Chapter 5

Characterizing *Tabebuia rosea* (Bertol.) DC. Using Microsatellites in Provenance and Progeny Trials in Colombia

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59289

1. Introduction

The area under forests is estimated to be approximately 3,870 million hectares worldwide, 95% of which corresponds to natural forests or native woodlands and the remaining 5% to planted forests [1]. Forests are dual purpose—commercial exploitation and environmental improvement [2]. Because of the pressure exerted by environmentalists, current global efforts strive to reduce timber extraction in natural forests. Forecasts are that the future increase in demand for timber will be covered by trees specifically planted for this purpose. Because of their capacity to fix carbon, forests will play an increasingly important role in view of current climate change attributed to greenhouse gas emissions [3].

Genetic improvement programs for forest species face diverse problems such as the long regeneration periods and the high costs involved in maintaining a forest population over a long period of time at different locations. Furthermore, compared with short-cycle crops, the returns to investment in forest plantations are definitively more delayed [4]. Molecular markers have a great impact on genetic improvement because they minimize the intervals of regeneration, increase the genetic gain per generation, and accumulate genetic information key for 'non-domesticated' species. Molecular markers are increasingly included in forest genetic breeding programs, where they are used to estimate polymorphism, establish parameters of relationship and mating systems, characterize genotypes, and assist in selection processes [5]. Microsatellites are the markers that have been most used in recent years and can be used to design seed orchards to estimate the contamination of pollen from external sources and to study mating models and variation of male fertility.

The development of microsatellite markers for forest species has been limited. The ratio of use of this type of marker in forest species, as compared its use in other cultivated species, is 6:1. Exceptions to the above are forest species used in the timber industry, such as *Pinus*, *Quercus,* and *Eucalyptus* [6, 7].

In Latin America, Brazil plays a pioneering role in the development of microsatellite markers for forest species of economic and ecological importance, for example *Caryocar brasiliense* [8], *Ceiba pentandra* [9], *Copaifera langsdorffii* [10], *Eugenia uniflora* [11], and several *Cariniana* species [12], among others.

In Colombia, coffee has been traditionally grown in association with forest species such as *Tabebuia rosea* and *Cordia alliodora*, which serve as shade, live fences, or perimeters [13]. Their characteristics, such as good wood quality, make them very appropriate to be used in agroforestry systems. *Tabebuia rosea,* a species with high-value, good-quality wood, is very important in Central America. Its lilac-pink flowers make it one of the most eye-catching trees of Central and South America, where it is mainly used as a shade and ornamental plant because of the beauty of its pink flower panicles [14, 15] (Figure 1). Work carried out with microsatellites for the *Tabebuia* genus includes studies carried out in *Tabebuia aurea* by Braga et al. [16], who developed 21 polymorphic microsatellites using a genomic library. This current study characterized, using microsatellite molecular markers, the best-performing *T. rosea* materials of provenance and progeny trials established by Colombia's National Center for Coffee Research (CENICAFE) in the country's coffee-growing region as well as the best materials established by the Santa Rosalía de Palermo Reforestation Company (REFOPAL) and the National Corporation for Forest Research and Promotion (CONIF) in the country's Caribbean region.

Figure 1. *Tabebuia rosea* tree in Colombia´s coffee region

http://dx.doi.org/10.5772/59289

2. Materials and methods

2.1. Plant material

Samples were taken from provenance and progeny trials carried out by CENICAFE, a REFOPAL-CONIF clonal orchard, and a CONIF progeny orchard. The provenance and progeny trial, established by CENICAFE in 1997, is located in Colombia's coffee-growing region. Seed from plus trees collected in Colombia as well as in El Salvador, Guatemala, and Nicaragua were introduced for this trial (Table 1).

The REFOPAL-CONIF clonal seed orchard for conservation purposes, established in 1999, is located in Colombia's Caribbean region (code SA). Several progeny of this trial are also located in the Caribbean region (code HI). The REFOPAL-CONIF trial involves 24 sites of origin, 21 of which correspond to clones originating from plus trees and 3 from commercial seed (absolute controls) (codes HIT1, HIT2, and HIT3) (Table 1). All samples were identified according to their origin: Population 1, REFOPAL and CONIF clonal orchards; Population 2, progenies of REFOPAL and CONIF clonal orchards; and Population 3, CENICAFE provenance and progeny trials.

