 10.5433/1679-0359.2016v37n4p1733

542 Silva FFS, Oliveira GM, Araújo MN, et al (2017) Rainfall events, high CO_2 concentration and
543 germination seeds of Caatinga. J Environ Anal Prog 2:258–265. doi:
544 10.24221/jeap.2.3.2017.1453.258-265

545 Stark LR (2017) Ecology of desiccation tolerance in bryophytes: A conceptual framework and
546 methodology. Bryologist 120:130–165. doi: 10.1639/0007-2745-120.2.130

547 Trovão DMBM, Fernandes PD, Andrade LA de, Dantas-Neto J (2007) Variações sazonais de
548 aspectos fisiológicos de espécies da Caatinga. Rev Bras Eng Agrícola e Ambient
549 11:307–311. doi: 10.1590/S1415-43662007000300010

550 Vargas G, Werden LK, Powers JS (2015) Explaining Legume Succes in Tropical Dry Forsts

551 Based on Seeding Germination Niches: A New Hypothesis. Biotropica 47:277–280. doi:
552 10.1111/btp.12210

553 Vieira CV, Edvaldo A. Amaral da S, Alvarenga AA, et al (2010) Stress-associated factors
554 increase after desiccation of germinated seeds of *Tabebuia impetiginosa* Mart. Plant
555 Growth Regul 62:257–263. doi: 10.1007/s10725-010-9496-3

556 Wyse S V, Dickie JB (2017) Predicting the global incidence of seed desiccation sensitivity. J
557 Ecol 105:1082–1093. doi: 10.1111/1365-2745.12725

CAPÍTULO 3 – Diversidade genética e estrutura de populações de *Anadenanthera* spp. em floresta tropical seca usando marcadores microssatélites. *Genetic diversity and population structure of* Anadenanthera *spp. in a tropical dry forest using microsatellite markers*

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Abstract: *Anadenanthera* is represented by two species, *A. colubrina* and *A. peregrina*, which are widely distributed trees in tropical dry forests. Tools used to study forest trees are important for the conservation of genetic resources of this group. We sought to answer two questions. What are the levels of genetic variability and population structure of *Anadenanthera* spp.? Can microsatellites help in the taxonomic identification of this group? A UPGMA dendrogram was generated using the Jaccard index based on the genetic distance of 39 alleles and nine loci. An analysis of molecular variance was conducted using total decomposition among and within the populations of *Anadenanthera* spp. Gene flow (Nm) was estimated by the number of migrants, based on the parameter Φ_{ST}. The size of the alleles varied from 175 bp to 794 bp. The averages for allelic frequency, polymorphism information content (PIC) and heterozygosity were 0.58, 0.52 and 0.45, respectively, demonstrating the high capacity for detecting genetic variability. The coefficient of similarity varied between 20 and 80%, with a cophenetic value of 0.81. The two Bayesian clusters divide *A. colubrina* and *A. peregrina*. The genetic variability among the population is high, $\Phi_{ST} = 0.217$ ($P < 0.001$), restricting the Nm to one migrant per generation (0.9). Population genetics can be studied using these markers, which also help in the taxonomic identification of *Anadenanthera* Speg.

Keywords: Population genetics; *Anadenanthera colubrina*; *Anadenanthera peregrina*; Angico; SDTF; SSR

3.1. Introduction

Anadenanthera Speg. (Fabaceae, Caesalpinioideae; LPWG 2017) is represented by two species and four varieties in Brazil (BFG 2015). *Anadenanthera colubrina* (Vell.) Brenan and *Anadenanthera peregrina* (L.) Speg. are trees with heights that range from 10 to 20 m. The former is culturally, economically and medicinally important in South America (Mirella et al. 2017) and both generally have an allogamous reproductive system (Costa et al. 2003; Borges et al. 2017). Regarding their ecological group, these species are classified as early secondary (Durigan and Nogueira 1990; Santos et al. 2004) and can be used to recuperate degraded areas. It is not recommended to use only anatomical features of the bark to distinguish *A. colubrina* and *A. peregrina*.

The chemical composition of the bark of these species is notable for its high levels of tannins (*A. peregrina*), total phenolic compounds and flavonoids (*A. colubrina*), and excellent antioxidant activity (both species) (Mota et al. 2017).

There are strong indications that the genus originated in Brazil, where the two species are well represented in seasonally dry tropical forest (SDTF) (Altschul 1964; Mogni et al. 2015). In Brazil, SDTFs (*i.e.*, *Chaco*, *Cerrado* and *Caatinga*) are rich in biodiversity. Genetic diversity is important and must be maintained to ensure the survival and sustainable use of forest species (Fageria and Rajora 2014; Guzmán et al. 2015).

The use of microsatellite markers (SSRs) as a tool to estimate allelic diversity can help in understanding genetic diversity of populations (Widiyatno et al. 2016) and can contribute to decisions about the management and conservation of species (Barrandeguy and Garcia 2016; Sharma et al. 2017). The high level of polymorphism in SSR markers allows for genetic diversity studies within and among populations (Park et al. 2009; Widiyatno et al. 2016). Over the last decade there have been significant advancements in the development of SSR markers for plants (Vieira et al. 2016); however, the high cost and amount of work (Santos et al. 2010) have made it difficult to use these markers for allelic diversity analyses. Until now, only 29 markers for *A. colubrina* have been developed (Barrandeguy et al. 2012; Feres et al. 2012).

Genetic variability and population structure studies can be used as tools in conservation programs of native species (Vinson et al. 2015). Works about the genetic variability of natural populations of *Anadenanthera* Speg. are limited to the *Chaco* region of northern Argentina (Barrandeguy et al. 2012, 2014, 2016; Calonga Solís et al. 2014; García et al. 2014; Goncalves et al. 2014; Mazo et al. 2014; Barrandeguy and García 2015; Mirella et al. 2017).

There are no records of genetic diversity studies of natural populations of *Anadenanthera* spp. in SDTF in Brazil. These studies are important for public policy and conservation projects (Mirella et al. 2017). Therefore, we sought to answer two questions. What are the levels of genetic variability and population structure of *Anadenanthera* spp. in SDTF? Can microsatellites help in the taxonomic identification of this group?

