Genetic diversity and structure in *ex situ* conserved populations of *Hevea brasiliensis*

Diversidade e estrutura genética em populações de *Hevea brasiliensis* conservadas ex *situ*

**Murilo da Serra Silva[1*], José Cambuim[2],
Thaisa Yuriko Kuboyama Kubota[2], Silvelise Pupin[2],
Rafael Moyses Alves[3], Mario Luiz Teixeira de Moraes[2], Paulo de Souza Gonçalves[4],
Alexandre Magno Sebbenn[5] and Miguel Luiz Menezes Freitas[5]**

Resumo

Locos microssatélites foram utilizados para investigar a diversidade e estrutura genética e o parentesco de duas populações de *Hevea brasiliensis* conservadas *ex situ*, uma em Selvíria, estado do Mato Grosso do Sul e uma em Marabá, estado do Pará e assim conhecer seu potencial para programas de melhoramento e conservação genética. A população de Selvíria apresentou número total de alelos (100) menor do que a população Marabá (179) com a média por locos de 6,7 e 11,9 respectivamente. A heterozigosidade esperada (H_e), heterozigosidade observada (H_o) e o índice de fixação (F) foram semelhantes entre populações, mas a riqueza alélica (R) foi maior em Marabá. A diferenciação genética entre as populações (G'_{ST}= 0,28) revelou que 72% da diversidade genética está distribuída dentro das populações. O coeficiente médio de coancestria dentro de ambas as populações foi positivo, mas os valores foram próximos de zero (< 0,08) e considerando indivíduos de ambas as populações, a coancestria media foi zero (-0,001). Portanto, os cruzamentos entre indivíduos dessas populações podem ser priorizados para promover uma maior heterose no programa de melhoramento. Os resultados sugerem que as populações são de interesse para programas de conservação e melhoramento genético.

Palavras-chave: genética de populações, microssatélites, seringueira.

Abstract

Using microsatellite loci, this study assesses the genetic diversity and structure and relatedness in two conserved *ex situ Hevea brasiliensis* populations in Selvíria, Mato Grosso do Sul State, and Marabá, Pará State, Brazil. The aim of the study was to determine the potential of these populations for breeding and conservation programs. The total number of alleles was lower in Selvíria (100) than in Marabá (179 alleles), with an average of 6.7 and 11.9 alleles per locus, respectively. The expected heterozygosity (H_e), observed heterozygosity (H_o), and fixation index (F) were similar between populations, but allelic richness (R) was higher in Marabá. Genetic differentiation between populations (G'_{ST}= 0.28) indicates that genetic diversity is mainly distributed within populations. The mean coancestry coefficient in each population was positive but close to zero (< 0.08), and when considering both populations together it was -0.001. Therefore, crosses between individuals of these populations can be encouraged to promote heterotic effects through breeding. The results suggest that the studied populations can be used in both conservation and breeding programs.

Keywords: microsatellite markers, population genetics, rubber tree

1. Instituto Federal de Educação, Ciência e Tecnologia do Pará – IFPA. Marabá / PA, Brazil. * Corresponding author: mserrasilva@yahoo.com.br

2. Faculdade de Engenharia de Ilha Solteira – FEIS, Universidade Estadual Paulista "Júlio de Mesquita Filho"-UNESP. Ilha Solteira / SP, Brazil.

3. Embrapa Amazônia Oriental, Empresa Brasileira de Pesquisa Agropecuária. Belém / PA, Brazil.

4. Instituto Agronômico de Campinas – IAC. Campinas / SP, Brazil.

5. Instituto Florestal de São Paulo – IF, Secretaria de Meio Ambiente do Estado de São Paulo. São Paulo / SP, Brazil.

INTRODUCTION

The rubber tree [*Hevea brasiliensis* (Willd, ex Adr, de Juss) Muell,-Arg] supplies the raw material used in the manufacture of a wide range of every-day objects. Despite being a native Amazonian species, most of the rubber consumed in the Brazilian market is imported (HOMMA, 2012). In order for Brazil to be self-sufficient in meeting its internal demand, hundreds of thousands of hectares of rubber trees must be planted, which offers opportunities to provide income for thousands of small-scale producers (HOMMA, 2012).

