

*Full Length Research Paper*

## **Transferability of microsatellite loci from *Croton floribundus* Spreng. to *Croton urucurana* Baill. (Euphorbiaceae)**

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***Croton urucurana*, known as “sangra-d’água”, is a tree species with timber and beekeeping potential, and also with medicinal potential. The use of microsatellite markers, simple sequence repeats (SSRs) is efficient in population genetic studies, with high transferability rate between correlated species. The present study aimed to optimize the polymerase chain reaction (PCR) protocol and to test the transferability of seven primers developed for *Croton floribundus* to *C. urucurana*. The amplification of 113 accessions from different Brazilian biomes (*Cerrado*, *Pantanal* and *Mata Atlântica*) was tested. For optimization, the concentration and label of the MgCl<sub>2</sub> reagent was varied in addition to annealing temperature. Four tested primers showed amplification products, guaranteeing 57% of transferability. This result confirms the efficiency of *C. floribundus* microsatellite loci for future studies of genetic variability in *C. urucurana*.**

**Key words:** Genetic variability, *sangra-d’água*, molecular markers, simple sequence repeats (SSRs), primers.

### **INTRODUCTION**

*Croton urucurana* Baill., commonly known by the names “sangra-d’água”, “sangre del dragón” or “dragon’s blood”, is a small tree species from the Euphorbiaceae family, pioneer, found mainly in riparian forests from various forest formations, widely distributed by Paraguay,

Argentina, Uruguay and Brazil (Caruzo and Cordeiro, 2007). It is a species of multiple uses: timber and beekeeping potential; used in urban afforestation and recovery of degraded riparian areas; and great prominence for medicinal potential (Lorenzi, 2002).

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The *Croton* genus is the second largest in the family, with about 1,300 species, where South America, the Antilles and Mexico are the main centers of diversity for this group (Burger and Huft, 1995). Its economic importance is increasingly recognized, due to its enormous potential for production of essential oils with active constituents such as flavonoids, alkaloids and terpenoids, in addition to its medicinal uses (Maciel et al., 2002).

Several species are used in folk medicine, and among them are some with scientifically proven therapeutic activities (Salatino, 2007). The species most used are *Campylocentrum sellowii* (antibiotic activity), *Croton tiglium* (antitumor), *C. cortesianos* (treatment of dermatitis), *C. macrostahys* (vermifuge), *C. lineares* (antipyretic), *C. sublyratus* (against ulcer) and *C. penduliflorus* (purgative) (Randau et al., 2004). Because of this great medicinal importance, genetic studies are essential to develop conservation strategies and also products (Alves et al., 2012). For *Croton* genus there are some research related to genetic diversity (Angelo et al., 2005; Silvestrini et al., 2015; Rinthong et al., 2011; Scaldaferrri, 2013, 2014; Oliveira et al., 2016; Almeida-Pereira et al., 2017; Silva et al., 2018), however, molecular information are still scarce; for species *C. urucurana* they are almost non-existent and it is necessary to advance in the genetic diversity characterization of this species.

Studies of within and among population genetic variation, are fundamental to know the populations' structures and can be performed efficiently by enzymatic or molecular genetic markers (Resende, 1999).

Microsatellites or Single Sequence Repeats (SSRs) are very efficient molecular markers in studies of population genetics, genetic variability, evolutionary studies and genetic mapping (Varshney et al., 2005; Hodel et al., 2016). Because of characteristics such as high polymorphism, codominant inheritance pattern, multiple alleles, abundance, and broad distribution throughout the genome, microsatellites are very powerful and popular tools to characterize genetic variation among individuals or closely related taxa (Guichoux et al., 2011).

SSR markers have a high transferability rate among related species. The decrease in expenses with genomic libraries, the cloning and sequencing for sequence isolation and the development of new primers, make the transferability test between species an advantageous strategy (Ferreira and Grattapaglia, 1998; Zucchi et al., 2002; Wang et al., 2009). Markers developed for a species can be transferred to related species, since flanking sequences from microsatellite regions can be conserved among phylogenetically related species (Varshney et al., 2005; Barbara et al., 2007). Thus, the transferability rate is higher among species of the same genus, and decreases among distant genera of a same family (Mnejja et al., 2010).

