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Short communication

Transferability of SSR markers from *Eugenia uniflora* L. to Myrtaceae species of the Atlantic Forest

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ABSTRACT

The Myrtaceae family is home to several species of economic and ecological importance, but many are in areas of constant environmental degradation, demanding studies aiming at the conservation status evaluation or other characteristics of interest. One of the widely used tools for these purposes is SSR markers. This study sought to evaluate the transferability of 11 new SSR markers characterized in *Eugenia uniflora* to 14 different species of the family Myrtaceae. Out of the 11 markers tested, nine were amplified in at least one of the examined species. For seven species, this is the first report of cross-species transfer of SSR markers. These markers may contribute to the characterization of the genetic diversity of the species and planning policies for the conservation and breeding of these genetic resources.

Keywords: genetic diversity, microsatellite markers, Myrtoideae, molecular diversity, molecular markers.

Introduction

The Myrtaceae family is widely represented among botanical families, dispersed in various regions of the world (Govaerts *et al.* 2008). In Brazil, it is present in several ecosystems, mainly in the Atlantic Forest (Guilherme *et al.* 2004), a biome suffering a historical decrease in its natural area. Despite the degradation and fragmentation, it is estimated that the Atlantic Forest holds about 20,000 species, including several endemic and endangered ones, being recognized as a region of high priority for the conservation of global biodiversity (Myers *et al.* 2000).

In general, Brazilian Myrtaceae are not used as a source of timber, like species of *Eucalyptus*, but are used to supply firewood, manufacture small objects (Costella *et al.* 2013), in popular medicine and provide a variety of eatable fruits. Despite the recognized economic, cultural, and ecological importance of several Brazilian Myrtaceae (e.g., Brazilian cherry, guava, jaboticaba, and feijoa), scientific data for the conservation of less prominent

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species is still incipient. In turn, molecular markers based on the direct analysis of the DNA are useful tools for generating sound information about the genetic diversity of such species.

Nuclear SSR markers are one of the predominant genetic markers for molecular ecology studies. However, researchers usually demand universal markers that can be used in different species. Such markers facilitate comparisons among closely related taxa for addressing the mechanisms involved in population divergence and speciation, as well as comparisons among multiple co-occurring species for studying the interaction of diversity patterns at the genetic and community levels (Barbará *et al.* 2007). Given the high polymorphism and codominant nature of nuclear SSRs, the potential of cross-species transfer of these markers has been gaining increased importance. Particularly for forest species with incipient commercial importance, the transferability of SSR markers may be the main tool for researchers accessing genetic diversity and the structure of natural populations. Aiming to provide a new set of molecular markers for genetic studies of Myrtaceae species from the Atlantic Forest, this study investigated the transferability of 11 newly characterized SSR markers from *E. uniflora* to 14 species of this family.

The SSR markers were prospected through the partial genome sequencing of *E. uniflora* (Sarzi *et al.* 2019; Stefenon *et al.* 2019), while the 14 species investigated correspond to six key genera of Myrtaceae native to the Atlantic Forest. Leaf samples were collected from adult individuals in forest fragments in the Central region of the Rio Grande do Sul State, Atlantic Forest domain. Species were identified based on morphological characteristics described in the specialized literature (Landrum & Kawasaki 1997; Souza & Lorenzi 2005; Lorenzi *et al.* 2006; Sobral *et al.* 2015). Vouchers of each species (Figure 1) were deposited in the Bruno Edgar Irgang Herbarium, Federal University of the Pampa, São Gabriel, Brazil. Total DNA was isolated from the leaves of two distinct trees of each species using the CTAB method (Doyle & Doyle 1987). The concentration, purity, and integrity of the DNA were verified by electrophoresis in 0.8% agarose gel and through spectrophotometry in a NanoVue™ spectrophotometer.

PCR amplifications were performed in 12.5 µL reaction mix containing 100 ng of DNA, 0.25 µM of buffer, 0.5 µM $MgCl_2$, 1 U of Taq DNA-Polymerase, 0.05 µM each dNTP, 0.125 µM of each primer, and 0.01% BSA. Amplification reactions were performed on a BIO-RAD C1000 Touch™ Thermal Cycler with an initial step of 94 °C for 3 minutes, followed by 39 cycles with a step of denaturation at 94 °C for 45 seconds, the specific annealing temperature of each primer (Beise *et al.* 2022) for 30 seconds, and extension at 72 °C for 1 minute, with a final extension of 72 °C for 10 minutes.

