Amplification test and selection of markers analogue to resistance genes in species and commercial varieties of *Passiflora* spp.

Larissa Neres Barbosa de Souza[1], Nátilla Deyse Souza Costa Dias[1], Vandrick de Oliveira Santana[1], Lucas Amorim Silveira[2], Messulan Rodrigues Meira[1], Elisa Susilene Lisboa dos Santos[1,2,3], Fábio Gelape Faleiro[4], Carlos Bernard Moreno Cerqueira-Silva [1,2,3*]

[1]*Postgraduate Program in Environmental Sciences, State University of Southwest Bahia, 45700-000; Itapetinga, BA, Brazil.*
[2]*Biochemistry and Molecular Biology Multicentric Program, State University of Southwest Bahia, 45700-000, Itapetinga, BA, Brazil.*
[3]*Department of Exact and Natural Sciences, State University of Southwest Bahia, 45700-000, Itapetinga, BA, Brazil.*
[4]*Embrapa Cerrados - CPAC, 73010-970, Planaltina, DF, Brazil.*

**Corresponding author. E-mail: csilva@uesb.edu.br*

ABSTRACT. Brazil is highlighted as the largest producer and consumer of passion fruit (*Passiflora*) and holder of representative biodiversity. Studies dedicated to the genetic characterization of the genus in order to understand the existing diversity are important for the advancement of genetic improvement and conservation programs. The aim of this study was to select combinations of RGA (Resistance Genes Analogs) primers for commercial passion fruit species and varieties. To this end, amplification tests were performed on a PCR platform in 17 combinations of RGA primers for eight species and 12 commercial varieties of passion fruit from the Banco Ativo de Germoplasma Flor da Paixão. All combinations of primers have generated amplification products in at least one of the *Passiflora* samples. The number of combinations that generated amplification products varied from seven to 12 in the species and from three to eight in the varieties. Three to 12 combinations generated amplification products for the samples, emphasizing that the RGA markers were efficient in accessing genomic loci in the species and varieties of passion fruit. The variation of efficient combinations in each of the species and varieties attested to the importance of this research in the preliminary stages of genetic studies as auxiliary tools in conservation and genetic improvement programs. However, this study made it possible to identify combinations of primers to be prioritized in inter and intraspecific genetic characterizations of passion fruit.

Keywords: Passion fruit, Selection, RGA, Genetic enhancement.

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INTRODUCTION

The genus *Passiflora* is the most representative of the Passifloraceae family. It has about 525 species; Brazil is one of the main centers of diversity of the genus with approximately 160 species of passion fruit, (Cervi & Imig, 2013). Brazil also stands out for the expressive number of endemic *Passiflora* species, with approximately 90 species, in addition to standing out as the largest producer and consumer of passion fruit in the world (Bernacci et al. 2016, Gonçalves & Souza, 2006).

Passion fruit show considerable intra and interspecific genetic variability (Meletti et al. 2005, Junqueira et al. 2005), giving the Passifloraceae family economic value added to different sectors such as food, medicinal and ornamental. As a food, the fruit is consumed both fresh and processed, while in the pharmaceutical industry, the entire plant is used (Castro, 2012, Cunha et al. 2004). With regard to ornamental use, the highlights are its beautiful foliage and the diversity of shapes and colors of its flowers. Genetic variability is generally considered fundamental for the maintenance and advancement of genetic improvement programs (Nass, 2011). Attributes like these are observed in passion fruit, which have improvement actions dedicated to the characteristics regarding the shape and size of the fruits, color and flavor of the pulp, content of glycosylated flavonoids as well as antioxidant agents, quantity, periodicity and durability of the flowers

(Costa &Tupinambá, 2005, Faleiro et al. 2006, Faleiro et al. 2008, Zeraik, 2010, Jesus et al. 2014, Cerqueira-Silva et al. 2018). Another attribute of the genus linked to variability is survival in environments unsuitable for annual crops such as grains. This genetic advantage gives the plant economic viability, as it survives under conditions of biotic and abiotic stress (Faleiro et al. 2011, Cerqueira-Silva et al. 2014).

Although the *Passiflora* genus has natural genetic diversity and potential economic value, the increase in human actions poses a threat to the maintenance and expansion of this diversity (Cerqueira-Silva et al. 2015, Jesus et al. 2014). To mitigate the loss of these resources, it is necessary to invest in *ex situ* conservation, with active germplasm banks being examples of strategies for the conservation and characterization of genetic heritage.

