



Characterization and selection of markers associated with resistance analogous genes as input for genetic analysis of *Prosopis juliflora* (Sw.) DC.

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ABSTRACT. The characterization and selection of molecular markers are important for genetic pre-breeding programs since they make it possible to choose the most appropriate markers to be used in future research. Therefore, enabling the generation of subsidies for genetic-molecular studies in algaroba (*Prosopis juliflora* (Sw.) DC). The amplification profile was characterized. It was generated from 17 pairs of RGA primers (*Resistance Gene Analogs*) in 20 samples of genomic DNA of *P. juliflora* extracted from specimens collected in the city of Itapetinga, Bahia. The amplifications were performed according to previously published laboratory routines and the amplification profiles analyzed from the photodocumentation of the electrophoresis results in 2% agarose gels. Based on the amplification profiles the primer pairs were classified as: Suitable: amplifications in the whole samples and with easy visualization; Reasonable: amplification in parts of the samples and/or difficult to visualize or Inadequate: absence of visible amplification products. Descriptive analyzes associated with the number of generated markers, percentage of polymorphism, expected heterozygosity (*He*) and the content of polymorphic information (PIC) were also performed. In a nutshell, 12 out of the 17 pairs of RGA primers generated amplification products with easy visualization and only two of these 12 pairs of primers were monomorphic. The percentage of polymorphism varied from 60% to 100%, *He* and PIC presented an average of 0.21 (ranging from 0 to 0.38) and 0.17 (ranging from 0 to 0.29), respectively. The results confirm that the RGA primers present adequate characteristics for genetic studies in *P. juliflora*, making it possible to prioritize 12 pairs of primers, which are subject to genetic improvement studies.

Keywords: RGA primers; Algaroba; PCR.

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Introduction

The species *Prosopis juliflora* (Sw.) DC, popularly known as algaroba, belongs to the family Fabaceae, subfamily Mimosoideae. It is a tree species, native to Central and South America, inserted in Brazil in the 1942s, adapting well to semi-arid regions (Leão, Almeida, Dechoum, & Ziller, 2011; Cunha & Gomes, 2012). Algaroba has diverse potential for human and animal feeding, as well as for wood production, soil protection, afforestation and pasture shading (Ribaski, Drumond, Oliveira, & Nascimento, 2009; Rodrigues, Silva, Silva, Oliveira, & Andrade, 2013).

Although the algaroba has ecological and economic importance, there are still few genetic and molecular studies related to estimates of its diversity and genetic structure. However, this knowledge is a prerogative both for the elaboration of strategies for sustainable management and conservation of genetic resources as well as for the advancement of breeding programs (Faleiro, Amabile, & Cerqueira-Silva, 2018).

The use of molecular markers in studies of genetic diversity is widely accepted and used, among other factors, due to the reliability and reproducibility of the results (Souza, 2015; Pomponio et al., 2015). Several molecular markers are used in genetic characterization studies, both for phylogenetic and evolutionary applications, as well as for practical purposes in breeding programs and conservation actions (Rai, Shekhawat, Kataria, & Shekhawat, 2017; Bessega et al., 2019). The genetic diversity study of *Prosopis cineraria* L. and *Prosopis juliflora* (Sw.) DC L. using ISSR and RAPD markers is an example of research of this nature that contributes to the understanding of the intra-specific diversity in the genus *Prosopis* (Elmeer & Almalki, 2011).

For genetic diversity studies, the crucial step, after identification and collection of the sample population, is the definition of the molecular markers to be used in it. In this context, the characterization and selection of primers contribute to cost reduction and to optimize the time spent before the evaluation of the entire population, thus avoiding the use of primers unsuitable for the target species (Silva, Freitas, Santos, Cardoso, & Cerqueira-Silva, 2018).

Among the molecular markers used in genetic studies of plants are the RGA (Resistance Gene Analogs). These are markers associated with regions considered rich in leucine and nucleotide binding sites (Nucleotide Binding Site Leucine Rich Repeat - NBS-LRR). In plants, these NBS-LRR domains have their function associated with disease resistance as a response to hypersensitivity and the LRR domain, specifically, they are responsible for the primary recognition of possible pathogens, causative agents of cell damage (Jones & Dangl, 2006).