Progeny/clone/plus tree (no.)	Code	Country of origin	Type of trial	Trial or collection site (plus trees)
1	SA2	Colombia (Atlántico)	Clonal orchard	San Antero (Córdoba)
2	SA16	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
3	SA32	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
4	SA3	Colombia (Atlántico)	Clonal orchard	San Antero (Córdoba)
5	SA17	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
6	SA33	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
7	SA4	Colombia (Bolívar)	Clonal orchard	San Antero (Córdoba)
8	SA18	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
9	SA34	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
10	SA6	Colombia (Atlántico)	Clonal orchard	San Antero (Córdoba)
11	SA19	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
12	SA35	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
13	SA7	Colombia (Atlántico)	Clonal orchard	San Antero (Córdoba)
14	SA20	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
15	SA36	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
16	SA8	Colombia (Bolívar)	Clonal orchard	San Antero (Córdoba)
17	SA22	Colombia (Sucre)	Clonal orchard	San Antero (Córdoba)

Progeny/clone/plus tree (no.)	Code	Country of origin	Type of trial	Trial or collection site (plus trees)
18	SA38	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
19	SA10	Colombia (Magdalena)	Clonal orchard	San Antero (Córdoba)
20	SA24	Colombia (Sucre)	Clonal orchard	San Antero (Córdoba)
21	SA41	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
22	SA11	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
23	SA25	Colombia (Sucre)	Clonal orchard	San Antero (Córdoba)
24	SA42	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
25	SA12	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
26	SA27	Colombia (Sucre)	Clonal orchard	San Antero (Córdoba)
27	SA43	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
28	SA13	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
29	SA28	Colombia (Sucre)	Clonal orchard	San Antero (Córdoba)
30	SA44	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
31	SA14	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
32	SA29	Colombia (Sucre)	Clonal orchard	San Antero (Córdoba)
33	SA46	Colombia (Magdalena)	Clonal orchard	San Antero (Córdoba)
34	SA15	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
35	SA31	Colombia (Córdoba)	Clonal orchard	San Antero (Córdoba)
36	HI31	Colombia (Córdoba)	Progeny of clonal orchards	La Independencia Hacienda
37	HI9	Colombia (Magdalena)	Progeny of clonal orchards	La Independencia Hacienda
38	HI33	Colombia (Córdoba)	Progeny of clonal orchards	La Independencia Hacienda
39	HI35	Colombia (Córdoba)	Progeny of clonal orchards	La Independencia Hacienda
40	H1T2	Check	Commercial material 2	La Independencia Hacienda
41	HIT3	Check	Commercial material 3	La Independencia Hacienda
42	HI19	Colombia (Córdoba)	Progeny of clonal orchards	La Independencia Hacienda
43	HI47	Colombia (Sucre)	Progeny of clonal orchards	La Independencia Hacienda
44	H1T1	Check	Commercial material 1	La Independencia Hacienda

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Progeny/clone/plus tree (no.)	Code	Country of origin	Type of trial	Trial or collection site (plus trees)
45	HI23	Colombia (Sucre)	Progeny of clonal orchards	La Independencia Hacienda
46	HI44	Colombia (Córdoba)	Progeny of clonal orchards	La Independencia Hacienda
47	HI5	Colombia (Bolívar)	Progeny of clonal orchards	La Independencia Hacienda
48	061/96	El Salvador	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
49	506/92	Guatemala	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
50	SO2386	Nicaragua	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
51	AGO95	Guatemala	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
52	CU-II-1*	Colombia (Cundinamarca)	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
53	MAR 96-09	Guatemala	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
54	AG 95-30	Guatemala	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
55	SO2386	Nicaragua	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
56	M-I-1*	Colombia	CENICAFE provenance and progeny trial	Floridablanca (Santander). Pueblo Bello (Cesar)
57	CU-I-1-10	Colombia	CENICAFE plus trees	Cundinamarca
58	CU-I-1-11	Colombia	CENICAFE plus trees	Cundinamarca
59	CU-I-1-12	Colombia	CENICAFE plus trees	Cundinamarca
60	CU-II-1-10	Colombia	CENICAFE plus trees	Cundinamarca
61	CU-II-1-11	Colombia	CENICAFE plus trees	Cundinamarca
62	ABR 95-24	Guatemala	CENICAFE plus trees	Floridablanca (Santander) Pueblo Bello (Cesar)
63	CU-II-*	Colombia	CENICAFE provenance and progeny trial	Floridablanca (Santander) Pueblo Bello (Cesar)
64	ABR 95-30	Guatemala	CENICAFE provenance and progeny trial	Floridablanca (Santander) Pueblo Bello (Cesar)