Transferability studies on *Croton* are scarce. One of them was performed by Mishra et al. (2011), who

developed microsatellite markers based on the EST (Expressed Sequence Tags) of *Catharanthus roseus* and analyzed transferability to other three medicinal species from different genera. One of the species tested was *Croton macrostachyus*, where tests indicated a transfer rate of 30%. For two species from the same genus as *C. urucurana* and *C. floribundus*, it is expected to find high transferability rates. However, rates can be lower since phylogenetic studies (Riina et al., 2009; Van Ee et al., 2011) indicate that *C. floribundus* and *C. urucurana* can be relatively distant from each other because they belong to two distinct subgenera: *Croton* subg. *Geiseleria* and *Croton* subg. *Adenohylli*, respectively (Van Ee et al., 2011).

Considering this and the lack of studies involving the genetic diversity of *C. urucurana*, the present study aimed to test the transferability of microsatellite markers (SSR) designed for *C. floribundus* species to *C. urucurana*, as well as to optimize a PCR protocol for the amplification of these primers.

## MATERIALS AND METHODS

### DNA extraction

Accessions were sampled from *C. urucurana* native populations located in riparian forest fragments. They were from three locals with different Brazilian biomes: savanna (*Cerrado*), wetlands (*Pantanal*) and Atlantic forest, more specifically semi-deciduous tropical forest (*Mata Atlântica*). Terminal leaflets were collected from 32 accessions belonging to an anthropically established population at the Nações Indígenas Park in the municipality of Campo Grande/MS, which is considered as *Cerrado* area (20°27'14"S, 54°34'26"W); 41 accessions from a population established in the Piraputanga district, municipality of Aquidauana/MS, considered as *Pantanal* area (20°27'21"S; 55°30'01"W), and 40 accessions from a population established in the municipality of Rio Brilhante/MS, considered as *Mata Atlântica* area (21°43'07"S, 54°30'20"W), totalling 113 sampled individuals. After sampling, leaves were sanitized with sodium hypochlorite solution 0.5% and distilled water, and stored at 4°C in individual bags properly identified for DNA extraction. The samples were macerated in a porcelain mortar with liquid nitrogen until obtaining a finely ground and uniform material which was posteriorly stored at -20°C.

Total DNA from samples was extracted following the CTAB method (Doyle and Doyle, 1990) with appropriate adaptations to improve the quality of the obtained material. In this protocol, extraction buffer containing CTAB 3%, 100 mM Tris-HCl (pH 8.0), 2M NaCl, 25 mM EDTA (pH 8.0) and 5% PVP was used.

In a 2 ml Eppendorf® tube, the macerated material was added until the mark of 0.5 ml of the tube (approximately 120 mg) together with 800 µl of buffer and 16 µl of β-mercaptoethanol. The sample was then incubated in a water bath at 65°C for 60 min. After incubation, 600 µl of chloroform and isoamyl alcohol (24:1) was added and the sample was shaken for 5 min. It was then centrifuged (6 min at 12,000 rpm) and the aqueous phase (supernatant) was transferred to a new tube. Then 600 µl of ice-cold isopropanol was added for the precipitation of DNA and the sample was incubated at -20°C overnight. Thereafter, the tube was centrifuged (12 min at 12,000 rpm) and the supernatant was discarded. The precipitate was washed with 70% ethanol and diluted in 40 µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA).

**Table 1.** Characteristics of microsatellite regions of primers used to test transferability from *C. floribundus* to *C. urucurana*.

Accession number (Genbank)	Primer code	Primers (5' to 3')	Repeat motif	Size range (bp)
JX236034	SSR-5	F: CGTGTGCTTCTCTTCACC R: GCCAAATGGGTTTCTCGTTA	(AAG) <sub>8</sub> N <sub>18</sub> (CT) <sub>4</sub>	142-183
JX236034	SSR-6	F: CCACATTCCACATCACTTCC R: ACGTGGCAAATGGGTAGTC	(GA) <sub>5</sub>	203-213
JX236035	SSR-8	F: CTCCTGAACCCACGCTAGAT R: AGAGGCTTCGTGTTGTTCTCGT	(AG) <sub>8</sub>	214-216
JX236036	SSR-10	F: AGACGGAAGGGAAGTGGAGT R: CGCAGCATCATATCACCAGA	(AAG) <sub>8</sub>	141-187
JX236037	SSR-12	F: TTGGGCAGATTCATGTAACG R: TTGGTTGAGGGAACAGATGA	(GAA) <sub>8</sub> N <sub>37</sub> (AG) <sub>3</sub>	157-198
JX236038	SSR-14	F: AGCACTCCTTCCACGACATT R: TGGTGAGACGCTTCATGTTG	(CA) <sub>11</sub>	192-198
JX236039	SSR-16	F: GGCAGGCATAAATCAGCAAT R: TATCGAATCGTCGGATTGG	(GAA) <sub>6</sub>	200-255

Source: Silvestrini et al. (2013).