Brazil has the largest collection of accessions and varieties of passion fruit distributed and preserved in Active Germplasm Banks (AGBs) (Meletti et al. 2005). This initiative, although not the only one, is effective in preventing the loss of genetic material and subsidizing improvement studies, since it allows the characterization, evaluation, exchange and documentation of germplasm (Jesus et al. 2014, Sousa, 2014). The characterization and evaluation of conserved materials in terms of biological, agronomic and genetic aspects is essential in the context of the action of species management and conservation plans (Faleiro et al. 2011).

Genetic characterization studies of the genus *Passiflora* have been carried out using different molecular markers for more than two decades (Cerqueira-Silva et al. 2014, Cerqueira-Silva et al. 2015). Molecular markers make it possible, in general, to differentiate genetically related individuals (Borém & Miranda, 2005). They generate genetic information with the most varied practical developments, ranging from the indication of preferential crosses, to the genetic mapping of genes of interest and or understanding of specific metabolic routes (Faleiro, 2018, Cerqueira-Silva et al. 2014).

The RGA markers have conserved regions and can represent sites of biological relevance in the phenotypic expression of resistance genes, thus presenting themselves as a useful tool for different genetic approaches (Bent, 1996). Based on phylogenetic conservation, different primers were developed in order to amplify regions analogous to resistance genes (Leister et al. 1996, Kanazin et al. 1996, Yu et al. 1996). Thus, markers of this nature make it possible to obtain a lot of polymorphism between different accessions of the same species or different species of genus, which may corroborate in systems of genetic characterization of accessions and cultivars (Hammond-Kosack & Jones, 1997).

A study carried out by Paula and collaborators in 2010, with these same initiators in the evaluation of wild passionflower, reported satisfactory results, which observed a wide genetic variety. According to the authors, such variability made it possible to establish levels of similarity between accessions, as well as to identify sequences related to the resistance gene to plant pathogens. Studies like this contribute to guide the selection of resistant specimens to be inserted in breeding programs.

Despite the complexity of actions and developments related to the use of molecular biology in general and molecular markers in particular, the characterization and selection of markers to be used is a basic step used in genetic studies, regardless of the class of marker to be used. Thus, the aim was to select combinations of RGA to be used as a priority in studies of genetic characterization of species and commercial varieties of passion fruit.

MATERIAL AND METHODS

DNA collection and sample storage

The plant material was collected at Embrapa Cerrados - Cerrados Agricultural Research Center - CPAC, located in Brasília-DF under the geographic coordinates (S15.6041265, W-47.7119669). 38 representative accessions of passion fruit species were collected at the Germoplasm Active Bank (BAG) Flor da Paixão da Embrapa Cerrados, available on the platform http://alelobag.cenargen.embrapa.br. Of the accessions used in the present study, eight are representatives of species and 12 are of varieties obtained in the genetic improvement programs of Embrapa (Table 1).

Access DNA was extracted using the CTAB 2% method (*Cationic Hexadecyl Trimethyl Ammonium Bromide*) as recommended by Faleiro et al. (2003) and quantified by spectrophotometry using the NanoDrop® equipment. Subsequently, the DNA samples extracted from the accessions were diluted in the proportion of 100 µl to 50 ng μL^{-1} for standardization, conditioned on dry ice and taken to the Laboratory of Applied Molecular Genetics, of the State University of Southwest Bahia (UESB), *Campus* Itapetinga-BA (LGMA-UESB) where they were submitted to the polymerase chain amplification reaction (PCR).

Polymerase chain amplification test (PCR)

For the amplification tests, 17 combinations of RGA primers (*Resistance Genes Analog*) (Table 2), previously evaluated for species of the genus *Passiflora* spp. (Pereira, 2012, Paula et al. 2010) and for the species *Cucumismelo* L. (Maciel, 2014).

The PCR reaction consisted of 16 µL, containing: 8 µL of DNA at 2 ng / µL; 1 µL of each RGA initiator; 0.11 µLTaq DNA Polymerase; 1 µL of

the dNTP mix; 1 µL of Magnesium Chloride (MgCl2); 1.7 of 10X Buffer; 2.19 µL of ultra-pure water (q.s.p). The programs adopted for the amplification reactions consisted of 5 minutes at 95 ° C for initial denaturation; followed by 34 cycles (30 seconds at 95 ° C, 1 minute at 37°C for annealing, 1 min and 20 sec at 72 ° C for extension); followed by a final 10 min extension. at 72°C.