The products of the amplifications were resolved using electrophoresis on 3% agarose gel, stained with GelRed®, and visualized using UV light (Figure 2). A 100 bp ladder was used as a reference. The sizing of the alleles was performed using the software TotalLab TL 120 1D v. 2009 (Nonlinear Dynamics, UK).

The cross-species transferability was effective in 11 species (Table 1), with successful PCR amplification within the expected size, based on the genome of *E. uniflora*. No marker was amplified in *E. verticillata, M. pungens,* and *P. rivularis*. From two (in *C. xanthocarpa* and *P. guajava*) to seven (in *E. involucrata* and *P. cattleyanum*) markers were transferred across the species included in this study. The PCR amplification of markers Pit57 and Pit115 failed for all species. The number of amplified alleles ranged from one to 11 (Table 1). Markers Pit26 and Pit138 are linked to genes *FRI* (involved in the regulation of flowering time) and *LECRKS7* (involved in resistance response to the pathogenic oomycetes) respectively. Gene-linked SSR markers are expected to show a high degree of transferability since they are found linked to conserved expressed regions (Petry *et al.* 2019). However, marker Pit26 cross-amplified only in *P. peruviana* and *P. cattleyanum*, while Pit138 amplified in nine species (Table 1).

It has been shown that SSR markers transferability is more likely to occur among taxonomically close species (65% between species within a genus, 43% between genera within a family, and 28% between families within an order; Barbará et al. 2007). Our results were comparatively higher with successful transferability reaching 73% of the markers to *Eugenia* species (ranging from 0% to 83.3% of the markers) and 63.6% of the markers to other genera within the family Myrtaceae (ranging from 0% to 75% of the markers).

The SSR markers transferability among species of Myrtaceae was reported by Santos *et al.* (2007), Ferreira-Ramos *et al.* (2014), Nogueira *et al.* (2016), and Petry *et al.* (2019). In this study, we add to the scientific literature, a set of nine SSR markers able to be used in genetic studies of nine Myrtaceae species of the Atlantic Forest. To the best of our knowledge, this is the first report of marker transfer to *E. ramboi, E. rostrifolia, E. uruguayensis, C. guazumifolia, C. xanthocarpa, M. pungens,* and *P. peruviana,* even though SSR markers were recently developed for *C. xanthocarpa* (Petry *et al.* 2019). These transferred markers may contribute to the characterization of the genetic diversity of the species and planning conservation policies and breeding of these genetic resources.