Progeny/clone/plus tree (no.)	Code	Country of origin	Type of trial	Trial or collection site (plus trees)
65	MAR96/09	Guatemala	CENICAFE provenance and progeny trial	Líbano (Tolima)
66	AGO25-30	Guatemala	CENICAFE provenance and progeny trial	Fredonia (Antioquia)
67	ABR 95-24	Guatemala	CENICAFE provenance and progeny trial	Fredonia (Antioquia)
68	AGO95-30	Guatemala	CENICAFE provenance and progeny trial	Fredonia (Antioquia)
69	MAR96-09	Guatemala	CENICAFE provenance and progeny trial	Fredonia (Antioquia)
70	061/96	El Salvador	CENICAFE provenance and progeny trial	Fredonia (Antioquia)
71	ABR95-30	Guatemala	CENICAFE provenance and progeny trial	Fredonia (Antioquia)

Table 1. Samples used, codes, country of origin, and type of trial to characterize *Tabebuia rosea* in Colombia.

2.2. Molecular characterization

Molecular characterization was performed using microsatellite markers in two ways:

1. Applying the principle of transferability: markers developed for *T. aurea* by Braga et al. [16] were used in *T. rosea.*

2. Developing specific microsatellites for *T. rosea* from genomic libraries enriched for microsatellite motifs.

In both cases, the same plant material was used as well as the same DNA extraction protocols and electrophoresis and silver nitrate staining procedures; however, amplification conditions varied depending on the group of microsatellite markers. Amplified products were separated by denatured 6% polyacrylamide gel electrophoresis, run in BioRad's Sequi-Gen GT Sequencing Cell vertical electrophoresis chambers. Gels were dyed with silver nitrate following the protocol of Benbouza et al. [17].

Details of the procedure are described below:

2.3. DNA extraction

The QIAGEN Plant DNeasy Mini Kit was used to extract DNA from 20 mg macerated dry leaf tissue following the manufacturer's recommendations. To improve quality, the DNA was purified using the protocol described by Castillo [18]. DNA quality and quantity were verified by agarose gel electrophoresis at 0.8%, dyed with ethidium bromide.

http://dx.doi.org/10.5772/59289

2.4. DNA amplification using microsatellites developed for *Tabebuia aurea*

Based on the principle of transferability, DNA amplification of *T. rosea* was performed using 21 microsatellites developed by Braga et al. [17] for *T. aurea* (Bignoniaceae), which were identified with the prefix 'Tau' (Table 2).

The amplification protocol was carried out in a final volume of 15 μl, with 0.9 μM of each primer, 1U Taq polymerase, 200 μM of each dNTP, 1X reaction buffer (10 mM Tri HCl, 50 mM KCl, 1.5 mM $MgCl_2$) and 10 ng DNA. The amplification profile consisted of 30, 1-minute cycles at 94 ˚C, 1 minute at melting temperature (˚C) (see Table 2), 1 minute at 72 °C, with a final 10-minute extension at 72 °C.