After extraction, total DNA was purified by treatment with ammonium acetate, following the protocol by Romano and Brasileiro (1999), and with the aim of minimizing the amount of interfering substances in the sample. The extracted DNA was dissolved in 100 µl of TE buffer and 50 µl of ammonium acetate (7.5 M), and then incubated for 15 min at 4°C. Subsequently, the sample was centrifuged for 30 min at 10,000 rpm at 4°C. Then, the supernatant was transferred to a new tube, where 400 µl of absolute ethanol was added and incubated for 60 min at -20°C. After incubation, sample tubes were centrifuged for 10 min at 5,000 rpm at 4°C. The supernatant was discarded and the precipitate washed with 70% ethanol and dissolved in 40 µl of TE buffer. In this way, final DNA showed a concentration around 40 ng.µL<sup>-1</sup> and, to evaluate the quality of the extracted material, DNA concentrations of samples were determined using a spectrophotometer (Biodrop® Denville Scientific Inc. Holliston) at an absorbance of 260 nm, and by electrophoresis (0.8% agarose gel).

### PCR optimization

Transferability was tested with SSR primers designed and developed for *C. floribundus* species (Silvestrini et al., 2013), totaling 7 pairs of primers (Table 1). The protocol used by Silvestrini et al., (2013) for primers amplification in *C. floribundus* was the following: final volume 25 µl, 40 ng DNA, 1x reaction buffer, 0.1 mmol.L<sup>-1</sup> dNTP, 2 mmol.L<sup>-1</sup> MgCl<sub>2</sub>, 10 pmol of each primer (forward and reverse) and 1.0 U of Taq DNA Polymerase; with thermocycler programmed with 5 min at 95°C as initial denaturation, followed by 30 amplification cycles (denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min), ending with a 5 min elongation at 72°C.

However, this amplification protocol was not efficient for these primers in *C. urucurana* and it was necessary to optimize some conditions. For this purpose, MgCl<sub>2</sub> concentrations at 1.0, 2.0 and 3.0 mmol.L<sup>-1</sup> from three distinct brands (Invitrogen™, Sigma-Aldrich and Ludwig Biotec) were tested, in addition to dNTP (Sigma-Aldrich) concentrations at 0.1 and 0.4 mol.L<sup>-1</sup>. These values were always tested with a final volume of 25 µl, 40 ng DNA, 1x reaction

buffer, 10 pmol of each primer (forward and reverse) and 1.0 U of Taq DNA Polymerase.