In addition, RGA primers are also useful in genetic studies related to diversity estimates, genetic mapping (Paula, Fonseca, Boiteux, & Peixoto, 2010; Carmo, Martins, Musser, Silva, & Santos, 2017) and genomic marker selection (Fritsche-Neto, Resende, Miranda, & DoVale, 2012). The main advantage of this marker is the possibility of using it without necessarily requiring previous information about the genome of the species to be evaluated, being easily manipulated and having greater reproducibility (Borém & Caixeta, 2009).

Considering the present context, the objective was to characterize the amplification profile of different combinations of RGA primers and to select the most appropriate combinations to support genetic studies in *Prosopis juliflora* (Sw.) DC.

Material and methods

Collection of samples

Leaf samples of 20 *Prosopis Juliflora* (Sw.) DC. specimens were collected at the Federal Institute of Bahia (IFBA) in Itapetinga, Bahia, Brazil. These specimens are located in the following geographical coordinates: Latitude 15° 14'38.57 "S and Longitude 40° 13 '38.95" W and are registered in the National System for Management of Genetic Heritage and Associated Traditional Knowledge - SisGen, under the registration number AFF0E34.

The vegetation of the region where the IFBA of Itapetinga is located is characterized as ecotone between Caatinga and Deciduous and Semi-deciduous Seasonal Forest. The plant material was taken to the Laboratory of Applied Molecular Genetics (LGMA) of the State University of the Southwest of Bahia (UESB, Juvino de Oliveira campus), in the municipality of Itapetinga, where they were stored in a freezer at -20°C until the extraction of the Genomic DNA.

Nucleic acid extraction

The extraction of the genomic DNA was performed by the method proposed by Sunnucks and Hales (1996), and 0.4g of *P. juliflora* young leaf tissue was macerated using 3.0 ml SDS (sodium dodecyl sulfate) buffer without the addition of liquid nitrogen and β -mercaptoethanol. DNA quantification was performed from horizontal electrophoresis on 1% (w v⁻¹) agarose gels stained with 0.2% w v⁻¹ of Red Invitrogen Gel Biotium®, compared to a number of known concentrations of DNA phage lambda Invitrogen® (λ). The purity of the DNA was estimated by spectrophotometric reading ($A_{260/230}$ and $A_{260/280}$ absorbance ratios) using BioDrop μ LITE (Whitehead Scientific). Subsequently samples of the genomic DNA were standardized in 2 ng. μ L⁻¹ and stored in a freezer -20°C.

Characterization of molecular markers

The characterization of the markers was performed in two sequential steps. The first step consisted in characterizing and classifying the amplification profiles of the 17 pairs of RGA primers (Table 1) for a set of five DNA samples, being: Adequate - amplifications in all the samples and with easy visualization; Reasonable - amplification in parts of the samples or difficult to visualize and Inadequate - absence of visible amplification products. As for the second step, the pairs of primers classified as 'Adequate' in the first step were selected, and the amplification profiles observed in 15 more *P. juliflora* samples were evaluated, totaling 20 individuals used.

Table 1. Description of the 17 pairs of RGA (Resistance Gene Analogs) primers tested on algaroba (*Prosopis juliflora* (Sw.) DC).