While the species continues to be exploited and rubber extracted in natural populations in the Amazon region, there are very few areas with high levels of productivity. These areas are based on the system proposed by Kageyama et al. (2002) and the increased productivity is due to the selection of genotypes, initially conserved *ex situ* (OLIVEIRA et al., 2015; SOUZA et al., 2017), that enabled planting in a variety of agroecosystems, such as agroforestry (RIGHI et al., 2008; VIRGENS FILHO, 2014) and monocultures (GONÇALVES et al., 2011). However, successful domestication occurred outside of the species' center of origin in ecosystems where the fungus *Microcylus ulei* (Henn,) Arx does not occur. This fungus causes leaf disease and can have significant impacts on rubber plantations.

Considering the context of deforestation in natural populations in the region where the species originated, *ex situ* conservation plans are important not only to maintain the genetic diversity of the species, but also to enable genetic improvement of genotypes adapted to different environments. As such, several studies have examined the dissimilarity between genotypes to obtain satisfactory heterotic effects through crosses, as discussed in Aguiar and Gonçalves (2006).

Understanding genetic diversity is crucial to the success of conservation and breeding programs. Molecular markers have been used to assess genetic diversity through indices such as expected and observed heterozygosity and fixation index, among others. Some studies have quantified the genetic diversity of rubber trees using these markers (CUBRY et al., 2014; PERSEGUINI et al., 2012; PETHIN et al., 2015), offering significant potential for genetic studies involving the species (SOUZA et al., 2009).

Several regions in Brazil have favorable edaphoclimatic conditions for the cultivation of rubber trees. Therefore, a range of genotypes are being studied for the purpose of genetic improvement in conserved *ex situ* populations. In the state of Pará, Brazil, areas suitable for rubber cultivation have been identified with satisfactory soil and climatic conditions, but without the occurrence of leaf disease. Marginal areas have also been identified that are predominantly strip E, or areas that could be suitable for rubber tree cultivation provided there is sufficient water availability and deep soils (CAMARGO; CAMARGO, 2014). Camargo and Camargo (2014) also highlight areas in Mato Grosso do Sul State and other regions across the country that range from suitable to optimal for rubber cultivation.

Using microsatellite markers, the present study aimed to investigate the genetic diversity and structure, inbreeding, and relatedness in two *H. brasiliensis* conserved *ex situ* populations in Selvíria, Mato Grosso do Sul State, and Marabá, Pará State, Brazil.

MATERIAL AND METHODS

Study site and sampling

From the two study populations, Selvíria (SEL) and Marabá (MAR), only *H. brasiliensis* adult trees capable of becoming mother trees in a progeny test that produced fruits in the 2016 reproductive event were sampled. SEL is located in the municipality of Selvíria, Mato Grosso do Sul State, at Fazenda de Ensino, Pesquisa e Extensão, UNESP Ilha Solteira Campus (FEIS/UNESP) (51° 22′ W, 20° 22′ S; altitude of 335 m). According to the Köppen-Geiger classification, the climate is humid tropical type Aw. In 2016, the average annual temperature was 24.7°C, average relative air humidity was around 75%, and average annual rainfall was 1355.2 mm (UNESP, 2016). The soil is classified as dystrophic Red Latosol (LVd), according to current soil nomenclature (SANTOS et al., 2018). This population consists of 18 adult trees from open-pollinated seeds, collected on the occurance lines of GT1 clones, in a stand that also includes clones PR107, RRIM600, IAN873 and IAN717. The species has explosive dehiscence, and there may be trees of these clones. The MAR population is located in the municipality of Marabá, Pará State, at the Marabá Rural Campus, Federal Institute of

Pará (IFPA) (05° 34′ 14.8″ S, 49° 06′ 02.3″ W; altitude of 100 m). The population includes 46 adult trees that are remnants of a plantation established in 1980 with seeds of unknown origin. The area of this population is 4.2 ha. According to the Köppen-Geiger climate classification, the municipality of Marabá is AW, occurring in the AM transition zone. Data from the IFPA Marabá Rural Campus and ITV weather station for 2016 shows an average annual temperature of 26.5°C (minimum of 14.7°C and maximum average of 27.5°C). The rainfall index was 1549 mm/year and the soil is classified as Red-Yellow Argisol (SANTOS et al., 2018).