Locus	GenBank accession number	Primer sequence F (5′-3′)	Primer Sequence R (5′-3′)	Annealing temperature (° C)	Number of loci	He[1]	Ho[1]	PIC[1]
Tau07	DQ666983	CCATAAGCTGCATCAACACA	ATCCTAAGATCGGTACTCCA	50	1	0.4259	0.478	0.385
Tau12	DQ666987	CATCATCAAGGTCAAGATCA	CATTCTAGTCTTCCATAAGT	52	1	0.6234	0.491	0.5513
Tau14	DQ666989	GGTAACGGATTGCTGGTTGT	CATTGCGAATGGCCTATGGT	55	1	0.8344	0.721	0.816
Tau17	DQ666992	TGGCCGTGTTGATGTTTATG	TGCCTCACGCTCTATGTGTC	52	1	0.8439	0.701	0.8269
Tau21	DQ666996	CTTTTGGGGGTCTTTGGAAT	TGAAAGAGACAGAGACAAAGATACA	55	1	0.5285	0.290	0.4911
Tau22	DQ666997	TATCTCTCCGCCGTACACCT	CCAATCGAAGAGCCCATTTA	52	2	0.7803	0.576	0.7502
Tau27	DQ667000	GGTAAATCATCTTCCGCTTCC	ACTGCAGAATCGCCTTTTGT	52	1	0.4081	0.508	0.3684
Tau30	DQ667002	TAGTTTAAGGGTGCCGTTGG	CGAACATAAAGAGGCAACCA	55	2	0.4558	0.588	0.3942
Tau31	DQ666982	TCGTGCAGCTTTTGAGTCTG	CTGCAAAACACAAAGCGAAA	52	1	0.6982	0.632	0.6492
TRA101	GU011737	CAAGACACATCCACGTACATAG	CTCACTCCCTTTAGTTTGTCAC	56	2	0.7749	0.568	0.7454
TRA3	GU011814	AGTAATTCCATCCAATCACATC	TGCATCAATCAAGTTGTAAGTC	56	2	0.4154	0.422	0.3715
TRA104	GU011690	CTCCCAAAGCCTTCTTTATATC	GTGGTAGTTGGAGAACATCATC	56	2	0.6185	0.984	0.5431

Locus	GenBank accession number	Primer sequence F (5′-3′)	Primer Sequence R (5′-3′)	Annealing temperature (° C)	Number of loci	He[1]	Ho[1]	PIC[1]
TRB109	GU011820	GCGCTGATGTTCATAATCTGA	CCATTGTTGGCCCTATCTTAT	56	2	0.7929	0.735	0.7606
TRB110	GU011754	GACCCAGGAAATGTTCTCG	AACGGTTGAGGAGCCATC	56	2	0.7308	0.375	0.6912
TRB103	GU011713	GAACGGGAAGACGCAGTC	GGCAGGTGGCAGAAGATC	56	2	0.8288	0.499	0.8070
TRB6	GU011721	TCATTGAGAGGAGCATTATACA	TTCAGTTGCGATGAGACAG	56	1	0.7783	0.590	0.7536
TRB8a	GU011719	GGTGGTGGAACGTCAGAT6AAG	GAGGGAATGCAAACACTTCAC	55	5	0.7686	0.576	0.7471
TRD1	GU011808	CCATCCATCACATCAAGC	GAAAGCAGTTCCCAGTAGTG	55	4	0.4267	0.459	0.3841
TRB104	GU011740	GTTCAATATGCGTCATCAATC	AACGAACTCAGAACTTTCGAC	55	1	0.8103	0.615	0.783
TRC8	GU011704	TTGGCTGACTGATACGATTG	GTGCTGGTCCTGTCCATC	55	1	0.5732	0.353	0.5557
TRA109	GU011728	GGAGAACGGATGTCTGTCAG	GCGTAGGATTTGGTGAAGTG	55	2	0.6998	0.576	0.6479
TRD110	GU011708	TGGATTAGAGAGCATGAGG	GCCATAATGATCCTGCATG	55	1	0.409	0.333	0.3916
TRC103	GU011717	TATTTCGCTCACGCATAAG	GCTTTGTCTCCTATCCAACTC	55	1	0.8433	0.750	0.8232
TRC105	GU011770	AAGCCCAGATTACTGTCTTCC	CGCGTGTGAGACTGTGAC	55	1	0.6885	1.000	0.6348

[1] He: Expected heterozygosity; Ho: Observed heterozygosity; PIC: polymorphic information content.

Table 2. Characteristics of the 24 microsatellite markers used in *Tabebuia rosea.*

2.5. Construction of genomic libraries to design primers for *Tabebuia rosea*

A genomic library enriched for microsatellite motifs was built to obtain a higher number of microsatellites. The microsatellites developed using this methodology was identified with prefix 'TR'. The procedure for building the library is described below:

Colony production. Recombinant plasmids were produced by linking digested fragments of *T. rosea* in the Hind *III* restriction site of plasmid pUC19. Fragments were enriched for microsatellite motifs. Digested products were introduced by electroporation within *Escherichia coli* DH5*α* races (ElectroMax™, Invitrogen). To isolate colonies for subsequent sequencing, cells

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were planted on Petri dishes with culture media containing Blue-gal/IPTG/ampicillin-LB agar, X-gal/IPTG/ampicillin-LB agar, or S-gal/IPTG/ampicillin-LB agar.