Pairs	Code	Sequences 5'- 3'	References
1	S1	GGTGGGGTTGGGAAGACAACGYCT	Leister, Ballvora, Salamini and Gebhardt (1996)
	NBSr1	AGTTGTRAYDATDAYYYTRC	
2	S2	GGIGGIGTIGGIAAIAACIAC	Yu, Buss and Maroof (1996)
	As1	CAACGCTAGTGGCAATCC	
3	S2	GGIGGIGTIGGIAAIAACIAC	Leister et al. (1996)
	As2	IAAIGCIAIGIGGIAAICC	
4	NBSf1	GGAATGGGNGGNGTNGGNAARAC	Yu et al. (1996)
	As2	IAAIGCIAIGIGGIAAICC	
5	S1	GGTGGGGTTGGGAAGACAACG	
	As1	CAACGCTAGTGGCAATCC	
6	S1	GGT GGGTT GGAAGACAACG	Leister et al. (1996)
	As2	IAAIGCIAIGIGGIAAICC	
7	S1	GGTGGGGTTGGGAAGACAACG	
	As3	IAGIGCIAIGIGGIAGICC	
8	NBSf1	GGAATGGGNGGNGTNGGNAARAC	Yu et al. (1996)
	NBSr1	YCTAGTTGTRAYDATDAYYYTRC	
9	NBSf1	GGAATGGGNGGNGTNGGNAARAC	Leister et al. (1996)
	As1	CAACGCTAGTGGCAATCC	
10	NBSf1	GGAATGGGNGGNGTNGGNAARAC	Yu et al. (1996)
	As3	IAGIGCIAIGIGGIAGICC	
11	RGA1f	AGTTTATAATTYSATTGCT	
	RGA2r	CACACGGTTTAAATTCTCA	
12	RGA1f	AGTTTATAATTYSATTGCT	Kanazin, Marek and Shoemaker (1996)
	RGA5r	TCAATCATTTCTTTGCACAA	
13	RGA1f	AGTTTATAATTYSATTGCT	
	RGA6r	AACTACATTTCTTGAAGT	
14	RGA1f	AGTTTATAATTYSATTGCT	
	RGA8r	CCGAAGCATAAGTTGGTG	
15	S2	GGIGGIGTIGGIAAIAACIAC	
	As3	IAGIGCIAIGIGGIAGICC	
16	As1	CAACGCTAGTGGCAATCC	Leister et al. (1996)
	As2	IAAIGCIAIGIGGIAAICC	
17	As1	CAACGCTAGTGGCAATCC	
	As3	IAGIGCIAIGIGGIAGICC	

I = A/T/G/C; D = A/G/T; E = C/G; R = A/G; Y = C/T.

Polymerase chain reaction (PCR) reactions were performed with the volume of 16 µL, containing 8 µL of DNA at 2 ng, 1 µL of each primer, 0.11 µL Taq DNA Polymerase, 1 µL of each dNTP mix, 0.25 µL each, 1 µL Magnesium Chloride (MgCl₂), 1.7 µL 10X Buffer (20 mM Tris-HCl [pH 8.4] and 50 mM KCl) and 2.19 µL sterile H₂O. The reactions were subjected to 35 cycles of amplification after initial denaturation at 95°C for 1 minute. Each cycle consisted of 40 seconds at 95°C, 1 minute at 37°C and 1 minute and 20 seconds at 72°C for annealing and extension. At the end of the cycles, a final extension of 5 minutes was performed at 72°C. PCR reactions were performed in triplicate on a Veriti 96 Well Thermal Cycler thermal cycler (Applied Biosystems®).

Reagents for the preparation of the genomic DNA extraction buffer were obtained from Sigma-Aldrich®. RGA markers and oligonucleotides for the PCR reaction, as well as intercalating and molecular weight markers, were obtained from Thermo Fisher Scientific®, supplied by Invitrogen®.

Electrophoresis in 2% (m v⁻¹) agarose gels with 0.5X TBE run buffer were performed from 6 µL aliquots of PCR products for 2 hours in 120 V electrical current. The gels were stained with Red Invitrogen Gel Biotium® which were exposed to the transilluminator (LPIX-EX, Loccus Biotecnologia, Cotias, São Paulo, Brazil) with incidence of ultraviolet light and photo documented.

Specifically for the second step of the characterization of the markers, descriptive analyzes of the amplification profiles were performed using Genes and GenAlex (Cruz, 2013; Peakall & Smouse, 2012) software, considering (i) the number of markers generated, the number and percentage of polymorphic markers (absent in at least one of the samples); the informative potential (PIC), calculated by the formula: $PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^n \sum_{j=1+1}^n 2p_i^2 p_j^2$ proposed by Botstein, White, Skolnick and Davis (1980); (iii) the expected heterozygosity (*He*) for each pair of primers, obtained according to Nei (1987) $He = 1 - \sum p_i^2$, where p_i = estimated allelic frequency of the *i* allele.