3.2. Material and methods

Plant material, extraction and quantification of DNA

Healthy leaves of 30 individuals of *Anadenanthera* Speg. were collected from 14 specimens in the Herbário do Trópico Semiárido (HTSA) and 16 plant matrices of natural populations in the *Caatinga* and *Cerrado* (Table 1). The fresh material was identified and stored in a freezer at -80 ºC until DNA extraction.

Table 1 Seasonally dry tropical forest (SDTF), reference city, identification code and voucher of *Anadenanthera* Speg., deposited in the Herbário do Trópico Semiárido, for the genetic diversity analysis based on the SSR markers

SDTF	Reference City	Id.	Species	Voucher
Caatinga	Casa Nova A	1	*Anadenanthera colubrina* (Vell.) Brenan	J.B. Silva s/n
	Casa Nova B	2	*Anadenanthera peregrina* (L.) Speg.	J. Paula-Souza 9854
	Casa Nova C	3	*Anadenanthera colubrina* var. *cebil* (Griseb.)	J. Paula-Souza 9860
	Casa Nova D	4	*Anadenanthera colubrina* var. *cebil* (Griseb.)	G. Fotius 3589
Caatinga	Petrolina A	5	*Anadenanthera colubrina* (Vell.) Brenan	L.H.P. Kiill s/n
	Petrolina B	6	*Anadenanthera colubrina* var. *cebil* (Griseb.)	J.L.S. Lima 19
	Petrolina C	7	*Anadenanthera colubrina* var. *cebil* (Griseb.)	G. Fotius 3237
	Petrolina M33	8	*Anadenanthera colubrina* var. *cebil* (Griseb.)	F.F.S. Silva 925
	Petrolina M34	9	*Anadenanthera colubrina* var. *cebil* (Griseb.)	F.F.S. Silva 926
	Petrolina M35	10	*Anadenanthera colubrina* (Vell.) Brenan	[a]
	Petrolina M36	11	*Anadenanthera colubrina* (Vell.) Brenan	[a]
	Petrolina M37	12	*Anadenanthera colubrina* (Vell.) Brenan	[a]
Caatinga	Juazeiro A	13	*Anadenanthera peregrina* (L.) Speg.	F.S. Gomes 1295
	Juazeiro B	14	*Anadenanthera colubrina* var. *cebil* (Griseb.)	C.T.V. Dias 141
	Juazeiro C	15	*Anadenanthera colubrina* (Vell.) Brenan	J.A. Siqueira-Filho 1589
Caatinga	Marizópolis A	16	*Anadenanthera colubrina* (Vell.) Brenan	A.P. Fontana 2031
	Marizópolis B	17	*Anadenanthera colubrina* (Vell.) Brenan	L.B. Pimentel 411
	Marizópolis C	18	*Anadenanthera colubrina* (Vell.) Brenan	L.B. Pimentel 770
	Marizópolis D	19	*Anadenanthera colubrina* (Vell.) Brenan	J.L. Costa-Lima 595
Cerrado	Uberlândia M1	20	*Anadenanthera peregrina* (L.) Speg.	F.F.S. Silva 922
	Uberlândia M2	21	*Anadenanthera peregrina* (L.) Speg.	[a]
	Uberlândia M3	22	*Anadenanthera peregrina* (L.) Speg.	[a]
	Uberlândia M4	23	*Anadenanthera peregrina* (L.) Speg.	[a]

	Uberlândia M5	24	*Anadenanthera peregrina* (L.) Speg.	[a]
	Uberlândia M8	25	*Anadenanthera peregrina* (L.) Speg.	[a]
Cerrado	Brasília M21	26	*Anadenanthera peregrina* (L.) Speg.	F.F.S. Silva 923
	Brasília M23	27	*Anadenanthera peregrina* (L.) Speg.	[a]
	Brasília M24	28	*Anadenanthera peregrina* (L.) Speg.	[a]
	Brasília M25	29	*Anadenanthera peregrina* (L.) Speg.	[a]
	Brasília M26	30	*Anadenanthera peregrina* (L.) Speg.	[a]

[a] Material was not archived in herbaria for these plant matrices; matrices Id. 8, 9, 20 and 26 represent the three populations of non-archived material

The method used to extract the DNA was proposed by Doyle and Doyle (1990), with the following modifications: after maceration of four pinnules in a mortar containing liquid nitrogen, each sample was transferred to an microtube (2 mL) containing 0.95 mL of 2X CTAB and incubated in a water bath at 60 ºC for 30 min, gently inverting the samples every 10 min; after this period, 0.95 mL of a chloroform:isoamyl alcohol mixture (24:1) was added, followed by centrifugation at 7500 rpm for 10 min; 0.7 mL of the supernatant was transferred to 1.5 mL microtubes and 0.466 mL of cold isopropyl alcohol was added, followed by gently inverting the tubes and keeping them on ice for 20 min; the samples were centrifuged at 12000 rpm for 10 min, until the formation of a pellet at the bottom of the tube; after discarding the supernatant, to precipitate the DNA 0.5 mL of 70% ethanol was added, followed by centrifugation at 12000 rpm for 5 min; the supernatant was discarded and 0.5 mL of 98% ethanol was added, the supernatant was gently poured out and the remaining material was dried at room temperature; after completely dry, the pellet was resuspended with 0.03 mL of Tris-EDTA; the DNA was quantified in agarose gel (0.8%), and the samples were then diluted to 50 ng/µL and stored at -20 ºC.

Reaction and amplification of DNA and resolution in polyacrylamide gels

All 20 SRR loci suggested by Feres et al. (2012) were evaluated for the diversity study of *Anadenanthera* Speg.: Acol 01 to Acol 20. PCR amplification was performed to a final volume of 10 µL, containing 2.5 ng of DNA, 0.3 µM of each primer, 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 1x of PCR buffer and 1 U of the enzyme *Taq* DNA

Polymerase. The amplification program follows Feres et al. (2012), with adjusted annealing temperature (Table 2) and an increase to 32 cycles. The PCR amplification products were separated on polyacrylamide gels (6%), according the method described by Costa and Santos (2013), stained with silver nitrate (Creste et al. 2001).