Clone selection for sequencing. Fragments containing microsatellite sequences were selected using biotinylated oligonucleotides complementary to the AG/CT repeat sequences and then amplified by PCR. DNA inserts were sequenced using the Amersham DYEnamic™ kit, following the manufacturer's instructions, and then run on electrophoresis in an ABI 377 sequencer (Applied BioSystems).

Microsatellite design. Before designing the primers, vector contamination was suppressed in the clone sequences and the percentage of redundancy in the genomic library was determined. PCR primers were designed using the software DesignerPCR, version 1.03 (Research Genetics, Inc). Table 2 presents the SSRs used in this study.

2.6. Amplification of microsatellites designed for *Tabebuia rosea*

Amplification reactions were conducted in a final volume of 10 μl with 0.6 μm of each primer, 200 μm of each dNTP, 1X reaction buffer, 1 U Taq polymerase, 2 mM $MgCl_2$, and 2 ng DNA. The amplification profile was 94 °C, 4 minutes of initial denaturation, 30 cycles, 40 seconds each, at 94 °C, melting temperature (° C) (see Table 2) for 40 seconds, 72 °C for 30 seconds, and a final 4-minute extension at 72 °C.

2.7. Statistical analysis

Statistical analyses were carried out using the program GENAlEX version 6.2 [19]. The analysis included the measurement of genetic variability, genetic diversity, and genetic distance.

3. Results and discussion

3.1. Transferability of Tau microsatellites

Of the 21 microsatellite markers evaluated, 11 presented positive amplification in samples of *T. rosea* and, of these, nine presented polymorphic amplifications (Table 2). The transferability of markers between species of the same genus was possible thanks to the synteny described in plant species and to comparative mapping in plants, such as that carried out by Bonierbale et al. [20] in Solanaceae. Other important synteny studies have been carried out in Gramineae to evaluate maize microsatellites in sugarcane [21] and rice microsatellites in sorghum [22]. Rice and sugarcane microsatellite sequences were used to characterize natural populations of *Guadua angustifolia* [23].

The transferability of microsatellites between species of the same genus, in particular tropical trees, has been addressed by Collevatti-Garcia et al. [8] for the *Caryocar* genus and by Braga et al. [16] for the *Tabebuia* genus. The latter study confirmed the transferability of microsatellite markers—the same used in this study—to other species of the *Tabebuia* genus (*T. ochracea, T. serratifolia, T. roseo-alba, T. impetiginosa)*. Braga et al. [16] described the primers

Tau 12, Tau 14, Tau 15, Tau 21, Tau 22, Tau 27, Tau 28, and Tau 31 as the most versatile primers because they presented positive amplification in all the aforementioned species of *Tabebuia*, whereas Tau 13, Tau 17, and Tau 30 presented amplification in at least three of the four *Tabebuia* species. Of the first group described by Braga et al. [16], Tau 12, Tau 14, Tau 21, Tau 22, and Tau 31 amplified samples of *T. rosea* in the present study and, of the second group, Tau 17 and Tau 30.

The nine polymorphic microsatellites used in the present study showed amplifications of one or two loci for a single microsatellite marker (Table 2), although polymorphic amplification was obtained with nine SSRs and 11 loci were observed. Markers presenting two loci were Tau 22 and Tau 30 (Table 2). The size of the products obtained ranged from 150 to 300 bp. These data agreed with those described for microsatellite development.

The distribution of the allele patterns in the three tree populations of *T. rosea* studied indicate that the average number of alleles in the three populations varied between four and five alleles. The number of frequent alleles ranged between three and four, whereas the number of informative alleles ranged between two and three (Table 2). The latter indicates the allelic diversity throughout all loci in each population. The average number of private alleles (whose frequency is less than 5%) is close to 1, with the CENICAFE population presenting the highest number of private alleles.