Results and discussion

The genomic DNA of the species *Prosopis juliflora* (Sx) PC showed average quality (purity) with ratio $A_{260}/A_{280}=1.919^{\pm 0.35}$ and $A_{260}/A_{230}=0.421^{\pm 0.15}$ and average concentration of $321.0^{\pm 0.15} \text{ ng } \mu\text{L}^{-1}$. The obtained value presented good purity even without having its RNA and proteins digested by RNase and proteinase enzymes. For this observation, the physiological nature of the species was considered, which holds many polysaccharides and phenolic compounds among other chemical constituents that favors low values of purity (Rathore, 2009). Although the absorbances showed values lower than 2.0, they corroborate the literature, because they were over 1.8. According to reports, values smaller than these are considered contaminated with proteins (Nelson & Cox, 2004). Therefore, this value is satisfactory, considering that we waived the treatment with RNase and proteinase to this species which contributed to the reduction of extraction and purification cost.

Another important factor that corroborates the result, is the operationalization of the extraction technique, it was also possible because we did not use β -mercaptoethanol and liquid nitrogen in order to make the extraction less harmful to health. It is known that the use of liquid nitrogen in the DNA extraction process allows for a better enzymatic neutralization and favors the disruption of the cell wrap. However, such use raises the procedure costs. Therefore, in the present study, the absence liquid nitrogen did not interfere with the quality of the extracted DNA, offering good results, which are also in agreement with the literature that advocates a low cost technique with fewer steps in the procedure (Michel-López et al., 2013; Santos & Araújo, 2017).

Regarding the characterization of the amplification results, 70% of the combinations tested RGA primers were classified as adequate (A) corresponding to primer number 7. In turn, they were classified as reasonable (R), corresponding to primer number 9 and inadequate (I) corresponding to primer number 2, with 12 and 18%, respectively, of the other amplifications observed. Considering that the large majority of RGA primer pairs (82%) made it possible to obtain molecular markers, it demonstrates the potential use of this technique in genetic studies of *P. juliflora* species (Figure 1).