The amplification products were subjected to a horizontal electrophoresis system in 2% agarose gel (m / v) and TBE buffer (0.5X) (Trisborate-EDTA) with a voltage of 120 W for 2 hours. Red Biotium® safe Gel (Uniscience) was used as an intercalant and the 1 Kb Plus DNA Ladder standard (Invitrogen, Carlsbad, CA, USA) was used as a size standard for the obtained amplification products. Subsequently, the gels were exposed to the ultraviolet transluminator and documented in a Kodak photo-documentation system (KODAK MI Software).

Descriptive analysis and selection of markers

The amplification patterns of the 17 combinations of RGA initiators for the eight species and 12 commercial varieties were analyzed by two researchers, aiming to increase the reliability and reproducibility of the readings / interpretations of the amplification patterns. In summary, the amplification results were classified as Adequate (+) or Inadequate (-), depending on the presence, or not, of bands (markers) in the 2% agarose gels (Figure 1).

The data were tabulated in order to have a description related to the totals and percentages of the classifications of the combinations of RGA starters in each of the 8 species and 12 commercial varieties of passion fruit, allowing both the identification of the combinations to be prioritized in each sample, as well as those to be prioritized in interspecific studies.

Table 1. Species of the genus *Passiflora* spp. classified according to subgenre, number of accesses used and their respective codes.

Species	Subgender	Accession code
P. alata Curtis	*Passiflora*	CPAC MJ -02-17
		CPAC MJ -02-09
		CPAC MJ-02-09
		CPAC MJ -02-19
P. cincinnata Mast	*Passiflora*	CPAC MJ -26-03 (PL1)
		CPAC MJ -26-03 (PL2)
		CPAC MJ -26-03 (PL3)
		CPAC MJ -26-03 (PL4)
P. coccínea Aubl.	*Passiflora*	CPAC MJ -08-05
		CPAC MJ -08-01
		CPAC MJ-08-02
P. edulis Sims	*Passiflora*	CPAC MJ-21-07
		CPAC MJ-21-07
		CPAC MJ-21-07
		CPAC MJ-21-06
P. incarnata L.	*Passiflora*	CPAC MJ-31-02
		CPAC MJ-31-02
P. quadrifaria Vanderpl	*Passiflora*	CPAC MJ-42-02
		CPAC MJ-42-03
		CPAC MJ-42-01
		CPAC MJ-42-01
P. setacea DC.	*Passiflora*	CPAC MJ-12-04
		CPAC MJ-12-06
		CPAC MJ-12-06
		CPAC MJ-12-04
P. tenuifila J. C Mikan	*Passiflora*	CPAC MJ-30-01
BRS Céu do Cerrado	Plants variety	PM GP CPAC
BRS Estrela do cerrado		PM GP CPAC
BRS Gigante amarelo		PM GP CPAC
BRS Mini maracuja roxo		PM GP CPAC
BRS Mel do Cerrado		PM GP CPAC
BRS Pérola do Cerrado		PM GP CPAC
BRS Rosa púrpura		PM GP CPAC
BRS Roseflora		PM GP CPAC
BRS Rubi do Cerrado		PM GP CPAC
BRS Rubiflora		PM GP CPAC
BRS Sol do Cerrado		PM GP CPAC
BRS Vitta		PM GP CPAC

Table 2. Description of combinations of RGA (Resistance Genes Analog) initiators used in the amplification tests with 8 species and 12 commercial varieties of passion fruit.