3.2. Molecular characterization with TR microsatellites

A total of 109 different microsatellites were identified in four of the enriched genomic libraries, which contained between 10,000-15,000 recombinant cells and allowed 74 microsatellite primers to be designed and 24 primers to be synthesized. The percentage of redundancy for this library was 11.4%. The latter were initially tested with the six samples that presented the highest polymorphism in analysis with Tau primers. The seven microsatellite primers presenting polymorphic amplification (Table 2) were then selected. Each of these seven primers presented amplification of two loci, except for the TRB6 primer pair, which presented the amplification of one locus (Table 2).

The results obtained based on the construction of the genomic library can be compared with those obtained by Braga et al. (2007), who obtained 271 positive colonies after constructing enriched libraries in the same genus, from which 31 polymorphic primers were synthesized.

For the most part, the microsatellites developed for *T. rosea* (TR primers) allowed the amplification of two loci per primer pair. Multi-allelic SSRs, such as TRB103 and TRB6, could be differentiated based on the allele frequencies of each locus in each population. With the primer pair TRB6, private alleles occur in the CENICAFE population (alleles 9, 10, and 11). The second locus of TRA3 presents a high frequency (over 80%) of allele 2 in the three populations. This allele could serve as marker for the species. The distribution of allele patterns indicates that the total number of alleles ranges between 4 and 6 and the number of informative alleles between 3 and 4.

Other studies carried out in forest species, such as the one conducted by Li et al. [24], report an average of 5-7 informative alleles for *Quercus aquifolioides*, using six microsatellites, after

having obtained 12 alleles on average. These results ratify the phenomenon observed in the present study, where after having an average of 4-6 alleles, the number of informative alleles declined drastically to only 3-4. Braga et al. [16] reported an average of 18.7 alleles for *T. aurea*, which differs from the value found in this study (average of 4-6 alleles). This difference could be attributed to the fact that Braga et al. [16] worked with natural populations, whereas the present study was conducted in selected populations where several alleles could have been lost. This hypothesis gains strength if the number of alleles obtained for the two groups of Tau and TR primers is compared. The results are similar. In addition, with both Tau and TR primers, the highest number of alleles is observed in selected REFOPAL-CONIF and CENICAFE populations.

3.3. Molecular characterization with both groups of SSRs (Tau and TR)

The results of the analyses of the 24 loci corresponding to both groups of microsatellites (Tau and TR) are analyzed and discussed below.

3.4. Measures of genetic variability (private alleles)

The evaluation of the presence of private alleles indicated a marked prevalence of this type of alleles in CENICAFE provenance and progeny populations and in REFOPAL-CONIF clonal orchards, indicating the need to preserve these orchards to perform directed crosses.

Lombardi [25] found a total of 85 alleles, with an average of 10.6 alleles per locus, when samples of *T. roseo-alba* were amplified with markers developed by Braga et al. [16]. Feres et al. [26] found, in turn, an average of 4.4 alleles per locus in *T. roseo-alba*, values very similar to those found in the present study. Averages of 13.5 alleles have been reported for other Bignoniaceae such as *Jacaranda copaia* [27].

3.5. Measures of genetic diversity

The genetic diversity parameters found when analyzing the 24 loci (Tau and TR primers) helped identify populations with low consanguinity, multi-allelic markers, and expected heterozygosities equal to observed heterozygosities. The mean expected heterozygosity is similar to the mean observed heterozygosity (Table 3). These values also indicate that the frequency of several alleles in the population is clearly concentrated in a few alleles. Values higher than 0.8 have been obtained in multi-allelic microsatellites in tropical forest species, those presenting between 9-10 alleles [8]. This finding differs from the results obtained in this study, where the average number of alleles for all loci and populations ranged between 4 and 5 with the microsatellites used. Findings suggest that these populations, although selected for genetic improvement, are balanced. Studies in *T. aurea* with microsatellites specifically developed for the species revealed an average of 26 alleles per locus and an observed heterozygosity of 0.587, a value similar to that reported for *T. rosea*. In addition, the probability of genetic identity was high (1.03×10^{-37}) and the probability of exclusion was 0.9889 [16].