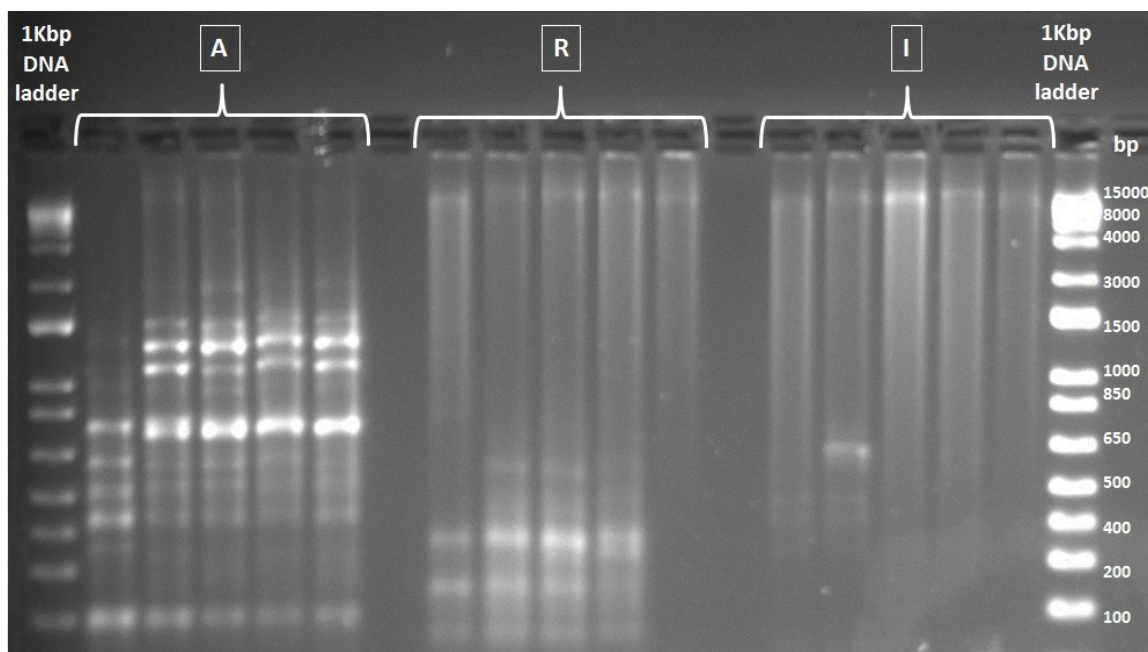


Figure 1. Amplification profiles obtained using Resistance Gene Analogs (RGA) pairs in *Prosopis juliflora* (Sw.) DC.

Notes: * A = Adequate; R = Regular; I = Inadequate.

The size of the fragments obtained with the RGA primers being considered in regard to two classifications (adequate and reasonable) ranged from 200 to 2.000 bp. These results corroborate Poolsawat, Kativat, Arsakit and Tantasawat (2017), who reported bands between 100 and 2.072 bp when associated with ISSR-RGA primers for *Vigna radiata* L. Wilczek. The 'A' pairs produced 68 markers in the 20 accessions studied, averaging 5.7 markers per primer pair. 10 out of these pairs showed 83% of polymorphic marks. The number of markers generated ranged from one for primer pairs 4 (NBsf1 and As2) and 10 (NBsf1 and As3) to 16 for primer pair 7 (S1 and As3) (Table 2). In turn, the percentage of polymorphism ranged from 60% for primer pair 14 (RGA1f and RGA8r) to 100% for primer pair 17 (As1 and As3) (Table 2).

Table 2. Characterization of the amplification profile of genomic regions of *Prosopis juliflora* (Sw.) DC. from 12 pairs of RGA (Resistance Gene Analogs) primers that presented adequate to 20 specimens.

Oligonucleotides Pairs	Markers generated	% Polymorphism	He	PIC
1	5	80	0.25	0.2
4	1	0	0	0
6	9	88.9	0.29	0.24
7	16	93.5	0.3	0.24
8	5	80	0.17	0.15
10	1	0	0	0
11	6	83.3	0.18	0.15
12	5	80	0.38	0.29
13	4	75	0.2	0.17
14	5	60	0.23	0.18
16	3	66.7	0.21	0.18
17	8	100	0.31	0.26
Total	68	-	-	-
Mean	5.7	67.3	0.21	0.17

He: Heterozigidade esperada; PIC: Conteúdo de Informação Polimórfica

As to the expected heterozygosity (*He*), considering the pairs of suitable primers selected for algaroba, a mean of 0.21 (ranging from 0 to 0.38) was observed. While the polymorphic information content (PIC) was 0.17 and ranged from 0 to 0.29. Variations of this nature aid in the selection of primers to be used in genetic studies. In algaroba for example, the combinations of these primers contributed to this selection, in which presented satisfactory values (Table 2). The variation observed in the efficiency of the amplifications, both with respect to identification of pairs of primers unsuitable for *P. juliflora*, and relative to the number of markers, percentage of polymorphism and other descriptive variables (Table 2), reinforce the importance of studies dedicated to the characterization and selection of primers.

In genotypes of *Passiflora* sp. (passion fruit) for example, Paula et al. (2010) used six pairs of RGA primers and obtained an average of 16.5 markers per primer pair.

Other studies with the same markers the RGAs of the present study were also carried out for passion fruit species. These described adequate amplification with 100% polymorphism for *Passiflora* Spp. (Souza et al., 2020). In *Passiflora setacea*, they registered polymorphism ranging from 99 to 100% (Pereira, Corrêa, & Oliveira, 2015). According to the authors, although the primers are adequate and with good amplification, the expected heterozygosity inferred in these two studies was low. This observation emphasizes that the RGA initiators can be used as a biomarker of genetic diversity, since the low heterozygosity is related to the domestication and adaptation of genotypes in an ex situ environment, to examples of accessions maintained in germplasms with good vegetative propagation and not the quality of molecular marker (Pires & Kageyama, 1985). Thus, the heterogeneity presented by the variation in the number of polymorphic loci between the pairs of RGA, favors studies of this nature, being useful in molecular genetic diagnostics.