Combination	Initiators	Nucleotide sequence 5 '- 3'
1	S1	GGTGGGGTTGGGAAGACAACG
	NBSr1	GGTGGGGTTGGGAAGACAACGYCT
2	S2	GGIGGIGTIGGIAAIACIAC
	As1	CAAGGCTAGTGGCAATCC
3	S2	GGIGGIGTIGGIAAIACIAC
	As2	IAAIGCIAGIGGIAAICC
4	NBSf1	GGAATGGGNGGNGTNGGNAARAC
	As2	IAAIGCIAGIGGIAAICC
5	S1	GGTGGGGTTGGGAAGACAACG
	As1	CAAGGCTAGTGGCAATCC
6	S1	GGTGGGGTTGGGAAGACAACG
	As2	IAAIGCIAGIGGIAAICC
7	S1	GGTGGGGTTGGGAAGACAACG
	As3	IAGIGCIAGIGGIAGICC
8	NBSf1	GGAATGGGNGGNGTNGGNAARAC
	NBSr1	GGTGGGGTTGGGAAGACAACGYCT
9	NBSf1	GGAATGGGNGGNGTNGGNAARAC
	As1	CAAGGCTAGTGGCAATCC
10	NBSf1	GGAATGGGNGGNGTNGGNAARAC
	As3	IAGIGCIAGIGGIAGICC
11	RGA1f	AGTTTATTATTYSATTGCT
	RGA2r	CACACGGTTTAAAATTCTCA
12	RGA1f	AGTTTATTATTYSATTGCT
	RGA5r	TCAATCATTTCTTTGCACAA
13	RGA1f	AGTTTATTATTYSATTGCT
	RGA6r	AACTACATTTCTTGCAAGT
14	RGA1f	AGTTTATTATTYSATTGCT
	RGA8r	CCGAAGCATAAGTTGGTG
15	S2	GGIGGIGTIGGIAAIACIAC
	As3	IAGIGCIAGIGGIAGICC
16	As1	CAAGGCTAGTGGCAATCC
	As2	IAAIGCIAGIGGIAAICC
17	As1	CAAGGCTAGTGGCAATCC
	As3	IAGIGCIAGIGGIAGICC

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

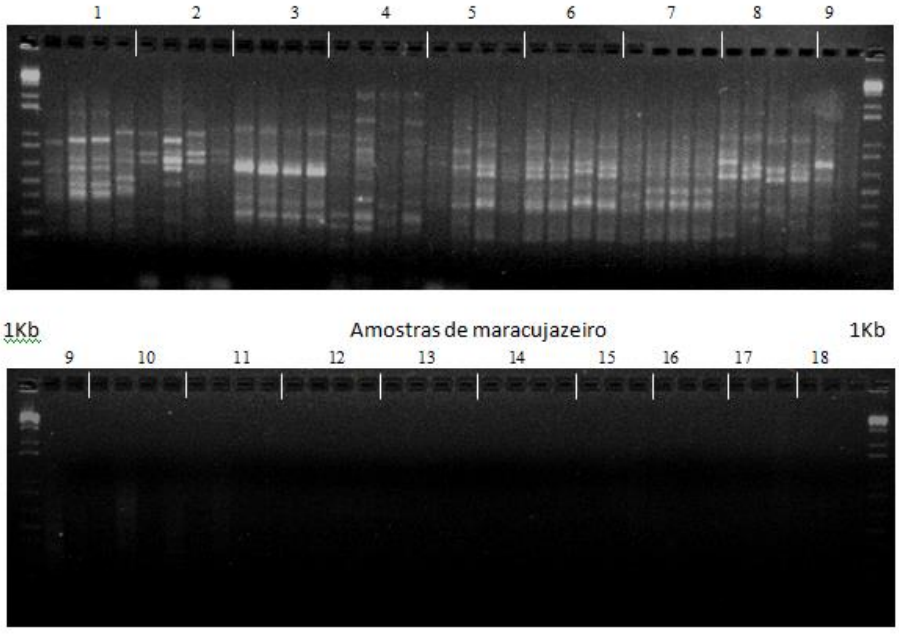

Figure 1. Amplification profiles (in 2% agarose gels) generated from combinations of RGA starters in commercial passion fruit species and varieties (*Passiflora*), being considered adequate, due to the presence of bands / markers, the superior and inappropriate profile, by the absence of bands / markers, the lower profile.1- *P. alata*; 2- *P. cincinnata*; 3- *P. edulis*; 4- *P. foetida*; 5- *P. maliformes*; 6- *P. nitida*; 7- *P. quadrangularis*; 8- *P. amethystina*; 9- *P. caerulea*; 10- *P. organensis*; 11- *P. quadrifaria*; 12- *P. setacea*; 13- *P.suberosa*; 14- *P tholosanii*; 15- *P.vitifolia*; 16- *P. actinea*; 17- *P. ambígua*; 18- *P. capsularis*.