Population	Na* (mean)	Ne* (mean)	He* (mean)	Ho* (mean)
REFOCOSTA-CONIF (clonal orchards)	5.333	3.374	0.630	0.608
REFOCOSTA-CONIF (progenies)	4.250	2.937	0.598	0.635
CENICAFE (provenance and progeny trials)	4.875	2.907	0.608	0.502
All populations/all loci	4.819	3.072	0.612	0.582

* Na=Number of alleles with frequencies higher than or equal to 5%; Ne=Number of informative alleles; He=Expected heterozygosity; Ho=Observed heterozygosity

Table 3. Genetic diversity in three populations for forest improvement programs of *Tabebuia rosea* and two groups of microsatellites (Tau and TR).

Other forest species of interest, such as pine species *Pinus elliottii* var. elliottii and *P. caribaea* var. hondurensis, yielded heterozygosity values of 0.52+/-0.2. Shepherd et al. [28] attribute these values to the use of transpecific microsatellites in pine species, a situation which is replicated in this study by using *T. aurea* microsatellites in *T. rosea*.

Lombardi [25], who also worked with Tau primers, reported a mean observed heterozygosity of 0.271, compared with a heterozygosity of 0.746. Feres et al. [26] attribute the differences between heterozygosities to the presence of null alleles that mask heterozygote individuals under the presence of one band. Null alleles occur more frequently in transferred primers than in primers designed specifically for a given species.

The analysis of molecular analysis (AMOVA) revealed that the highest percentage of variation could be attributed to differences between individuals (71%), followed by the variation between populations (25%) and variation within populations (4%). Miller and Schaal [29] analyzed the structure of natural and cultivated populations of a forest species presenting an edible fruit (*Spondias purpurea*). The percentage of variation between natural populations was 35.65% and, within populations, 64.35%, whereas the corresponding values in cultivated materials were 30.19% and 69.81%.

3.6. Genetic distance measurements

The genetic distance between populations indicates that the populations that are closest genetically (0.114) are those of REFOPAL–CONIF (both clonal orchards and progenies). This value was expected because of the higher inter-relationship of the trees of this group. The CENICAFE population, on the other hand, is distant from populations on Colombia's Atlantic Coast.

Principal coordinates analysis, based on the genetic distances between individuals, revealed greater proximity between the REFOPAL-CONIF populations—clonal orchards and progenies—and a greater distance regarding the CENICAFE provenance and progeny trial (Figure 2). The genetic distance between the populations of the CENICAFE trials and the REFOPAL–CONIF trials (both clonal orchards and progenies) is evident. CENICAFE trials present greater variability than REFOPAL–CONIF trials and this could be attributed to the introduction of Central American materials to CENICAFE's genetic breeding program. Figure 2 endorses the

http://dx.doi.org/10.5772/59289

idea that both breeding programs are complementary, and that the exchange of material and information can prove advantageous for both entities. If plans are to begin controlled pollinations, then it would be practical to cross materials from both programs. Likewise, tree clones of both programs should be established in open-pollinated orchards.