Table 2 Allelic pattern, in base pairs, estimated for 30 accessions of *Anadenanthera* genotyped with 9 microsatellite markers; annealing temperatures (Ta)

	Locus Acol								
	4	5	7	9	11	14	16	18	20
Casa Nova A	765/765	467/467	422/422	175/210	579/599	423/423	616/616	484/512	310/316
Casa Nova B	765/794	459/459	408/422	-	579/599	-	-	427/427	301/310
Casa Nova C	794/794	459/459	422/422	175/175	569/579	415/431	616/616	427/427	310/316
Casa Nova D	794/794	459/467	422/422	175/182	569/579	423/431	594/616	427/427	310/316
Petrolina A	765/765	459/459	422/422	175/175	569/579	423/431	594/594	484/484	310/316
Petrolina B	-	459/459	422/422	175/175	569/579	423/423	594/616	455/455	310/316
Petrolina C	794/794	459/467	408/422	175/182	569/579	423/431	616/616	427/484	329/336
Petrolina M33	635/765	459/459	422/422	175/210	569/569	423/423	575/575	427/427	301/310
Petrolina M34	-	459/459	422/422	175/182	569/579	415/415	556/594	427/427	301/310
Petrolina M35	765/765	459/459	422/483	175/175	569/579	431/431	575/594	427/427	310/316
Petrolina M36	765/765	459/459	422/422	175/175	579/599	415/423	556/556	427/427	329/336
Petrolina M37	-	459/459	422/422	175/175	579/599	423/431	575/575	427/427	310/316
Juazeiro A	765/765	459/459	422/422	175/175	569/579	423/431	594/594	427/451	310/316
Juazeiro B	765/765	459/459	422/422	175/175	569/579	431/439	616/616	427/427	-
Juazeiro C	765/765	467/467	422/422	175/175	569/579	415/431	594/616	427/451	310/316
Marizópolis A	-	459/459	422/422	175/210	579/599	423/423	594/594	-	310/316
Marizópolis B	765/765	459/459	422/422	175/175	569/579	423/439	575/594	-	310/316
Marizópolis C	765/765	459/459	422/422	175/182	569/579	431/431	594/594	427/427	301/310
Marizópolis D	765/765	459/459	422/422	175/182	579/599	431/431	594/594	427/427	310/316
Uberlândia M1	635/655	467/467	422/483	-	569/579	423/423	575/627	427/455	301/310
Uberlândia M2	635/655	459/459	422/483	175/175	-	423/423	575/575	455/455	301/310
Uberlândia M3	635/655	459/459	422/483	182/210	569/579	423/423	594/616	427/427	310/316
Uberlândia M4	765/765	459/459	-	175/210	569/579	423/423	575/594	427/427	301/310
Uberlândia M5	-	459/459	-	175/201	569/579	423/423	575/594	427/484	310/316
Uberlândia M8	-	459/459	422/483	201/210	579/599	423/423	594/594	512/512	301/310
Brasília M21	655/794	484/484	422/422	210/224	599/621	431/431	556/556	-	-
Brasília M23	-	459/459	422/483	210/210	579/599	423/423	575/616	427/427	301/310
Brasília M24	655/655	484/484	422/422	210/210	599/621	431/431	-	427/427	301/310
Brasília M25	635/655	484/484	422/422	175/210	579/579	431/431	556/556	455/455	301/310
Brasília M26	655/655	484/493	422/422	210/210	579/579	431/431	-	-	301/301
Ta (ºC)	56	56	56	56	60	58	56	52	56

Polymorphism and cluster analysis

To analyze the allelic pattern for each individual of *Anadenanthera* Speg., the number of base pairs (bp) for each allele was estimated using the inverse mobility method, based on the regression of products of known size of 50 bp molecular marker (Ludwig Biotec ®). The microsatellites were analyzed for allelic presence (1) and absence (0) to construct a Jaccard index similarity matrix. A dendrogram with the distance of each individual was generated using the UPGMA clustering method (unweighted, based on the arithmetic mean). The significance of the dendrogram was tested using the cophenetic correlation. The program NTSYSpc (Rohlf 2009) was used to conduct these tests.

The allelic frequency, number of genotypes and alleles, genetic diversity, heterozygosity and polymorphism information content (PIC) were calculated for each microsatellite, for the 30 individuals of *Anadenanthera* spp., using the program Power Marker version 3.25 (Liu and Muse 2005).

Population structure analysis

Genotypes were also grouped using the program STRUCTURE 2.3.4 (Pritchard et al. 2000). The analysis was conducted using the Markov chain Monte Carlo (MCMC) method with a burnin of 100,000 steps, as well as 10,000 steps for clustering inference. Ten runs were conducted for each *K* value (number of possible clusters); when executions on the same *K* values produced discrepant results, the majority rule was used to select the ideal result (Friedlaender et al. 2008). The Δ*K* value was used to detect the most likely number of clusters (Evanno et al. 2005), which was calculated using STRUCTURE HARVESTER (Earl and vonHoldt 2012) that is available online to view outputs. Of the 20 independent executions, the one with the highest Ln Pr (*X*|*K*) value (probability of log or likelihood) was chosen and represented as a line graph.

The analysis of molecular variance (AMOVA) was analyzed by the decomposition of the total variation of the components among and within populations, using squared Euclidean distance (Excoffier et al. 1992). The significance of these genetic parameters was determined using the randomization method (999 permutations). The gene flow (Nm) was estimated by the number of migrants, based on the parameter Φ_{ST}, which is analogous to F_{ST} (Wright 1949; Meirmans and Hedrick 2011). The program GenAlEx 6.5 (Peakall and Smouse 2012) was used for these tests.

3.3. Results

SSR polymorphism

The polymorphic amplification was visible and easy to interpret for only 9 of the 20 microsatellite loci, including the following: Acol 04, Acol 05, Acol 07, Acol 09, Acol 11, Acol 14, Acol 16, Acol 18 and Acol 20. For these markers, 39 alleles were detected. The size of the alleles varied from 175 bp for marker Acol 09 to 794 bp for marker Acol 04 (Table 2).

The average allelic frequency was 0.577, demonstrating a high capacity of detecting genetic variability, with values between 0.389 and 0.857 for the loci Acol 16 and Acol 7, respectively. Locus 7 had the smallest number of genotypes and alleles, three for each parameter, as well as the smallest values of genetic diversity and PIC. The largest number of genotypes and alleles occurred together in the locus Acol 16, which also had larger genetic diversity and PIC among all the loci. The frequency of heterozygosity was lowest for locus Acol 5 (0.1) and highest for locus Acol 20 (0.96) (Table 3).