Microsatellite analysis

DNA extraction and PCR procedures followed the protocol described by Faria et al. (2011). The initial data were generated on the ABI 3100XL sequencer and exported using the Genotyper software. The markers were selected based on microsatellite loci from a study by Le Guen et al. (2009), who analyzed the genetic structure of several *H. brasiliensis* populations. However, for better quality and optimization of the analysis, the primers were redesigned by the Hereditas/Genomax laboratory, based on the sequence available in GenBank (NCBI, Table 1).

Table 1. *Primers* used in the characterization of 15 microsatellite loci of *Hevea brasiliensis*, with locus code, NCBI accession number, *primer* sequence, average estimated primer size, and multiplex system code used.

Tabela 1. *Primers* utilizados na caracterização de 15 locos microssatélites em *Hevea brasiliensis*, com o código do loco, número de acesso ao NCBI, sequência do *primer*, estimativa do tamanho médio do primer e código do sistema multiplex empregado.

Locus	NCBI accession number	Primer	Mean size (bp)	Multiplex
A2365	AY486666	[6-fam]-cattcggtagcgcattatca gtttcgcctctctcctcctgctat	144	A
A2368	AY486668	[6-fam]-tcaaggttcaataaagcttcaca gtttaaattcagttcacaatgctgtca	240	A
A2387	AY486690	[vic]-gtgaggacccaggactttga gtttagctgcagagagccagagac	227	A
A2389	AY486679	[ned]-tcttttggtctttctcaataaattttc gtttcatccaccacagtttcgag	180	A
A2406	AY486697	[vic]-cactgacccatagggtatatctca gttttgcttttgttctgatgc	148	A
A2413	AY486681	[ned]-aatagagggaatgcccacag gtttccgaagagcttaaggacgtg	241	A
A2508	AY486740	[6-fam]-gcagatctaggcccatagaaa gtttgccttaggtagcttgtcgat	244	B
A2684	AY486821	[ned]-aaaggaagaggttgctgctg gtttcctttctctttcattttcttga	142	B
A2689	AY486822	[6-fam]-aagccacagccattcttgat gtttaactcaaaacccacaaagaaaaa	110	A
A2376	AY486841	[vic]-gcaaaactttgcaatttctcaa gtttggaaagaacaggaaatcatca	238	B
A2762	AY486852	[ned]-tccatgagcacaagttaccaa gtttgcgctcacaaattctcaaaa	247	B
TA2163	AY486617	[vic]-aaaaatttctggatgatgttatactttt gtttgctaacatcagacatgcctagaga	144	B
TAs2558	AY486760	[6-fam]-tcctctactcaatacaaaggaagg gtttaaccctggtaacccaacg	202	B
BAC55B02	DQ115609	[6-fam]-cccaacaggtcaaggtttct gtttgtcagaatatgtgatttctcttctc	136	B
T2083	AY486904	[ned]-cctcacctgcaatgagaaca gtttgctctctctttcccctcttt	129	B

Data analysis

Genetic diversity was estimated using the FSTAT program (GOUDET, 2002). The estimated indices include: number of alleles per locus (k), total alleles across loci (K), allelic richness (R), observed heterozygosity (H_o), expected heterozygosity in Hardy-Weinberg equilibrium within populations