Acknowledgments

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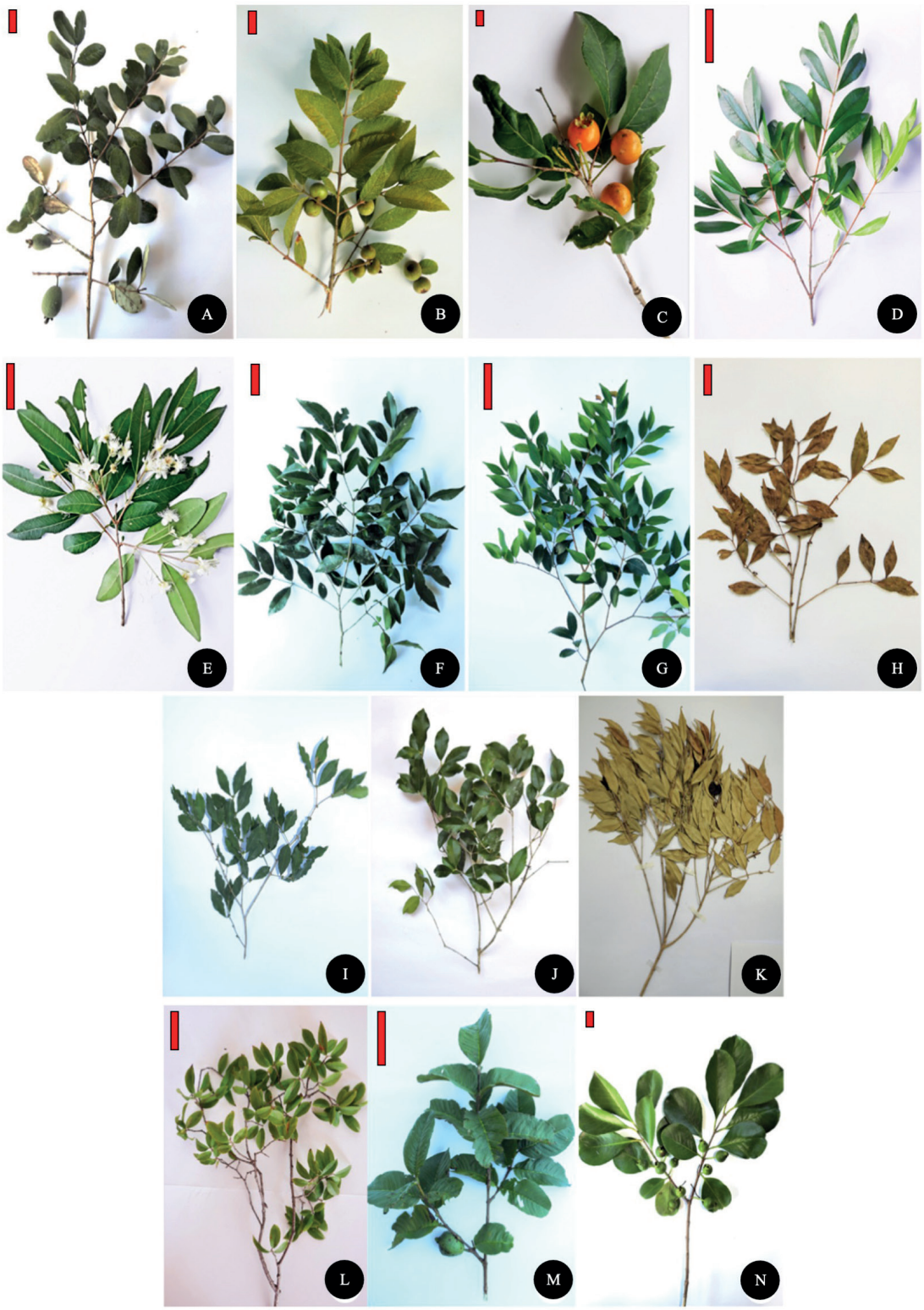

Figure 1. Vouchers of each species used in this study, deposited in the Bruno Edgar Irgang Herbarium, Federal University of the Pampa, São Gabriel, Brazil, and their corresponding herbarium ID. (**A**) *Acca sellowiana* (O. Berg) Burret - HBEI 1626. (**B**) *Campomanesia guazumifolia* (Cambess.) O. Berg - HBEI 1625. (**C**) *Campomanesia xanthocarpa* Mart. ex O. Berg - HBEI 1632. (**D**) *Eugenia involucrata* DC. - HBEI 1627. (**E**) *Eugenia pyriformis* Cambess. - HBEI 1628. (**F**) *Eugenia ramboi* D. Legrand. - HBEI 1629. (**G**) *Eugenia rostrifolia* D. Legrand. - HBEI 1630. (**H**) *Eugenia uruguayensis* Cambess. - HBEI 1621. (**I**) *Eugenia verticillata* (Vell.) Angely - HBEI 1631. (**J**) *Myrcianthes pungens* (O. Berg) D. Legrand - HBEI 1633. (**K**) *Plinia rivularis* (Cambess.) Rotman - HBEI 1566. (**L**) *Plinia peruviana* (O.Berg) Kausel - HBEI 1634. (**M**) *Psidium cattleyanum* Sabine - HBEI 1635. (**N**) *Psidium guajava* L. - HBEI 1636. The red bar above each voucher corresponds to the number of amplified SSR markers in the species.