RESULTS AND DISCUSSION

The wide variation in the efficiency of the different combinations of RGA initiators in generating amplification products in the commercial passion fruit species and varieties (Table 3) reinforces the importance of amplification tests as a preliminary step in the effective conduct of genetic studies. The importance of such studies, as a preliminary step to save costs and enhance the efficiency of choosing molecular markers in genetic studies, was also recognized by Silva et al. (2018) in the genus *Croton*, Vieira et al. (2019) and Amaral et al. (2019) in the genus *Melocacatus*, in addition to Paula et al. (2010) in genus *Passiflora*.

The number of combinations that generated amplification products ranged from seven (for *P. tenuifila*) to 12 (for *P. edulis*), in the passion fruit species, and from three (for the BRS roseflora, sol do cerrado and vitta varieties) to eight (for the BRS giant yellow variety), in the varieties evaluated (Table 3).The greater number of RGA combinations classified as appropriate among the passion fruit species, to the detriment of the varieties can, among other possibilities, be related to the greater genetic diversity expected for wild accessions at the expense of germplasms resulting from selection and improvement, as it is the case for natural varieties. In this context, germplasm banks and especially wild species are recognized as an essential source of genetic variability for the maintenance and advancement of breeding programs. (Nass, 2011, Faleiro et al. 2011, Cerqueira-Silva et al. 2015).

On average, the combinations of RGA primers were suitable for approximately eight species and, or, varieties, enhancing the use of these markers in interspecific studies. Only two combinations of primers, namely: combinations 13 (RGA1f and RGA6r) and 16 (As1 and As2) proved to be suitable for a single species (*P. incarnata*). All other combinations are suitable for a minimum of three species, such as combinations 12 (RGA1f and RGA5r) and 15 (S2 and AS3), with combinations with common amplification for all species and varieties tested (combination 5, primers S1 and As1). It was possible to identify three to 12 combinations of primers that generated amplification products in the evaluated passion fruit, which proved to be efficient in accessing genomic loci in the species and varieties of passion fruit.

In a study by Pereira (2012), 85% of the tested RGA starter combinations amplified in the yellow passion fruit varieties, in addition, for a total of 15 starter combinations, five showed a greater number of reading regions with clear visualization. The combinations of S1 + As2 and RGA1f + RGA2r primers, corresponding to combinations 6 and 11 of this study, were tested by Pereira (2012), with no amplification products being observed. Whereas for the species *P. setaceae*, Pereira et al. (2015) tested four combinations and obtained 100% polymorphism with high quality.

Table 3. Descriptive presentation of the classification, considering the occurrence (+) or not (-) of amplification in the eight species and 12 commercial varieties of passion fruit (*Passiflora*) evaluated.

Accessions	RGA primer combinations																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total	(%)
Pal	-	+	+	+	+	-	+	+	+	+	-	-	-	-	+	-	+	10	58,8
Pci	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	+	9	52,9
Ped	+	+	+	+	+	-	+	+	+	+	+	-	-	+	-	-	+	12	70,5
Pqu	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	10	58,8
Pse	+	+	+	+	+	-	-	+	+	+	-	-	-	+	-	+	+	11	64,7
Pco	+	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	8	47
Pin	+	-	+	-	+	-	+	+	+	-	-	+	+	-	+	-	+	10	58,8
Pte	+	-	-	-	+	+	+	-	+	-	+	-	-	-	-	-	+	7	41,1
BRS1	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	6	35,2
BRS2	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	6	35,2
BRS3	+	-	+	-	+	+	+	-	-	-	+	+	-	-	-	-	+	8	47
BRS4	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	6	35,2
BRS5	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	6	35,2
BRS6	+	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	+	5	29,4
BRS7	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-	5	29,4
BRS8	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	3	17,6
BRS9	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	4	23,5
BRS10	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	4	23,5
BRS11	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	3	17,6
BRS12	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	3	17,6
Total	19	6	6	5	20	10	13	4	7	6	9	3	1	4	3	1	19		
(%)	95	30	30	25	100	50	65	20	35	30	45	15	5	20	15	5	95		

* Species code: Pal: *P. alata* Curtis; Pci: *P. cincinnata* Mast .; Ped: *P. edulis* Sims; Pqu: *P. quadrifaria* Vanderpl; Pse: *P. setacea* DC; Pco: *P. coccinea* Aubl .; Pin: *P. incarnata* L .; Pte: *P. tenuifila* Killip; BRS Céu do Cerrado; BRS Estrela do Cerrado; BRS Gigante amarelo; BRS Mini maracujá roxo; BRS Mel do Cerrado; BRS Pérola do Cerrado; BRS Rosa púrpura; BRS Roseflora; BRS Rubi do Cerrado; BRS Rubiflora; BRS Sol do Cerrado; BRS Vitta.