Table 3 Statistical parameters of genetic diversity of 9 microsatellite markers for *Anadenanthera* Speg.

Locus	Allelic frequency	No. of genotypes	No. of alleles	Genetic diversity	Heterozygosity	PIC [a]
Acol_4	0.522	7	4	0.647	0.304	0.600
Acol_5	0.733	5	4	0.431	0.100	0.396
Acol_7	0.857	3	3	0.253	0.286	0.234
Acol_9	0.589	8	5	0.577	0.500	0.523
Acol_11	0.483	5	4	0.640	0.897	0.574
Acol_14	0.500	8	4	0.598	0.345	0.519
Acol_16	0.389	9	5	0.724	0.407	0.677
Acol_18	0.673	8	5	0.515	0.231	0.485
Acol_20	0.446	4	5	0.682	0.964	0.627
Average	0.577	6	4	0.563	0.448	0.515

[a] PIC: Polymorphism information content

UPGMA dendrogram suggests the stratification of the two species

The cophenetic correlation was 0.81, which indicates that the clustering is consistent in the dendrogram of the 30 individuals of *Anadenanthera* spp. analyzed based on the 39 alleles and 9 SSR loci. The individuals had a similarity coefficient between 20 and 80%. The individuals Brasília M21, M24, M25 and M26 had the greatest dissimilarity in relation to the other individuals evaluated. The individuals Juazeiro A and Petrolina A were the most similar (Fig. 1).

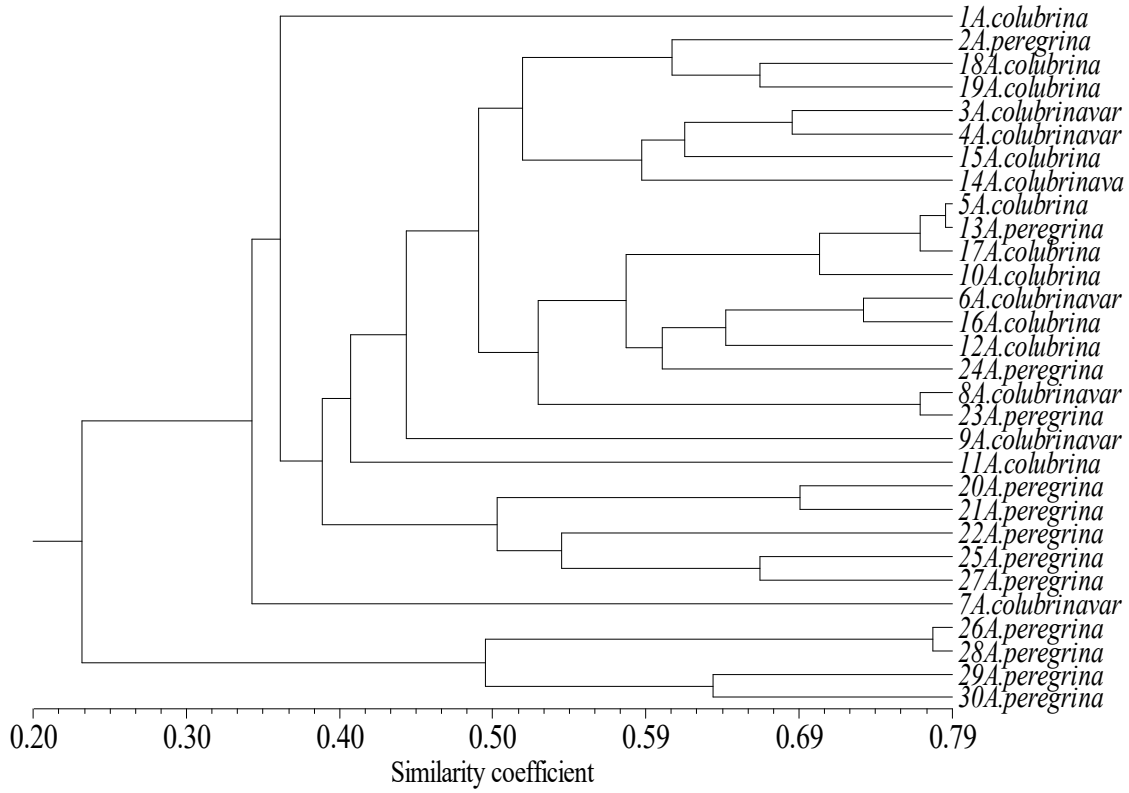

Fig. 1 UPGMA dendrogram based on the Jaccard coefficient for 30 accessions of *Anadenanthera* sampled from 7 subpopulations and analyzed using 9 microsatellite loci. Cophenetic correlation = 0.81

In the dendrogram, at approximately 0.4 similarity, the individuals cluster into five groups. The individuals of group 1, group 2 and group 4 are *Anadenanthera* spp. from the *Caatinga* (except 23 *A. peregrina* and 24 *A. peregrina*). However, the individuals of group 3 and group 5 are *Anadenanthera peregrina* from the *Cerrado*, characterizing this species well for this environment (Fig. 1). The groups correspond to the following: group 1, individual 1 *A. colubrina*; group 2, from 2 *A. peregrina* to 11 *A. colubrina*; group 3, 20 *A. peregrina* to 27 *A. peregrina*; group 4, 7 *A. colubrina* var. *cebil*; and group 5, 26 *A. peregrina* to 30 *A. peregrina*.

Bayesian analysis separates the populations of the two species into specific clusters

The Bayesian cluster analysis, conducted with the programs STRUCTURE and STRUCTURE HARVESTER, separated the two species of *Anadenanthera* into two populations, with a higher ΔK in $K = 2$ (Fig. 2; Fig. 3). The two clusters are related to geographic distribution; cluster 1 includes most individuals of *A. colubrina* from the

Caatinga and cluster 2 contains most individuals of *A. peregrina* from the *Cerrado*. There were small divergences in the Bayesian analysis for the individuals 23 *A. peregrina*, 24 *A. peregrina*, 13 *A. peregrina* and 7 *A. colubrina* (Fig. 3). The number of UPGMA clusters would be the same in the Bayesian analysis at a cutoff point of around 0.3 in the dendrogram (Fig. 1 and Fig. 3).