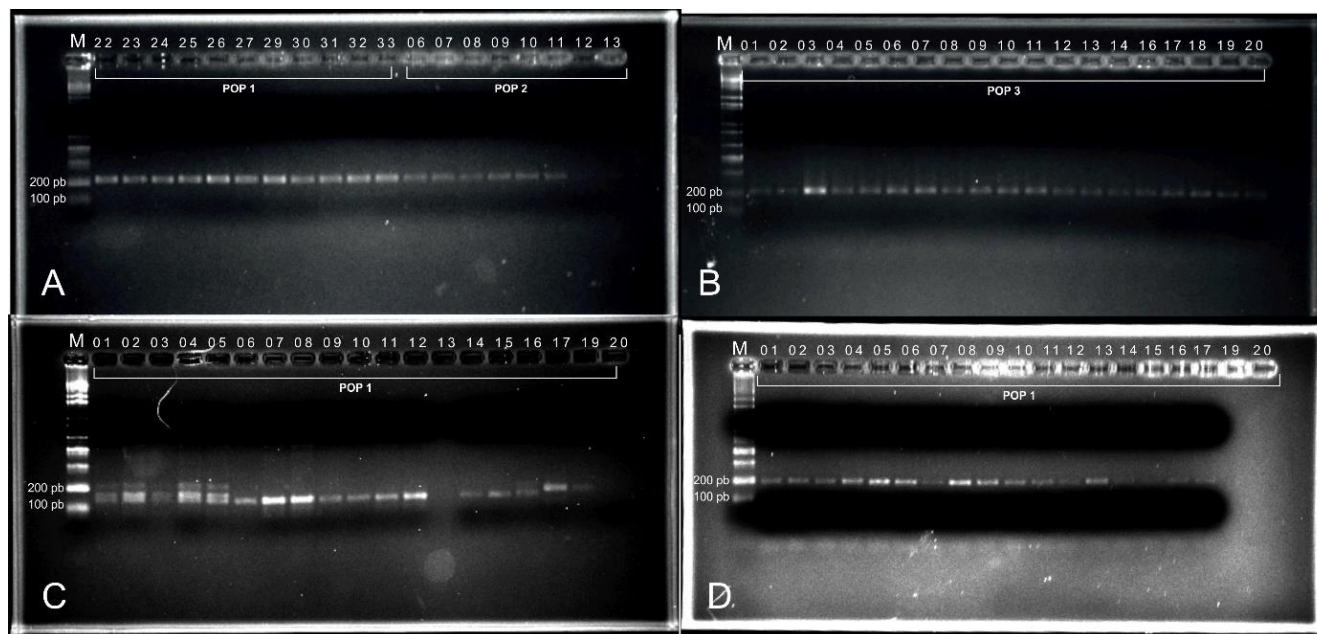
Optimum primer temperature in the PCR annealing step was also tested using the following temperatures: 50, 55 and 60°C. Conditions in the steps of denaturation and extension remained the same in all tests, that is, 95 and 72°C, respectively. Initially, 5 individuals from each population were chosen to perform the tests with the seven primers. After protocol optimization and the confirmation of transferred primers, all individuals collected from the 3 different populations were amplified (total of 113 accessions). DNAs were amplified using the thermocycler GeneAmp PCR System 9700 (Applied Biosystems).

### Transferability

To confirm the transferability of primers, products were subjected to electrophoresis at 100 V for 40 min on a 2% agarose gel which was stained with ethidium bromide and visualized under an ultraviolet transilluminator. The marker *1 Kb Plus DNA Ladder* (Invitrogen™) ranging from 100 to 12,000 bp was used to evaluate the presence or absence of bands and the respective molecular weight of the amplified fragments. The percentage of transferability was calculated relating the number of primers that showed transfer of loci and the total number of primers tested.

## RESULTS AND DISCUSSION

Unlike the extract reported in *C. floribundus* (Silvestrini et al., 2013) and what is expected for pioneer species (Coley, 1993), the DNA extracts obtained in the present study needed to be purified with ammonium acetate for secondary compounds. Concerning literature, there is greater difficulty in obtaining intact DNA sampling from tropical species, due to the presence of secondary compounds, especially of the phenol class (Rath et al.,



**Figure 1.** Patterns of bands amplified in 2% agarose gel in *C. urucurana*. **A:** Primer SSR-06; **B:** Primer SSR-08; **C:** Primer SSR-10; **D:** Primer SSR-16. M: Molecular marker 1 Kb Plus DNA Ladder (100-12,000 bp).

1998; Deshmukh et al., 2007). During genetic material extraction in *Croton antispyhiliticus*, Oliveira et al. (2016) observed viscosity and oxidation in the samples. Scaldaferrri et al. (2013), evaluating different extraction protocols of *Croton linearifolius*, observed the presence of polysaccharides in samples and found that methods using CTAB were the most efficient. Likewise, the DNA extraction method used in this study has proved it to be quite adequate based on the quality of samples visualized on the agarose gel, DNA concentration and spectrophotometer's absorbance reading.

In the PCR protocol optimization, *C. urucurana* DNA samples were amplified only using the following conditions: final volume of 25  $\mu$ l, 40 ng DNA, 1x reaction buffer, 0.4 mmol.L<sup>-1</sup> dNTP (Sigma-Aldrich), 3 mmol.L<sup>-1</sup> of MgCl<sub>2</sub> (Invitrogen™), 10 pmol of each primer (forward and reverse) and 1.0 U of Taq DNA Polymerase. Thermal cycle was programmed as follows: 5 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 55°C, 1 min at 72°C, ending with 10 min at 72°C.