(H_e), mean heterozygosity among populations (H_s), total expected heterozygosity (H_t) and fixation index (F) for the mean and each locus. Because our sample size was less than 50 individuals, F values were estimated based on Weir (1996) to avoid bias. The statistical significance of the F values were estimated by jackknifing across loci. We estimated the coefficient of genetic differentiation (G'_{ST}) based on Hedrick (2005), as indicated for microsatellite loci:

$$G'_{ST} = \frac{G_{ST}(1+H_s)}{1-H_s},$$

where, G_{ST} is Nei (1973) index of genetic differentiation. We also estimated the coancestry coefficient (θ_{xy}) between pairwise individuals within populations using the method described in Loiselle et al. (1995) and implemented in the SPAGEDI program (HARDY; VEKEMANS, 2002).

RESULTS AND DISCUSSION

Considering both populations, the total number of alleles identified was 197, ranging among loci from 3 to 20, and an average of 9.3 alleles. The total number of alleles was lower in Selvíria (100) than in Marabá (179), with an average of 6.7 and 11.9 alleles per locus, respectively (Table 2). Based on an unpaired t-test, MAR presented significantly (t= 2.75, P= 0.010) higher allelic richness (R = 9.20) than SEL (R = 6.67). However, when considering each locus, there were more heterozygous loci in SEL (eight loci) than in the MAR population (six loci). These results indicate that through crossing the SEL population has significant potential to produce new genotypes that can successfully adapt to different environments. However, the two studied populations have similar levels of genetic diversity when considering the means of observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F).

Table 2. Genetic diversity in the POP SEL and POP MAR populations of *Hevea brasiliensis* for 15 microsatellite loci. k is the number of alleles per locus; R is the allelic richness; H_o is the observed heterozygosity; H_e is the expected heterozygosity; F is the fixation index; G'_{ST} is the genetic differentiation between populations; and SD is the standard deviation; *P< 0.05.

Tabela 2. Diversidade genética em duas populações de *Hevea brasiliensis*, POP SEL e POP MAR. k é o número de alelos por loco; R é a riqueza alélica; H_o é a heterozigosidade observada; H_e é a heterozigosidade esperada; F é o índice de fixação; G'_{ST} é a diferenciação genética entre populações; SD é o desvio padrão; *P< 0.05.

Locus	POP SEL(n=18)					POP MAR (n=46)					G'_{ST}
	k	R	H_o	H_e	F	k	R	H_o	H_e	F	
A2689	8	8.00	0.833	0.851	0.133	12	9.68	0.739	0.836	0.160	0.508
A2365	7	7.00	0.888	0.794	0.143	11	8.19	0.803	0.785	0.169	0.494
A2406	8	8.00	0.833	0.799	0.138	11	7.33	0.630	0.617	0.169	0.308
A2387	7	7.00	0.722	0.680	0.139	12	10.66	0.717	0.887	0.154	0.273
A2413	5	5.00	0.777	0.724	0.140	8	7.34	0.456	0.796	0.137	0.296
A2558	6	6.00	0.499	0.541	0.129	16	11.30	0.609	0.869	0.147	0.978
A2508	4	4.00	0.111	0.758	0.075	14	9.82	0.543	0.856	0.142	0.027
A2163	11	11.00	0.666	0.830	0.121	16	12.14	0.913	0.879	0.171	0.469
A2376	9	9.00	0.833	0.851	0.133	14	11.52	0.733	0.851	0.158	0.422
A2684	9	9.00	0.889	0.809	0.142	15	11.74	0.913	0.888	0.170	0.110
A2762	4	4.00	0.111	0.758	0.075	10	7.77	0.543	0.818	0.144	0.008
BAC55	6	6.00	0.833	0.706	0.148	9	7.05	0.847	0.766	0.175	0.085
A2368	7	7.00	0.388	0.634	0.107	20	14.06	0.804	0.909	0.160	0.427
T2083	3	3.00	0.499	0.395	0.154	3	2.99	0.522	0.517	0.169	0.010
A2389	6	6.00	0.667	0.644	0.137	8	6.35	0.390	0.698	0.136	0.015
Mean	6.7	6.7	0.637	0.718	0.142*	11.9	9.20	0.677	0.798	0.162*	0.284*
SD	2.2	2.2	0.262	0.125	0.024	4.1	2.84	0.164	0.110	0.013	0.268