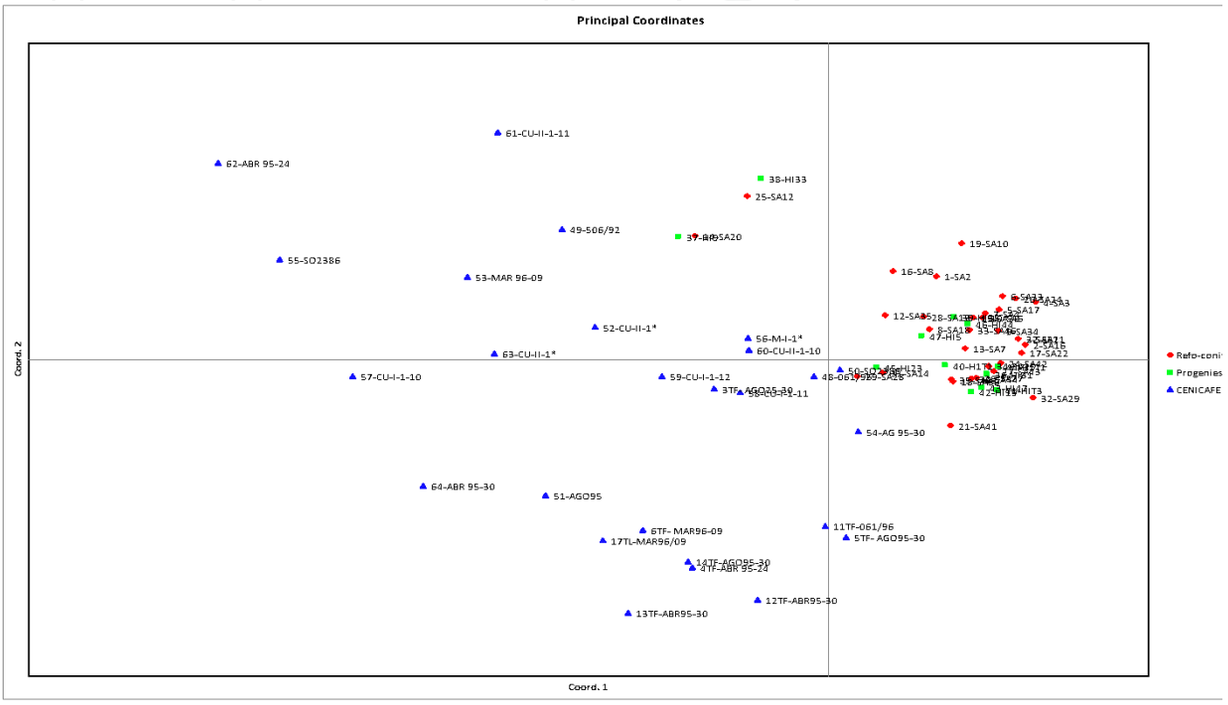

Figure 2. Principal coordinates analysis of samples of *Tabebuia rosea*.

Probability of identity: Given the need to identify each individual and assign each a probability of unequivocal identity, the study showed that this identity was established by combining eight different loci. More multi-allelic markers, with higher indexes of heterozygosity, are preferred. The optimal combination to characterize accessions of Vitis vinifera has also been reported to be eight primers [30].

Kirst et al. [31] determined the probability of identity of *Eucalyptus grandis* using microsatellites. The probability of finding identical genotypes in a population was found to be 2.3 x 10^{-9} using six EMBRA microsatellites developed in Brazil. This probability is considered practically null. Similar results were obtained with an improved population of *Eucalyptus dunnii*, when 46 individuals from a seed orchard were identified using four microsatellites [32].

4. Conclusions

This study verifies the transferability of microsatellites existing in the *Tabebuia* genus and sets forth the principle of synteny as an economically viable alternative for other *Tabebuia* species

for which information on the development of molecular markers is not available. The ample use of the microsatellites developed for *T. aurea* (Tau microsatellites) in several species of *Tabebuia* proves useful for the exchange of information between genetic breeding and conservation groups of *Tabebuia* species at the national and international levels.

Both groups of microsatellites amplified 24 polymorphic loci that could be useful in genotyping individuals of *T. rosea*. The measurement of genetic variability yielded an average of 4-5 alleles and between 3-4 frequent alleles. Expected heterozygosities equal to observed heterozygosities indicate that there is equilibrium within the populations, despite dealing with selected materials. The low indices of consanguinity of the three populations originating from improvement programs confirm the allogamous behavior of the species and, with the previous results, the hypothesis that the flower is self-incompatible gains strength. Meanwhile, private alleles were more frequent in the CENICAFE provenance and progeny trials and the REFOPAL-CONIF clonal orchards. The introduction of Central American materials influenced the high variability of CENICAFE materials. As a result, both selection processes are non-exclusive; on the contrary, they are complementary and the exchange of plant materials and information will prove beneficial to both.

Acknowledgements

The authors express their sincere thanks to Dr. Marco Cristancho (CENICAFE) for the technical revision of the present manuscript, to the Ministry of Agriculture and Rural Development of the Republic of Colombia, to the National Federation of Coffee Growers of Colombia, to the Government of the Department of Risaralda, and to the Technical University of Pereira for funding this research; to REFOPAL and CONIF for providing plant materials and morphological information on the evaluated materials. Our sincere thanks also go to Juliana Arias Villegas for planning and managing the project and to the staff of the Plant Biotechnology Lab of the Technical University of Pereira for their valuable contributions throughout the present study.

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http://dx.doi.org/10.5772/59289

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