In order to confirm the amplified products, samples were subjected to electrophoresis in 2% agarose gel and the amplification was considered positive when bands have shown fragments with the size of 141-255 bp according to SSR primer information (Table 1). Among the seven pairs of primers tested, four showed amplification products, which confirm the transferability efficiency of the primer (Figure 1). Primers that showed transferability were the following: SSR-6 (203-213 bp), SSR-8 (214-216 bp), SSR-10 (141-187 bp) and SSR-16 (200-255 bp). The microsatellite markers transferred are

composed of repeat motifs ranging from two nucleotides (SSR-06 and SSR-08 primers) to three nucleotides (SSR-10 and SSR-16 primers).

Thus, the transferability rate of SSR primers was 57.14%. The presence of transferred loci from the genome of *C. floribundus* to *C. urucurana* was expected for them being species from the same genus, but this value could be even higher. According to Kalia et al., (2011), the more phylogenetically related the species, the greater chance of conserved regions among them. Table 2 shows that the obtained percentage of microsatellite markers transferred is in the range of the ones found in other studies that looked for the transferability of markers in population genetics studies. The intermediate value found for transferability from *C. floribundus* to *C. urucurana* seems to be due to the phylogenetic distance between the species: same genus but different and not closely related subgenera (Van Ee et al., 2011).

By analyzing Table 2, we see that studies performed to test the transferability between species of the same genus generally presented the highest rates. Bressan et al., (2012) observed a 67% transferability rate when they tested 9 pairs of primers designed for *Jatropha curcas* in *Jatropha podagrica*, *Jatropha pohliana* and *Jatropha gossypifolia*. Similarly, Sousa et al., (2009) obtained an average of 73% transferability from *Centrosema pubescens* primers in eleven species of the genus *Centrosema*. However, Zucchi et al., (2002) reported the transferability of only 2.8% from primers designed for the genus *Eucalyptus* to the species *Eugenia dysenterica*. It is noticed that the transferability rate among individuals

**Table 2.** Studies on the transferability of microsatellite markers.

Species (developed markers)	Species (transferred markers)	Number of tested markers	Percentage of transferred markers (%)	Reference
<i>Anacardium occidentale</i> (Anacardiaceae)	<i>Anacardium humile</i> (Anacardiaceae)	14	86	Soares et al. (2013)
<i>Centrosema pubescens</i> (Fabaceae)	<i>Centrosema</i> spp. (Fabaceae)	26	73	Sousa et al. (2009)
<i>Jatropha curcas</i> L. (Euphorbiaceae)	<i>Jatropha</i> spp. (Euphorbiaceae)	9	67	Bressan et al. (2012)
<i>Cocos nucifera</i> (Arecaceae)	<i>Butia odorata</i> (Arecaceae)	50	56	Mistura et al. (2012)
<i>Glycine</i> spp. (Fabaceae)	<i>Phaseolus</i> spp. (Fabaceae)	48	41.6	Nascimento et al. (2013)
<i>Mercurialis huetii</i> (Euphorbiaceae)	<i>Mercurialis perennis</i> (Euphorbiaceae)	65	18.4	Pfeiffer et al. (2011)
<i>Eucalyptus</i> spp. (Myrtaceae)	<i>Campomanesia</i> spp. (Myrtaceae)	120	10	Miranda et al. (2016)
<i>Eucalyptus</i> spp. (Myrtaceae)	<i>Eugenia klotzschiana</i> (Myrtaceae)	120	9.2	Siqueira (2014)
<i>Eucalyptus</i> spp. (Myrtaceae)	<i>Eugenia dysenterica</i> (Myrtaceae)	365	2.8	Zucchi et al. (2002)

from different genera decreases as a result of the higher genetic distance between them. Peakall et al., (1998), in their work on the transferability of soybean primers (*Glycine max*), found a 65% rate of amplification among species from the same genus (*Glycine*), but in species of different genera this rate decreased to 3-13%.