The results observed herein for both populations show levels of genetic diversity similar to other studies of *H. brasiliensis*. For example, Le Guen et al. (2009), studying 307 individuals from 19 different collection points, found H_o of 0.59, while Perseguini et al. (2012) reported H_o of 0.25 in rubber tree accessions. Our estimates for SEL and MAR are also comparable with results obtained by Souza et al. (2015) who analyzed more than 1000 accessions from *ex situ* collections throughout South America, including several states in the Brazilian Amazon, and found a mean H_o of 0.64 and H_e of 0.76.

For the majority of loci in both populations, the H_e was greater than the H_o, resulting in positive F values and a mean significantly greater than zero in SEL (0.142) and MAR (0.162), indicating inbreeding. Phumichai et al. (2015), analyzing populations of the same species, found positive F values in two populations (0.015 and 0.254), while Souza et al. (2015) found a mean of 0.160. As these studies were based on samples of adult trees, it suggests that inbreeding in some *H. brasiliensis* populations may not result in inbreeding depression. This could be explained by the fact that the genetic load or deleterious alleles, the cause of inbreeding depression, is a population characteristic; while some populations may present inbreeding (homozygosis for identical by descent alleles), there is no inbreeding depression due to the absence or low frequency of deleterious alleles. Alternative explanations could include the occurrence of null alleles in some loci and/or the Wahlund effect (BITENCOURT; SEBBENN, 2007). Microsatellite loci are known to preset null alleles due to the non-amplification of some alleles. This can result in the misclassification of genotypes as homozygous when in the fact they are heterozygous, resulting in excess homozygous individuals. Furthermore, the Wahlund effect can occur if individuals of different populations, or the sampled population, are composed of mixtures of family structures (BITENCOURT; SEBBENN, 2007).

The coancestry coefficient (θ_{xy}) indicates genetic similarity or divergence between pairs of individuals and determines their degree of relatedness. Pairwise individuals can be considered unrelated ($\theta_{xy} = 0$), first cousins ($\theta_{xy} = 0.0625$), half-sibs ($\theta_{xy} = 0.125$), full-sibs or parent-child ($\theta_{xy} = 0.25$), self-sibs or clones ($\theta_{xy} = 0.5$). The mean coancestry (θ_{xy}) across all pairwise individuals for both populations (-0.001) indicates an absence of relatedness. For SEL, the mean coancestry (0.08) was near to that expected for first cousins and for MAR it was near zero (0.012). The MAR population showed a high frequency of unrelated individuals, followed by cousins, half-sibs, and full-sibs (Figure 1). SEL followed the same distribution pattern, except for half-sibs that showed a similar frequency to unrelated individuals. Considering the two populations together, a high percentage of unrelated individuals were observed, suggesting lower levels of relatedness between individuals from different populations than within populations.
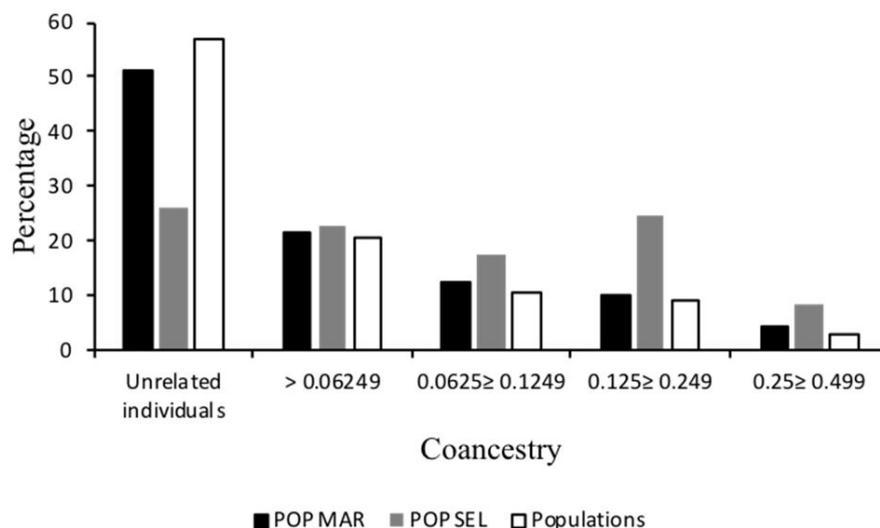