These records by Pereira et al. (2015) show that the efficiency of the RGA markers varies according to the species, the physiological stage of the plant as well as the quality of the genomic material, since the same primers present a distinct amplification pattern between accessions. Variations in amplification patterns may be related to the fact that RGA primers, in general, amplify regions associated with resistance genes (Paula et al. 2010). As observed among the accessions of the 12 varieties (BRS), in which most combinations of RGA starters were classified as inadequate. The difference in classification of the amplification product between species and cultivars, makes different combinations of RGA potential for detailing in studies of molecular genetic diagnostics of wild, domesticated and previously improved species.

Therefore, the preliminary selection of these accessions contributes to optimize the maintenance of germplasms as well as to identify promising accessions to be conserved and to select them for future genetic improvement studies (Paula et al. 2010).The results presented in this research are important for the characterization and genetic screening of passion fruit, since there are few studies with RGA combinations for the Passifloraceae family.

CONCLUSION

The studied RGA primers generated passion fruit amplification products in all combinations. Although all are potentially useful to support molecular genetic studies, their applicability will depend on the objective in question. However, the initiators classified as adequate, will enhance information at the population level *in situ*, collections of germplasm *ex situ* and even genotypes inserted in genetic improvement programs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Amaral, D. O. J., Araújo, D. R. C., Freitas, J. G., Batista, F. R. C (2019). Seleção de primers polimórficos para estudo de diversidade genética em cactaceas. Multi-ScienceJournal, 2(3), 9-13.

Barbosa, N. C. S. (2016). Anatomia foliar e diversidade genética em Passiflora spp. (Passifloraceae l.) resistentes ao cowpeaaphid-borne mosaicvirus (CABMV). (Dissertação de mestrado). Universidade Federal da Bahia, Brasil.

Bellon, G., Faleiro, F. G., Junqueira, K. P., Junqueira, N. T. V., Santos, E. C., Braga, M. F., Guimarães, C. T. (2007). Variabilidade genética de acessos silvestres e comerciais de Passiflora edulissims. com base em marcadores RAPD. RevistaBrasileira de Fruticultura. 29(1), 124-127.

Bent, A. F. (1996). Plant Disease Resistance Genes: Function Meets Structure. Plant Cell, Rockville, 8(10), 1757-1771.

Bernacci, L. C., Cervi, A. C., Milward-De-Azevedo, M. A., Nunes, T. S., Imig, D. C., Mezzonato, A. C. (2016). Passifloraceae. In: lista de espécies da flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponível em: <http://reflora.jbrj.gov.br/jabot/floradobrasil/FB12534> Acesso em: 13/02/2017.

Borém, A., Miranda, G. V. (2005). Marcadores Moleculares. In: Melhoramento de Plantas. (4º Ed.). (pp. 441-463). Viçosa: UFV.

Castro, J. A. (2012). Conservação dos recursos genéticos de Passiflora e seleção de descritores mínimos para caracterização de maracujazeiro. (Dissertação de Mestrado). Universidade Federal do Recôncavo Da Bahia, Brasil.

Cerqueira-Silva, C. B. M., Faleiro, F. G., Jesus, O. N., Santos, E. S. L., Souza, A. P. (2018). Passion Fruit (Passiflora spp.) Breeding. In: Al-Khayri, J., Jain, S., Johnson, D. (1º Ed.). Advances in Plant Breeding Strategies: Fruits. Springer.

Cerqueira-Silva, C. B. M, Faleiro, F. G., Nunes, O., Santos, E. S. L., Souza, A. P. (2015). The Genetic Diversity, Conservation, and Use of Passion Fruit (Passiflora spp.). Switzerland: Springer InternationalPublishing, 8, 215-231.

Cerqueira-Silva, C. B. M., Jesus, O. N., Santos, E. S. L., Corrêa, R. X., Souza, A. P. (2014). Genetic Breeding and Diversity of the Genus *Passiflora*: Progress and Perspectives in Molecular and Genetic Studies. Int. J. Mol. Sci. 15, 14122-14152.