Results obtained in the present study have shown to be promising, since transferability was positive in four of the seven pairs of SSR primers tested (57.14%). It was also possible to develop an efficient PCR protocol for the amplification of these primers for the species *C. urucurana*. Considering the high cost, and the amount of time and labour required to develop specific microsatellite markers for *C. urucurana*, results obtained in the present work may serve as a basis for future genetic studies, since transferred primers may be used for the characterization of genetic diversity among populations and also for the molecular characterization of the species, enabling the adoption of strategies for the genetic improvement and conservation of native populations of this species.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## REFERENCES

- Almeida-Pereira CS, Silva AVC, Alves RP, Feitosa-Alcantara RB, Arrigoni-Blank MF, Alvares-Carvalho SV, Costa TS, White LAS, Pinto VS, Sampaio TS, Blank AF (2017). Genetic diversity of native populations of *Croton tetradenius* Baill. using ISSR markers. *Genetics and Molecular Research* 16(2):gmr16029602. <https://doi.org/10.4238/gmr16029602>
- Alves M, Araújo MFL, Gusmão CLS, Lira-Neto AC, Carvalho R, Benko-Iseppon AM (2012). Diversity and Uses of the Genus *Croton* (Euphorbiaceae) in Northeastern Brazil. In: Rai MK, Cordell GA, Martinez JL, Marinoff M, Rastrelli L (eds) *Medicinal Plants: Biodiversity and Drugs*, 1st edn. CRC Press, New York, pp. 106-141.
- Angelo PCS, Chaves FCM, Xavier JJBN, Cruz JC, Lira MPS (2005). Avaliação inicial do RAPD como forma de acessar a variabilidade genética na espécie rica em linalol *Croton cajuçara* Benth. (sacaca). Manaus: Embrapa Amazônia Ocidental 28:1-4. Available in: [http://ainfo.cnptia.embrapa.br/digital/bitstream/CPAA-2009-09/12537/1/com\\_tec28.pdf](http://ainfo.cnptia.embrapa.br/digital/bitstream/CPAA-2009-09/12537/1/com_tec28.pdf)
- Barbara T, Palma-Silva C, Paggi GM, Bered F, Fay MF, Lexer C (2007). Cross- species transfer of nuclear microsatellite markers: potential and limitations. *Molecular Ecology* 16:3759-3767. <https://doi.org/10.1111/j.1365-294X.2007.03439.x>
- Bressan EDA, Scotton DC, Ferreira RR (2012). Development of microsatellite primers for *Jatropha curcas* (Euphorbiaceae) and transferability to congeners. *American Journal of Botany* 99:237-239. <https://doi.org/10.3732/ajb.1100532>
- Burger WC, Huft M (1995). *Flora Costaricensis: Family #113 Euphorbiaceae*. *Fieldiana Botany* 36:1-169.
- Caruzo MBR, Cordeiro I (2007). Synopsis of the tribe Crotoneae Dumort. (*Euphorbiaceae* s.s.) in the State of São Paulo, Brazil.