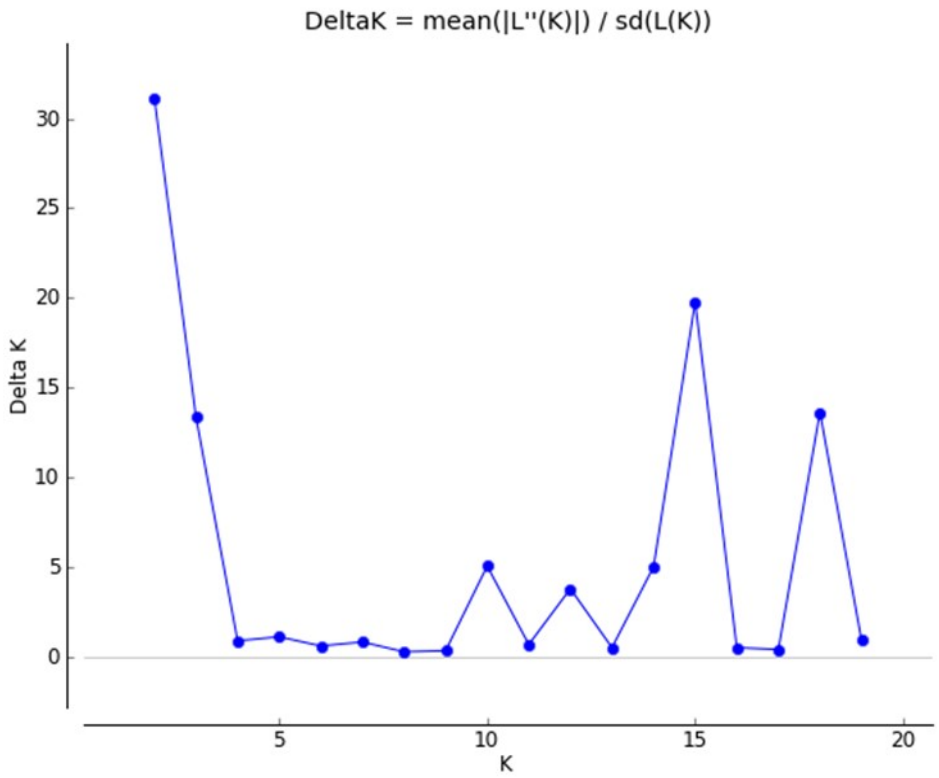

Fig. 2 Delta *K*, calculated with the average second order rate of change of the probability of *K* divided by the standard deviation of the probability *K*

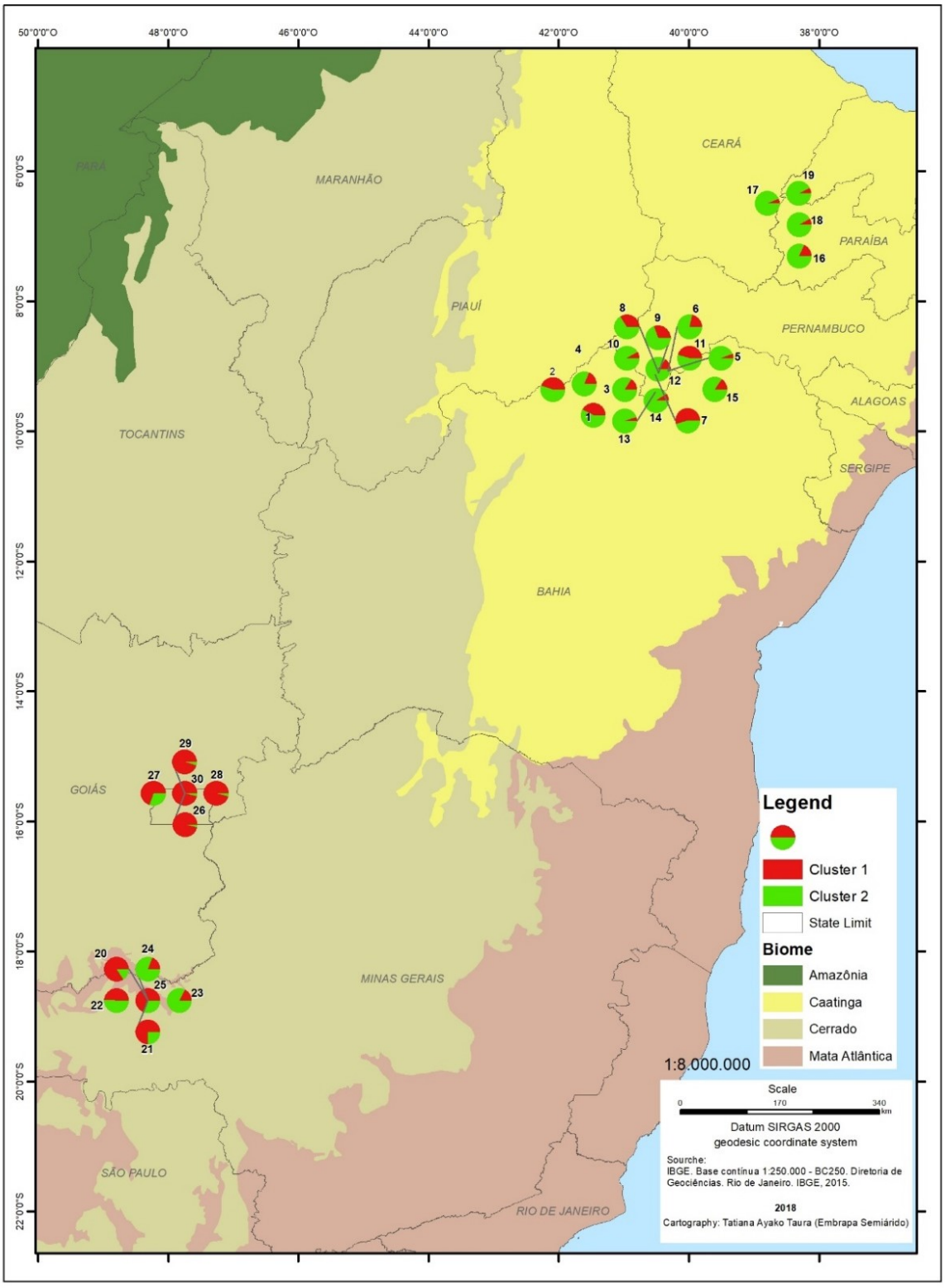

Fig. 3 Population structure of the 30 adult individuals of *Anadenanthera* Speg. based on 9 SSR markers (*K*=2)

Molecular analysis shows moderate divergence between the populations of the two species

The analysis of molecular variance shows a moderate divergence of 22% between the populations of the two species; the coefficient of variation between the populations of *Anadenanthera* spp. is $\Phi_{ST} = 0.217$ ($P < 0.001$), restricting the gene flow to one migrant per generation (Nm = 0.9) (Table 4).