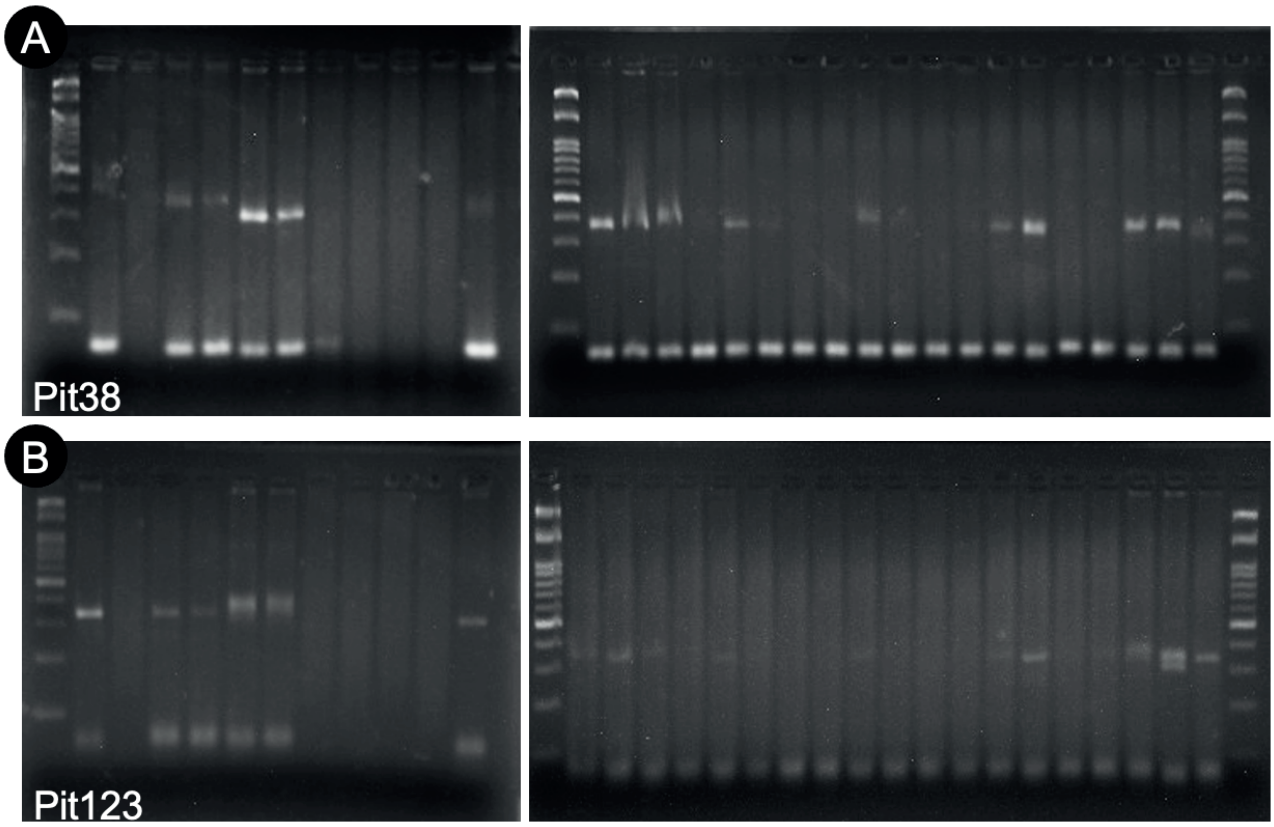

Figure 2. Example of the electrophoresis gel (3% agarose) visualized using UV light. (**A**) Transferability test of the SSR marker Pit38. (**B**) Transferability test of the SSR marker Pit123. Two different samples from each species were employed.

Table 1. SSR markers prospected in *E. uniflora* and tested for cross-species transferability to species of Myrtaceae. +: successfully transferred; -: failed cross-species transferability; Alleles: number of alleles amplified overall species.

Species	Marker										
	Pit26	Pit38	Pit53	Pit57	Pit71	Pit98	Pit115	Pit119	Pit123	Pit138	Pit140
E. uniflora	+	+	+	+	+	+	+	+	+	+	+
E. involucrata	-	+	+	-	+	-	-	+	+	+	+
E. pyriformis	-	+	+	-	+	-	-	-	+	+	+
E. ramboi	-	+	-	-	-	+	-	-	+	+	-
E. rostrifolia	-	+	+	-	-	+	-	-	+	+	+
E. uruguayensis	-	+	-	-	-	-	-	-	+	+	+
E. verticillata	-	-	-	-	-	-	-	-	-	-	-
A. *sellowiana*	-	-	+	-	-	-	-	-	+	+	-
C. guazumifolia	-	+	-	-	-	-	-	-	+	+	-
C. xanthocarpa	-	+	-	-	-	-	-	-	-	+	-
M. pungens	-	-	-	-	-	-	-	-	-	-	-
P. rivularis	-	-	-	-	-	-	-	-	-	-	-
P. peruviana	+	+	+	-	-	-	-	+	+	-	-
P. cattleyanum	+	+	+	-	-	-	-	+	+	+	+
P. guajava	-	-	+	-	-	-	-	-	-	-	+
Alleles	4	11	5	1	4	4	1	4	9	10	7

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