Variations of the percentage of polymorphic profiles in RGA primers are also observed in other species, such as in *Croton linearifolius* MULL. ARG, where a total of 24 pairs of RGA primers generated 73 marks and 14 combinations showed polymorphic marks (Silva et al., 2018). Studies with other species of *Prosopis* sp. were also realized with other classes of molecular markers. From the selection of 80 pairs of RAPD, in study of the natural population of *Prosopis cineraria* (L.) Druces, only nine pairs were polymorphic (Sivalingam, Samadia, Singh, & Chauhan, 2016). Another study also analyzed the diversity between *Prosopis cineraria* (L.) Druce and *Prosopis juliflora* Sw. DC. populations collected at different sites in Qatar in Asian continent using other primers like ISSR and RAPD markers, where Elmeer and Almalki (2011) found a total of 190 bands in 29 ISSR primers and 19 bands of 7 RAPD primers with an average polymorphism of more than 99% in all genotypes, being able to distinguish between the two species. Nevertheless Alves et al. (2018), researching *Prosopis rubriflora* Hassl. and *Prosopis ruscifolia* Griseb. with microsatellites reported similar genetic diversity between species (*He* = 0.59 and *He* = 0.60, respectively). Already to the specie in study, the *P. juliflora*, Pires and Kageyama (1985) mentioned the possibility of inbreeding in this species during its adaptation, justifying the low expected heterozygosity compared to the species above. Despite the widespread use of molecular markers and the already proven efficiency of RGA markers in different genetic approaches, the results presented are pioneering for *Prosopis juliflora* Sw. DC.. Thus, it is expected that the 12 pairs of RGA primers used in this study will subsidize genetic research, especially actions to characterize diversity that contribute to the improvement and conservation of the naturalized species. Finally, it is suggested for future works the

sequencing for primers design of the fragments obtained by 7 and 12 pairs of primers which presented the highest indexes of polymorphic bands and information (Table 2) (George, Manoharan, Li, Britton, & Parida, 2017). Thus it will be possible to better understand the distribution, adaptation as well as the resistance of the algaroba specie to abiotic factors.

Conclusion

The twelve pairs of Resistance Gene Analogs (RGA) primers (S1 + NbSr1 = 1); Bsf1 + As2 = 4; S1 + As2 = 6; S1 + As3 = 7; NBsf1 + NBrs1 = 8; NBsf1 + As3 = 10; RGA1f + RGA2r = 11; RGA1f + RGA5r = 12; RGA1f + RGA6r = 13; RGA1f + RGA8r = 14; As1+As2 = 16 e As1 + As3 = 17) presented satisfactory polymorphism and pairs 7 and 12 can be used to genetic study and genetic improvement.