Table 4 Analysis of molecular variance (AMOVA) and estimated gene flow (Nm) of the populations of *Anadenanthera* spp., calculated using the method of Wright (1949)

Source of variation	GL	SQ	QM	Total variance (%)	P value [a]	Φ statistic	Nm[b]
Among populations	6	79.567	13.261	22	<0.001	$\Phi_{ST} = 0.217$	0.9
Within populations	23	140.133	6.093	78	<0.001	$1- \Phi_{ST} = 0.783$	
Total	29	219.700	-	100			

[a] Probability based on 999 permutations; [b] $Nm = [(1-\Phi_{ST})/(4\ \Phi_{ST})]$

3.4. Discussion

We analyzed the genetic diversity and population structure of *Anadenanthera*, using SSR markers, with the goal of guiding conservation studies about the genetic resources of this group. The data generated show high genetic variability and will aid taxonomic botanists and public policy managers by helping prioritize locations that should be conserved. *Anadenanthera colubrina* and *A. peregrina* are very phenotypically similar so works like the present study are needed to correctly identify the species.

The SSR markers developed for *Anadenanthera* (Feres et al. 2012) showed a polymorphism in 14 of the 20 markers evaluated for *A. colubrina*. In addition, these authors confirmed the transferability of these markers to *A. peregrina* for 18 loci, which helps distinguish the two species. Our work, like Feres et al. (2012), also showed the that loci Acol 5 and Acol 16 had the lowest and highest values of genetic diversity, respectively.

The populations of groups 1, 2 and 4 include most individuals of *A. colubrina* from different regions of the *Caatinga*. A riparian forest environment might be the determining factor for the presence of the 2 *A. peregrina* and 13 *A. peregrina* individuals in an area of *Caatinga*. Rainfall indices could be limiting factors in the distribution of this species; during the rainy season, some areas of *Caatinga* receive less

than 200 mm of rain, making it difficult for both species to become established (Silva et al. 2017).

Branches 3 and 5 include individuals of *A. peregrina* from the *Cerrado*. The *Cerrado* is a dry tropical environment, like the *Caatinga*, but it receives more rainfall (up to 1600 mm; Macena et al. 2008) in the locations where the two populations of *A. peregrina* occur. The correlations between actual and estimated distances for the dendrogram have values from good to bad, with a cophenetic correlation of 0.81 (Fig. 1). There are two varieties of *A. peregrina*, var. *falcata* and var. *peregrina* (Altschul 1964), which probably resulted in the small divergences present in the UPGMA dendrogram and Bayesian analysis for individuals 23 *A. peregrina* and 24 *A. peregrina*.

The selected ΔK identified the different populations and helped in the taxonomic identification of *A. colubrina* and *A. peregrina*. Many studies have used the program STRUCTURE for native species, for example, *Spondias tuberosa* Arruda (Balbino et al. 2018), *Juglans hopeiensis* Hu (Hu et al. 2017) and *Eugenia dysenterica* (Mart.) DC. (Boaventura-Novaes et al. 2018). According to Porras-Hurtado et al. (2013), this tool is important to evaluate population genetic structure because it detects differences in allelic frequencies between individuals.

Of the nine works in the literature that cover the genetic variability of *Anadenanthera* spp., only two (Calonga Solís et al. 2014; García et al. 2014) archived material in herbaria for future studies. Although important, in our study some collections of individuals were not archived due to the close proximity of the plant matrices in the same population (Table 1; Fig. 3). The herborization process is very important so there are dried voucher collections that are permanently archived in herbaria and can be used to corroborate the results of a study. It is also extremely important to include basic information on voucher labels, such as coordinates, location name, collection date and collector name (Siqueira et al. 2012).

In a study conducted in northern Argentina with populations of *A. colubrina* an inbreeding depression was not observed, and the effective population size (Ne) represented 90% of the populations analyzed, demonstrating there was no genetic relation for most individuals evaluated (Mazo et al. 2014). The self-incompatibility present in the reproductive system of *A. colubrina* and *A. peregrina* favors genetic diversity during the selection of progenies of both species (Costa et al. 2003; Borges et

al. 2017). Thus, the ideal Ne to rescue germplasm of these allogamous species would be to collect the greatest number of seeds from the fewest number of plants, optimizing the collection time of the germplasm.

The *Cerrado*, as of 2009, had been reduced to nearly half its original area and suffers from intense forest fragmentation (Ganem et al. 2013). In the *Caatinga*, the extraction of adult individuals of *A. colubrina* is becoming more common, mainly to produce charcoal (Silva and Barbosa 2000). Fragmentation, linked to degradation of areas where *Anadenanthera* spp. occur, is one the greatest threats to the genetic diversity of these species (Barrandeguy et al. 2011; Athayde and Morellato 2014).

The use of SSR markers helped identify *A. colubrina* and *A. peregrina*. Further, the archived material of *Anadenanthera* spp. can be used to extract DNA for future studies about the genetic similarity of these species. Thus, we hope that the results of this work will be used in more detailed studies about the diversity, phylogeny and conservation of germplasm of *Anadenanthera* spp.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Data Archiving Statement

Population names, geographic location of *Anadenanthera* spp. populations, and microsatellite data for 30 *A. colubrina* genotypes were submitted as private data to the ResearchGate Database and named "Structure of populations of *Anadenanthera* spp. at dry forests of Brazil" https://www.researchgate.net/publication/331023501_Structure_of_populations_of_Anadenanthera_spp_at_dry_forests_of_Brazil. These will be made public prior to the final acceptance of this manuscript

References

Altschul S von R (1964) A taxonomic study of the genus *Anadenanthera*. Contrib from Gray Herb Harvard Univ 193:3–65. www.jstor.org/stable/41764816

Athayde EA, Morellato LPC (2014) Anthropogenic edges, isolation and the flowering time and fruit set of *Anadenanthera peregrina*, a cerrado savanna tree. Int J Biometeorol 58:443–454. doi: 10.1007/s00484-013-0727-y