Cervi, A. C., Imig, D. C. (2013). A new species of Passiflora (passifloraceae) from. Mato Grosso do Sul, Brasil. Phytotaxa, 103(1), 46-50

Costa, A. M., Tupinambá, D. D. (2005). O maracujá e suas propriedades medicinais- estado da arte. In: Faleiro, F. G., Junqueira, N. T. V., Braga, M. F. (Eds.) Maracujá: germoplasma e melhoramento genético. (pp. 475-508). Planaltina: Embrapa Cerrados.

Costa, J. L., Oliveira, E. J., Jesus, O. N. De, Oliveira, G. A. F., Neves, C. G., Castro, J. A. (2010). Estudo de diversidade genética em Passiflora edulissims com o uso de marcadores ISSR. In: Congresso Brasileiro de Fruticultura, 17-22 Outubro de 2010, Natal, RN (pp. 1-5). Natal: Sociedade Brasileira de Fruticultura.

Cunha, M. A. P., Barbosa, L. V., Faria, G. A. (2004). Botânica. In: Lima, A. De A.; Cunha, M. A. P. Maracujá: Produção e qualidade na passicultura. (pp. 13-36). Cruz das Almas: Embrapa Mandioca e Fruticultura.

Faleiro, F. G., Amabile, R. F., Silva, C. B. M. C. (2018). Marcadores Moleculares Aplicados ao Melhoramento Genético de Plantas. In: Amabile, R. F., Vilela, M. S., Peixoto, J. R. (1º Ed). Melhoramento de plantas: variabilidade genética, ferramentas e mercado. (pp. 53-76). Brasília: Sociedade Brasileira de Melhoramento de Plantas.

Faleiro, F. G., Junqueira, N. T. V., Braga, M. F., Peixoto, J, R. (2011). Pré-melhoramento do maracujá. In: Lopes, M. A., Fávero, A. P., Ferreira, M. A. J. F., Faleiro, F. G., Folle, S. M., Guimarães, E. P. (Eds). Pré-melhoramento de plantas. Estado da arte e experiências de sucesso. (pp 549–570). Brasília: Embrapa Informações Tecnológicas.

Faleiro, F. G., Junqueira, N. T. V., Braga, M. F., Oliveira, E. J., Peixoto, J. R., Costa, A. M. (2011). Germoplasma e melhoramento genético do maracujazeiro: histórico e perspectivas. (pp. 10-18). Planaltina: Embrapa Cerrados.

Faleiro, F. G., Junqueira, N. T. V., Braga, M. F., Peixoto, J. R. (2008). Caracterização de germoplasma e melhoramento genético do maracujazeiro assistidos por marcadores moleculares: resultados de pesquisa 2005-2008. (pp. 59). Planaltina: Embrapa Cerrados. (Boletim de Pesquisa e Desenvolvimento, Nº 207).

Faleiro, F. G., Junqueira, N. T. V., Braga, M. F. (2006). Maracujá: demandas para a pesquisa. (pp. 54). Planaltina, DF: Embrapa

Cerrados.

Faleiro, F. G., Faleiro, A. S. G., Cordeiro, M. C. R., Karia, C. T. (2003). Metodologia para operacionalizar a extração de DNA de espécies nativas do cerrado. (pp. 1-5). Planaltina: Embrapa Cerrados.

Gonçalves, J. S., Souza, S. A. M. (2006). Fruta da paixão: panorama econômico do maracujá no Brasil. Informações Econômicas, São Paulo, 36(12), 29-35.

Hammond-Kosack, K. E., Jones, J. D. G. (1997). Plant Disease Resistance Genes. Annual Review of Plant Physiology and Plant Molecular Biology, 48, 575-607.

Jesus, O. N., Machado, C. F., Soares, T. L., Junghans, T. G., Oliveira, E.J.,Faleiro, F. G. (2013). Recursos genéticos de Passiflora na Embrapa Mandioca e Fruticultura. In: Congreso Latinoamericano de Passifloras, Neiva, Huila, Colombia, 20-22 novembro de 2013 (pp. 24-35). Libro de Memorias. Neiva: CEPASS HUILA.

Junqueira, N. T. V., Braga, M. F., Faleiro, F. G., Peixoto, J. R., Bernacci, L. C. (2005). Potencial de espécies silvestres de maracujazeiro como fonte de resistência a doenças. In: Faleiro, F. G., Junqueira, N. T. V., Braga, M. F. (Eds.). Maracujá: germoplasma e melhoramento genético. (pp. 81-107) Planaltina: Embrapa Cerrados.