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References

- Alves, F. M., Sartori, Â. L., Zucchi, M. L., Azevedo- Tozzi, A. M. G., Tambarussi, E. V., Alves-Pereira, A., & Souza, A. P. (2018). Genetic structure of two *Prosopis* species in Chaco areas: a lack of allelic diversity diagnosis and insights into the allelic conservation of the affected species. *Ecology and Evolution*, 8(13), 6558-6574. doi: 10.1002/ece3.4137
- Bessegga, C., Cony, M., Saidman, B. O., Aguiló, R., Villagra, P., Alvarez, J. A., ... & Vilardi, J. C. (2019). Genetic diversity and differentiation among provenances of *Prosopis flexuosa* DC (Leguminosae) in a progeny trial: Implications for arid land restoration. *Forest Ecology and Management*, 443, 59-68. doi: 10.1016/j.foreco.2019.04.016
- Borém, A., & Caixeta, E. T. (2009). *Marcadores moleculares* (2a ed.). Viçosa, MG: UFV.
- Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32(3), 314-331.
- Carmo, T. V. B., Martins, L. S. S., Musser, R. D. S., Silva, M. M. D., & Santos, J. P. O. (2017). Genetic diversity in accessions of *Passiflora cincinnata* Mast. based on morphoagronomic descriptors and molecular markers. *Revista Caatinga*, 30(1), 68-77. doi: 10.1590/1983-21252017v30n108rc
- Cruz, C. D. (2013). Genes: a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy*, 35(3), 271-276. doi: 10.4025/actasciagron.v35i3.21251
- Cunha, L. H., & Gomes, R. A. (2012). A trajetória da algaroba no semiárido nordestino: dilemas políticos e científicos. *Raízes: Revista de Ciências Sociais e Econômicas*, 32(1), 72-95. doi: 10.37370/raizes.2012.v32.349
- Souza, L. N. B., Dias, N. D. S. C., Oliveira Santana, V., Silveira, L. A., Meira, M. R., Santos, E. S. L., ... Cerqueira-Silva, C. B. M. (2020). Amplification test and selection of markers analogue to resistance genes in species and commercial varieties of *Passiflora* spp. *Multi-Science Journal*, 3(1), 65-71. doi: 10.33837/msj.v3i1.1183
- Santos, E. M., & Araújo, R. R. (2017). Testes de comparação de protocolos de extração de DNA e de maceração de tecido de *Platonia insignis* Mart.(Clusiaceae). *Revista Brasileira de Biociências*, 15(4), 199-202.
- Elmeer, K., & Almalki, A. (2011). DNA finger printing of *Prosopis cineraria* and *Prosopis juliflora* using ISSR and RAPD techniques. *American Journal of Plant Sciences*, 2(4), 527-534. doi: 10.4236/ajps.2011.24062
- Faleiro, F. G., Amabile, R. F., & Cerqueira-Silva, C. B. M. (2018). *Marcadores moleculares aplicados ao melhoramento genético de plantas* (Livro Técnico - INFOTECA-E, p. 44-66). Planaltina, DF: Embrapa Cerrados.
- Fritsche-Neto, R., Resende, M. D. V., Miranda, G. V., & DoVale, J. C. (2015). Seleção genômica ampla e novos métodos de melhoramento do milho. *Ceres*, 59(6). doi: 10.1590/S0034-737X2012000600009
- George, S., Manoharan, D., Li, J., Britton, M., & Parida, A. (2017). Transcriptomic responses to drought and salt stress in desert tree *Prosopis juliflora*. *Plant Gene*, 12, 114-122. doi: 10.1016/j.plgene.2017.09.004

- Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323-329. doi: 10.1038/nature05286
- Kanazin, V., Marek, L. F., & Shoemaker, R. C. (1996). Resistance gene analogs are conserved and clustered in soybean. *Proceedings of the National Academy of Sciences*, *93*(21), 11746-11750. doi: 10.1073/pnas.93.21.11746
- Leão, T. C., Almeida, W. D., Dechoum, M. D. E. S., & Ziller, S. R. (2011). *Espécies exóticas invasoras no Nordeste do Brasil: contextualização, manejo e políticas públicas*. Recife, PE: Centro de Pesquisas Ambientais do Nordeste e Instituto Hórus de Desenvolvimento e Conservação Ambiental.
- Leister, D., Ballvora, A., Salamini, F., & Gebhardt, C. (1996). A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. *Nature Genetics*, *14*(4), 421-429. doi: 10.1038/ng1296-421
- Michel-López, C. Y., González-Mendoza, D., & Grimaldo-Juarez, O. (2013). Fast protocol for extraction of DNA from *Prosopis* spp leaves (plant adapted to arid environment) without liquid nitrogen. *Genetics and Molecular Research*, *12*(3), 4090-4094. doi: 10.4238/2013.September.27.10
- Nei, M. (1987). *Molecular evolutionary genetics*. New York, NY: Columbia University Press.
- Nelson, D. L., & Cox, M. M. (2004). *Lehninger, Principles of Biochemistry*. New York, NY: Worth Publishers.
- Paula, M. D. S., Fonseca, M. D. N., Boiteux, L. S., & Peixoto, J. R. (2010). Caracterização genética de espécies de *Passiflora* por marcadores moleculares análogos a genes de resistência. *Revista Brasileira de Fruticultura*, *32*(1), 222-229. doi: 10.1590/S0100-29452010005000021
- Peakall, R. O. D., & Smouse, P. E. (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
- Pereira, D. A., Corrêa, R. X., & Oliveira, A. C. (2015). Molecular genetic diversity and differentiation of populations of 'somnus' passion fruit trees (*Passiflora setacea* DC): Implications for conservation and pre-breeding. *Biochemical Systematics and Ecology*, *59*, 12-21. doi: 10.1016/j.bse.2014.12.020
- Pires, I. E., & Kageyama, P. Y. (1985). Caracterização da base genética de uma população de algaroba – *Prosopis juliflora* (Sw) DC- existente na região de Soledade – PB. *Instituto de Pesquisas e Estudos Florestais*, *30*, 1-8. Recuperado de <https://bitlybr.com/Bz13P>
- Pomponio, M. F., Acuña, C., Pentreath, V., Lauenstein, D. L., Poltri, S. M., & Torales, S. (2015). Characterization of functional SSR markers in *Prosopis alba* and their transferability across *Prosopis* species. *Forest Systems*, *24*(2), 2171-9845. doi: 10.5424/fs/2015242-07188
- Poolsawat, O., Kativat, C., Arsakit, K., & Tantasawat, P. A. (2017). Identification of quantitative trait loci associated with powdery mildew resistance in mung bean using ISSR and ISSR-RGA markers. *Molecular Breeding*, *37*, 150. doi: 10.1007/s11032-017-0753-2
- Rai, M. K., Shekhawat, J. K., Kataria, V., & Shekhawat, N. S. (2017). Cross species transferability and characterization of microsatellite markers in *Prosopis cineraria*, a multipurpose tree species of Indian Thar Desert. *Arid Land Research and Management*, *31*(4), 462-471. doi: 10.1080/15324982.2017.1338791
- Rathore, M. (2009). Nutrient content of important fruit trees from arid zone of Rajasthan. *Journal of Horticulture and Forestry*, *1*(7), 103-108.
- Ribaski, J., Drumond, M. A., Oliveira, V. R., & Nascimento, C. D. S. (2009). *Algaroba (Prosopis juliflora): árvore de uso múltiplo para a região semiárida brasileira* (Comunicado Técnico - INFOTECA-E). Colombo, PR: Embrapa Florestas.
- Rodrigues, L. C., Silva, A. A., Silva, R. B., Oliveira, A. F. M., & Andrade, L. H. C. (2013). Conhecimento e uso da carnaúba e da algaroba em comunidades do Sertão do Rio Grande do Norte, Nordeste do Brasil. *Revista Árvore*, *37*(3). doi: 10.1590/S0100-67622013000300008
- Silva, T. S. S., Freitas, J. S., Santos, E. S. L., Cardoso, T. S., & Cerqueira-Silva, C. B. M. (2018). Caracterização e seleção de marcadores moleculares em *Croton linearifolius* Mull. Arg. como subsídio para estudos genéticos. *Multi-Science Journal*, *1*(10), 4-8. doi: 10.33837/msj.v1i10.354
- Sivalingam, P. N., Samadia, D. K., Singh, D., & Chauhan, S. (2016). Molecular markers to distinguish 'Thar Shoba', a variety of khejri [*Prosopis cineraria* (L.) Druce], from trees in natural populations. *The Journal of Horticultural Science and Biotechnology*, *91*(4), 353-361. doi: 10.1080/14620316.2016.1160545
- Souza, D. C. L. (2015). Técnicas moleculares para caracterização e conservação de plantas medicinais e aromáticas: uma revisão. *Revista Brasileira de Plantas Medicinais*, *17*(3), 495-503. doi: 10.1590/1983-084X/13_071

- Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, 13(3), 510-524. doi: 10.1093/oxfordjournals.molbev.a025612
- Yu, Y. G., Buss, G. R., & Maroof, M. A. (1996). Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. *Proceedings of the National Academy of Sciences*, 93(21), 11751-11756. doi: 10.1073/pnas.93.21.11751