Balbino E, Caetano B, Almeida C (2018) Phylogeographic structure of *Spondias tuberosa* Arruda Câmara (Anacardiaceae): seasonally dry tropical forest as a large and continuous refuge. Tree Genet Genomes 14:1–8. doi: 10.1007/s11295-018-1279-4

Barrandeguy ME, Garcia MV (2016) Microsatellites as a Tool for the Study of Microevolutionary Process in Native Forest Trees. In: Abdurakhmonov IY (ed) Microsatellite Markers. Intech Open, pp 47–72

Barrandeguy ME, García MV (2015) Back to the future : Evidence of ancestral polymorphism in current populations of *Anadenanthera colubrina* var. *cebil* by

means of Population Graphs. Plant Sci Today 2:65–68. doi: 10.14719/pst.2015.2.2.102

Barrandeguy ME, García MV, Argüelles CF, Cervigni GDL (2011) Genetic diversity of *Anadenanthera colubrina* Vell. (Brenan) var *cebil*, a tree species from the South American subtropical forest as revealed by cpSSR markers. Silvae Genet 60:123–132. doi: 10.1515/sg-2011-0017

Barrandeguy ME, García MV, Prinz K, et al (2014) Genetic structure of disjunct Argentinean populations of the subtropical tree *Anadenanthera colubrina* var. *cebil* (Fabaceae). Plant Syst Evol 300:1693–1705. doi: 10.1007/s00606-014-0995-y

Barrandeguy ME, Prado DE, Goncalves AL, García M V. (2016) Demografía histórica de *Anadenanthera colubrina* var. *cebil* (Leguminosae) en Argentina. Boletín la Soc Argentina Botánica 51:689–703. doi: 10.31055/1851.2372.v51.n4.16358

Barrandeguy ME, Prinz K, García M V., Finkeldey R (2012) Development of microsatellite markers for *Anadenanthera colubrina* var. *cebil* (Fabaceae), a native tree from South America. Am J Bot 99:372–374. doi: 10.3732/ajb.1200078

BFG (2015) Growing knowledge: An overview of Seed Plant diversity in Brazil. Rodriguesia 66:1085–1113. doi: 10.1590/2175-7860201566411

Boaventura-Novaes CRD, Novaes E, Mota EES, et al (2018) Genetic drift and uniform selection shape evolution of most traits in *Eugenia dysenterica* DC. (Myrtaceae). Tree Genet Genomes 14:1–14. doi: 10.1007/s11295-018-1289-2

Borges LA, Machado IC, Lopes AV (2017) Bee pollination and evidence of substitutive nectary in *Anadenanthera colubrina* (Leguminosae-Mimosoideae). Arthropod Plant Interact 11:263–271. doi: 10.1007/s11829-017-9514-8

Calonga Solís V, Barrandeguy ME, García MV (2014) Historical divergence in *Anadenanthera colubrina* var. *cebil* (leguminosae) analyzing an intronic region of chloroplast DNA. Bol la Soc Argentina Bot 49:547–557

Costa RB, Contini AZ, Melo ESP (2003) Sistema reprodutivo de *Anadenanthera peregrina* (L.) Speg e *Vochysia haenkiana* (Spreng.) Mart. em fragmento de cerrado na Chapada dos Guimarães - MT. Ciência Rural 33:305–310. doi: 10.1590/S0103-84782003000200019

Costa SR, Santos CAF (2013) Allelic database and divergence among *Psidium* accessions by using microsatellite markers. Genet Mol Res 12:6802–6812. doi: 10.4238/2013.december.16.6

Creste S, Tulmann-Neto A, Figueira A (2001) Detection of Single Sequence Repeat Polymorphisms in Denaturing Polyacrylamide Sequencing Gels by Silver Staining. Plant Mol Biol Rep 19:299 – 306. doi: https://doi.org/10.1007/BF02772828

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus (Madison) 12:13–15

Durigan G, Nogueira JCB (1990) Recomposiçao de matas ciliares. IF Série Regist 4:1–14

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

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genet Soc Am 131:479–491. doi: 10.1007/s00424-009-0730-7

Fageria MS, Rajora OP (2014) Effects of silvicultural practices on genetic diversity and population structure of white spruce in Saskatchewan. Tree Genet Genomes 10:287–296. doi: 10.1007/s11295-013-0682-0

Feres JM, Monteiro M, Zucchi MI, et al (2012) Development of microsatellite markers for *Anadenanthera colubrina* (leguminosae), a neotropical tree species. Am J Bot 99:154–156. doi: 10.3732/ajb.1100446

Friedlaender JS, Friedlaender FR, Reed FA, et al (2008) The genetic structure of Pacific Islanders. PLoS Genet 4:0173–0190. doi: 10.1371/journal.pgen.0040019

Ganem RS, Drummond JA, Franco JL de A (2013) Conservation polices and control of habitat fragmentation in the Brazilian Cerrado biome. Ambient Soc 16:99–118.

doi: 10.1590/S1414-753X2013000300007

García MV, Prinz K, Barrandeguy ME, et al (2014) A unifying study of phenotypic and molecular genetic variability in natural populations of *Anadenanthera colubrina* var. *cebil* from Yungas and Paranaense biogeographic provinces in Argentina. J Genet 93:123–132. doi: 10.1007/s12041-014-0347-2

Goncalves AL, Barrandeguy ME, García MV (2014) Chloroplast genetic structure and differentiation in natural populations of *Anadenanthera colubrina* var. *Cebil* (Leguminosae) from Argentina. Bol la Soc Argentina Bot 49:235–245

Guzmán B, Rodríguez López CM, Forrest A, et al (2015) Protected areas of Spain preserve the neutral genetic diversity of *Quercus ilex* L. irrespective of glacial refugia. Tree Genet Genomes 11:1–18. doi: 10.1007/s11295-015-0950-2

Hu Y, Dang M, Feng X, et al (2017) Genetic diversity and population structure in the narrow endemic Chinese walnut *Juglans hopeiensis* Hu: implications for conservation. Tree Genet Genomes 13:1–14. doi: 10.1007/s11295-017-1172-6

Liu K, Muse S V. (2005) PowerMaker: An integrated analysis environment for genetic maker analysis. Bioinformatics 21:2128–2129. doi: 10.1093/bioinformatics/bti282