Kanazin, V., Frederick, M. L., Shoemaker, R. C. (1996). Resistance Gene Analogs are Conserved and Clustered in Soybean. Proceedings of the National Academy of Sciences of the U S A, 93, 11746-11750.

Leister, D., Ballvora, A., Salamini, F. E., Gebhardt, C. A. (1996). A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. NatureGenetics, 14, 421-429.

Maciel, C. C. (2014). Diversidade de genótipos de melão por meio de descritores morfológicos e marcador RGA. (Dissertação de Mestrado). Universidade Estadual de Santa Cruz, Brasil.

Meletti, L. M. M., Soares-Scott, M. D., Bernacci, L. C., Passos, I. R. S. (2005). Melhoramento genético do maracujá: passado e futuro. In: Faleiro, F. G., Junqueira, N. T. V., Braga, M. F. Maracujá: Germoplasma E MelhoramentoGenético. (pp. 55-78). Planaltina: Embrapa Cerrados.

Nass, L. L. (2011). Pré-melhoramento vegetal. In: Lopes, M. A., Fávero, A. P., Ferreira, M. A. J. F., Faleiro, F. G., Folle, S. M., Guimarães, E. P. (Eds). Pré-melhoramento de plantas. (pp. 23-38). Brasília: Embrapa Informações Tecnológicas.

Paula, S. M., Fonseca, M. E. 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.

Pereira, A. S. (2012). Genética e pós-melhoramento em variedades de maracujazeiro amarelo: variabilidade acessada por ferramentas biométricas e moleculares. (Dissertação De Mestrado). Universidade Estadual Santa Cruz, Brasil.

Pereira, D. A., Corrêa, R. X., Oliveira, A. C. (2015). Molecular genetic diversity and differentiation of populations of 'somnus' passion fruit trees (*Passiflorasetacea* DC): Implications for conservation and pre-breeding. BiochemicalSystematics e Ecolog, 59, 12-22.

Pinheiro, L. R., Rabbani, A. R. C., Silva, A. V. C, Ledo, A. S., Pereira, K. L. G., Diniz, L. E. C. (2012). Genetic diversity and population structure in the Brazilian cattleyalabiata(orchidaceae) using RAPD and ISSR markers. Plant System aticsand Evolution, 12, 1815-1828.

Silva, T. S. S., Freitas, J. S., Dos Santos, E. S. L., Cardoso, T. Dos. S., Cerqueira-Silva, C. B. M. (2018). Caracterização e seleção de marcadores moleculares em *Crotonlinearifolius*.Mull. Arg. Como subsídio para estudos genéticos. Multi-ScienceJournal, 1(10), 4-8.

Sousa, A. G. R., Souza, M. M., Melo, C. A. F., Sodré, G. A. (2015). ISSR markers in wild species of Passiflora l. (passifloraceae) as a tool for taxon selection in ornamental breeding. Genetics And Molecular Research, 14(4), 18534-18545

Viana, A. P., Pereira, T. N. S., Pereira, M. G., Souza, M. M., Aldonado, J. F. M., Amaral Jr, A. T. (2003). Genetic diversity among yellow passion fruit comercial76 genotypes and among passiflora species using RAPD. Revista Brasileira de Fruticultura, 25(3), 489-493.

Vieira, A. C., Cardoso, T. dos S., Silva, T. S. S., Cerqueira-Silva, C. B. M., Santos, E. S. L. (2019). Seleção de primers Inter SimpleSequenceRepeat em melocactusconoideusbuin. &bred. (cactaceae), espécie endêmica do sudoeste da Bahia, Brasil. Enciclopédia biosfera, 16(29), 1365-1374.

Yu, Y. G., Buss, G. R., SaghaiMaroof, M. A. (1996). Isolation of a Super Family of Candidate Disease-Resistance Genes in Soybean Based on a Conserved Nucleotide-Binding Site. Proceedings of the National Academy of Sciences of the USA, 93, 11751-11756.

Zeraik, M. L. (2010). Estudo analítico dos flavonoides dos frutos do maracujá (Passiflora edulis sims f. flavicarpa Degener). (Tese de Doutorado). Universidade de São Paulo, Brasil.

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