- Hoehnea 34(4):571-585. <https://doi.org/10.1590/S2236-89062007000400011>
- Coley PD (1993). Gap size and plant defenses. *Trends in Ecology & Evolution* 8(1):1-2. [https://doi.org/10.1016/0169-5347\(93\)90119-A](https://doi.org/10.1016/0169-5347(93)90119-A)
- Deshmukh VP, Thakare PV, Chaudhari US, Gawande PA (2007). A simple method for isolation of genomic DNA from fresh and dry leaves of *Terminalia arjuna* (Roxb.) Wight and Arnot. *Electronic Journal of Biotechnology* 10:468-472. <https://doi.org/10.4067/S0717-34582007000300014>
- Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.
- Ferreira ME, Grattapaglia D (1998). Introducción al uso de marcadores moleculares en el análisis genético. Brasília: Embrapa-Cenargen.
- Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, Petit RJ (2011). Current trends in microsatellite genotyping. *Molecular Ecology Resources* 11:591-611. <https://doi.org/10.1111/j.1755-0998.2011.03014.x>
- Hodel RGJ, Segovia-Salcedo MC, Landis JB, Crowl AA, Sun M, Liu X, Gitzendanner MA, Douglas NA, Germain-Aubrey CC, Chen S, Soltis DE, Soltis PS (2016). The report of my death was an exaggeration: A review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences* 4(6):apps.1600025. <https://doi.org/10.3732/apps.1600025>
- Kalia RJ, Rai MK, Kalia S, Singh R, Dhawan AK (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica* 177(3):309-334. <https://doi.org/10.1007/s10681-010-0286-9>
- Lorenzi H (2002). Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. 2nd edn. Nova Odessa: Instituto Plantarum.
- Maciel MAM, Pinto AC, Veiga Jr. VF (2002). Plantas Mediciniais: a necessidade de estudos multidisciplinares. *Química Nova* 25(3):429-438. <https://doi.org/10.1590/S0100-40422002000300016>
- Miranda EAGC, Boaventura-Novae CRD, Braga RS, Reis EF, Pinto JFN, Telles MPC (2016). Validation of EST-derived microsatellite markers for two Cerrado-endemic *Campomanesia* (Myrtaceae) species. *Genetics and Molecular Research* 15(1):gmr.15017658. <https://doi.org/10.4238/gmr.15017658>
- Mishra RK, Gangadhar BH, Yu JW, Kim DH, Park SW (2011). Development and characterization of EST based SSR markers in Madagascar periwinkle (*Catharanthus roseus*) and their transferability in other medicinal plants. *Plant Omics* 4(3):154-161. Available in: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.457.5371&rep=rep1&type=pdf>
- Mistura CC, Barbieri RL, Castro CM, Priori D, Villela JCB (2012). Transferibilidade de marcadores microsatélites de coco (*Cocos nucifera*) para butiá (*Butia odorata*). *Magistra* 24(4):360-369. Available in: <https://www.alice.cnptia.embrapa.br/bitstream/doc/966416/1/RosaLia13Artigo1012Claudeterrevisado1.pdf>
- Mnejra M, Garcia-Mas J, Audergon JM, Arus P (2010). *Prunus* microsatellite marker transferability across rosaceous crops. *Tree Genetics & Genomes* 6(5):689-700. <https://doi.org/10.1007/s11295-010-0284-z>
- Nascimento ATB, Silva CB, Costa MMR, Silva RLO, Kido EA (2013). Transferibilidade de marcadores moleculares de soja para aplicação no melhoramento de feijão caupi. In: XII Jornada de Ensino, Pesquisa e Extensão - JEPEX, 2013. Anais. Recife/PE: UFPE. Available in: <http://www.eventosufpe.com.br/2013/cd/resumos/R0555-1.pdf>
- Oliveira TG, Pereira AMS, Coppede JS, França SC, Ming LC, Bertoni BW (2016). Genetic diversity analysis of *Croton antisiphiliticus* Mart. using AFLP molecular markers. *Genetics and Molecular Research* 15(1):gmr.15017461. <https://doi.org/10.4238/gmr.15017461>
- Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998). Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. *Molecular Biology and Evolution* 15(10):1275-1287. <https://doi.org/10.1093/oxfordjournals.molbev.a025856>
- Pfeiffer T, Roschanski AM, Pannell JR, Korbecka GY, Schnittler M (2011). Characterization of microsatellite loci and reliable genotyping in a polyploid plant, *Mercurialis perennis* (Euphorbiaceae). *Journal of Heredity* 102(4):479-488. <https://doi.org/10.1093/jhered/esr024>
- Randau KP, Florêncio DC, Ferreira CP, Xavier HS (2004). Pharmacognostic study of *Croton rhamnifolius* H.B.K. and *Croton rhamnifolioides* Pax & Hoffm. (Euphorbiaceae). *Revista Brasileira de Farmacognosia* 14(2):89-96. <https://doi.org/10.1590/S0102-695X2004000200001>
- Rath P, Rajaseger G, Goh CJ, Kumar PP (1998). Phylogenetic analysis of Dipterocarps using random amplified polymorphic DNA markers. *Annals of Botany* 82:61-65. <https://doi.org/10.1006/anbo.1998.0652>