LPWG (2017) A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny – The Legume Phylogeny Working Group (LPWG). Taxon 66:44–77. doi: 10.12705/661.3

Macena F, Assad E, Steinke ET, Gustavo Müller A (2008) Clima do Bioma Cerrado. Agric Trop Quatro décadas inovações tecnológicas, Inst e políticas 93–148

Mazo TM, Barrandeguy ME, García M V. (2014) Endocría y coancestría en poblaciones naturales de *Anadenanthera colubrina* var. *cebil*. RECyT 22–27

Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. Mol Ecol Resour 11:5–18. doi: 10.1111/j.1755-0998.2010.02927.x

Mirella C, Roberta R, Franco P, et al (2017) A genetic study on subtropical *Anadenanthera colubrina* (Vell.) Brenan var. cebil (Griseb.) Altschul tree from Northwestern Argentina. J For Res 22:191–194. doi: 10.1080/13416979.2017.1283975

Mogni VY, Oakley LJ, Prado DE (2015) The distribution of woody legumes in neotropical dry forests: The pleistocene arc theory 20 years on. Edinburgh J Bot 72:35–60. doi: 10.1017/S0960428614000298

Mota GS, Sartori CJ, Miranda I, et al (2017) Bark anatomy, chemical composition and ethanol-water extract composition of *Anadenanthera peregrina* and *Anadenanthera colubrina*. PLoS One 12:1–14. doi: 10.1371/journal.pone.0189263

Park YJ, Lee JK, Kim NS (2009) Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. Molecules 14:4546–4569. doi: 10.3390/molecules14114546

Peakall R, Smouse PE (2012) GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539. doi: 10.1093/bioinformatics/bts460

Porras-Hurtado L, Ruiz Y, Santos C, et al (2013) An overview of *STRUCTURE*: Applications, parameter settings, and supporting software. Front Genet 4:1–13. doi: 10.3389/fgene.2013.00098

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genet Soc Am 155:945–959. doi: 10.1111/j.1471-8286.2007.01758.x

Rohlf FJ (2009) NTSYSpc: Numerical Taxonomy and Multivariate Analysis System. New York

Santos CAF, Oliveira VR, Rodrigues MA, Ribeiro HLC (2010) Caracterização molecular de cultivares de cebola com marcadores microssatélites. Pesqui Agropecu Bras 45:49–55. doi: 10.1590/S0100-204X2010000100007

Santos JHDS, Ferreira RLC, Silva JAA Da, et al (2004) Distinção de grupos ecológicos de espécies florestais por meio de técnicas multivariadas. Rev Árvore 28:387–396. doi: 10.1590/S0100-67622004000300010

Sharma SS, Islam MA, Singh VK, et al (2017) Genetic diversity, population structure and association study using TE-AFLP markers in *Pongamia pinnata* (L.) Pierre germplasm. Tree Genet Genomes 13:1–10. doi: 10.1007/s11295-016-1088-6

Silva FFS, Oliveira GM, Araújo MN, et al (2017) Rainfall events, high CO_2

concentration and germination seeds of Caatinga. J Environ Anal Prog 2:258–265. doi: 10.24221/jeap.2.3.2017.1453.258-265

Silva LMB, Barbosa DC (2000) Crescimento e sobrevivência de *Anandenanthera macrocarpa* (Benth.) Brenan (Leguminosae), em uma área de Caatinga, Alagoinha, PE. Acta Bot Brasilica 14:. doi: 10.1590/S0102-33062000000300003

Siqueira AA, Santos Filho JV dos, Siqueira Filho JA de (2012) Carolus : A new system for handling electronic botanical collections. Rodriguésia 63:715–732. doi: 10.1590/S2175-78602012000300016

Vieira MLC, Santini L, Diniz AL, Munhoz C de F (2016) Microsatellite markers: What they mean and why they are so useful. Genet Mol Biol 39:312–328. doi: 10.1590/1678-4685-GMB-2016-0027

Vinson CC, Dal'Sasso TCS, Sudré CP, et al (2015) Population genetics of the naturally rare tree *Dimorphandra wilsonii* (Caesalpinioideae) of the Brazilian Cerrado. Tree Genet Genomes 11:1–10. doi: 10.1007/s11295-015-0876-8

Widiyatno, Indrioko S, Na'iem M, et al (2016) Effects of different silvicultural systems on the genetic diversity of *Shorea parvifolia* populations in the tropical rainforest of Southeast Asia. Tree Genet Genomes 12:1–12. doi: 10.1007/s11295-016-1030-y

Wright S (1949) Genetical structure of populations. Ann Eugen 15:323–354. doi: 10.1038/166247a0

CONSIDERAÇÕES FINAIS

As plantas matrizes de *Anadenanthera colubrina* e *Cenostigma pyramidale* caracterizadas no presente estudo poderão ser utilizadas em futuras excursões de coleta de sementes. Os dados dendrométricos de cada planta auxiliará em programas de manejo destas espécies e na avaliação de crescimento das mesmas em condições naturais. O tipo de solo influencia diretamente no desenvolvimento de plantas matrizes, sendo aquelas marcadas na Caatinga de menor altura total em relação as plantas marcadas no Pantanal e Cerrado.

As plântulas de ambas as espécies emitiram raízes adventícias após à dessecação. O intervalo em que ocorre a tolerância à dessecação pós-germinativa em plântulas de *A. colubrina* e *C. pyramidale*, mostra-se como um importante auxílio no recrutamento e distribuição destas espécies em florestas tropicais sazonalmente secas (SDTF). Nosso trabalho revela um importante mecanismo de adaptação ecológica para espécies SDTF, em que as plântulas com maior comprimento de raiz inicial, mesmo que dessecadas por um período mais longo, tiveram aproximadamente 40% de sobrevivência.

A alta plasticidade de *A. colubrina* frente à dessecação pós-germinativa, aliada a alta diversidade genética do gênero, explica em parte a distribuição de *A. colubrina* e *Anadenanthera peregrina* em SDTF. O uso de marcadores microssatélites de *Anadenanthera colubrina* auxiliará na identificação correta do gênero, tendo em vista a alta similaridade fenotípica de *A. colubrina* e *A. peregrina*. Além disso, para otimizar o tempo em programas de resgate de germoplasma de *Anadenanthera* spp. o ideal seria coletar o maior número de sementes do menor número de plantas.