- Resende MDV (1999). Melhoramento de essências florestais. In: Borém A. Melhoramento de espécies cultivadas. UFV: Viçosa, MG, Brazil, pp. 589-647.
- Riina R, Berry PE, Van Ee BW (2009). Molecular phylogenetics of the dragon's blood *Croton* section *Cyclostigma* (Euphorbiaceae): a polyphyletic assemblage unraveled. *Systematic Botany* 34(2):360-374. <https://doi.org/10.1600/036364409788606415>
- Rinthong PO, Zhu S, Komatsu K, Chanama S, De-Eknamkul W (2011). Genetic variation of *Croton stellatopilosus* Ohba based on non-coding DNA sequences of ITS, *trnK* and *trnL-F* regions. *Journal of Natural Medicines* 65(3-4):641-645. <https://doi.org/10.1007/s11418-011-0536-8>
- Romano E, Brasileiro ACM (1999). Extração de DNA de plantas: Soluções para problemas comumente encontrados. *Biocologia Ciência e Desenvolvimento: Brasília* 2(9):40-43.
- Salatino A, Salatino MLF, Negri G (2007). Traditional uses, chemistry and pharmacology of *Croton* species (Euphorbiaceae). *Journal of the Brazilian Chemical Society* 18(1):11-33. <https://doi.org/10.1590/S0103-50532007000100002>
- Scaldfarri MM, Freitas JS, Santos ESL, Vieira JGP, Gonçalves ZS, Cerqueira-Silva CBM (2013). Comparison of protocols for genomic DNA extraction from velame pimenta (*Croton linearifolius*), a native species to the Caatinga, Brazil. *African Journal of Biotechnology* 12(30):4761-4766. <https://doi.org/10.5897/AJB2013.12182>
- Scaldfarri MM, Freitas JS, Vieira JGP, Gonçalves ZS, Souza AM, Cerqueira-Silva CBM (2014). Comparison of methods for estimates of molecular genetic diversity in genus *Croton*: influence of coefficients, clustering strategies and data projection. *Genetics and Molecular Research* 13(3):5566-5573. <https://doi.org/10.4238/2014.July.25.11>
- Silva TSS, Freitas JS, Santos ESL, Cardoso TS, Cerqueira-Silva CBM (2018). Caracterização e seleção de marcadores moleculares em *Croton linearifolius* Mull. Arg. como subsídio para estudos genéticos. *Multi-Science Journal* 1(10):4-8. Available in: <https://www.ifgoiano.edu.br/periodicos/index.php/multiscience/article/viewFile/354/397>
- Silvestrini M, Pinto-Maglio CAF, Zucchi MI, Santos FAM (2013). Cytogenetics and characterization of microsatellite loci for a South American pioneer tree species, *Croton floribundus*. *Genome* 56(12):743-751. <https://doi.org/10.1139/gen-2013-0159>
- Silvestrini M, McCauley DE, Zucchi MI, Santos FAM (2015). How do gap dynamics and colonization of a human disturbed area affect genetic diversity and structure of a pioneer tropical tree species? *Forest Ecology and Management* 344:38-52. <https://doi.org/10.1016/j.foreco.2015.01.026>
- Siqueira MN (2014). Transferibilidade e variabilidade genética de marcadores microsatélites gênicos em *Eugenia klotzschiana* Berg. (Myrtaceae). MSc Thesis, Federal University of Goiás, UFG. Available in: <https://repositorio.bc.ufg.br/tede/handle/tede/>
- Soares TN, Sant'Ana LL, Oliveira LK, Telles MPC, Collevatti RG (2013). Transferability and characterization of microsatellite loci in *Anacardium humile* A. St. Hil. (Anacardiaceae). *Genetics and Molecular Research* 12(3):3146-3149. <https://doi.org/10.4238/2013.January.4.24>
- Sousa ACB, Carvalho MA, Boaventura LR (2009). Microsatellite markers in tropical legume (*Centrosema pubescens* Benth): development, characterization, and cross-species amplification in *Centrosema* sp. *Conservation Genetics Resources* 1:347-352. <https://doi.org/10.1007/s12686-009-9080-1>
- Van Ee BW, Riina R, Berry PE (2011). A revised infrageneric classification and molecular phylogeny of New World *Croton*

- (Euphorbiaceae). Taxon 60(3):791-823. <https://doi.org/10.1002/tax.603013>
- Varshney RK, Graner A, Sorrells ME (2005). Genic microsatellite markers in plants: features and applications. Trends in Biotechnology 23(1):48-55. <https://doi.org/10.1016/j.tibtech.2004.11.005>
- Wang ML, Barkley NA, Jenkins TM (2009). Microsatellite markers in plants and insects. Part I: Applications of biotechnology. Genes, Genomes and Genomics 3(1):54-67. Available in: <https://pubag.nal.usda.gov/download/44058/PDF>
- Zucchi MI, Brondani RPV, Pinheiro JB, Brondani C, Vencovsky R (2002). Transferability of microsatellite markers from *Eucalyptus* spp. to *Eugenia dysenterica* (Myrtaceae family). Molecular Ecology Notes 2:512-513. <https://doi.org/10.1046/j.1471-8286.2002.00297.x>