Figure 1. Estimates of coancestry coefficient (θ_{xy}) among 64 *Hevea brasiliensis* genotypes from two populations: Marabá (MAR) and Selvíria (SEL).
Figura 1. Estimativa de coancestria (θ_{xy}) em 64 genótipos de *Hevea brasiliensis* em duas populações: Marabá (MAR) e Selvíria (SEL).

Considering the estimated values for coancestry (θ_{xy}), MAR has a higher percentage of unrelated individuals in comparison to SEL, as well as a lower percentage of individuals that are first cousins, half-sibs, and full-sibs. This is consistent with the common maternal origin of the SEL population and may also explain the inbreeding detected in this population, which could be due to Wahlund effect, as discussed above. The negative value of coancestry when considering all individuals reveals that there is high genetic dissimilarity between populations. Therefore, crosses among individuals of these populations can be encouraged to promote greater heterotic effects through breeding programs.

Mating between related individuals must be avoided as it can result in inbreeding, and possibly inbreeding depression, in the resulting descendent individuals.

The result for genetic differentiation (G'_{ST} = 0.28) indicates that the gene frequencies between populations are different, but the largest proportion of genetic diversity is distributed within populations [100(1-0.284) = 72%]. Furthermore, the pairwise coancestry values including both populations suggest that all individuals originated from seeds. Both populations have individuals with no or low levels of coancestry, indicating genetic diversity within populations. This result is significant for the genetic conservation of the species and provides useful information for breeding programs.

CONCLUSION

The *Hevea brasiliensis* populations studied herein present genetic diversity and differences in gene frequencies. As such, they are suitable for inclusion in *ex situ* conservation and breeding programs. However, both populations show inbreeding, which is likely due to mating among related individuals. Nevertheless, inbreeding can be managed by implementing crosses between unrelated individuals of the same or different populations. To ensure successful breeding programs, crossing among related individuals within populations must be avoided as it can result in mortality due the occurrence of inbreeding depression, resulting in increase of cost to replanting.

ACKNOWLEDGEMENTS

REFERENCES

AGUIAR, A. T.; GONÇALVES, P. S. Diversidade genética em genótipos de Hevea de origens amazônica e asiática. **Revista Ceres**, Viçosa, v. 53, n. 307, p. 285-291, 2006.

BITENCOURT, J. V. M.; SEBBENN, A. M. Patterns of pollen and seed dispersal in a small, fragmented population of the wind-pollinated tree *Araucaria angustifolia* in southern Brazil. **Heredity**, Edinburgh, v. 99, p. 580, 2007.

CAMARGO, A. P.; CAMARGO, M. B. P. Aptidão climática da heveicultura no Brasil. In: Alvarenga, A. P.; Carmo, C. A. F. S. (Ed.). **Seringueira**. Viçosa: Epamig, 2014. cap. 2, p. 793-840.

CUBRY, P.; PUJADE-RENAUD, V.; GARCIA, D.; ESPEOUT, S.; LE GUEN, V.; GRANET, F.; SEGUIN, M. Development and characterization of a new set of 164 polymorphic EST-SSR markers for diversity and breeding studies in rubber tree (*Hevea brasiliensis* Müll. Arg.). **Plant Breeding**, v. 133, n. 3, p. 419-426, 2014.

FARIA, D. A.; MAMANI, E. M. C.; PAPPAS, G. J.; GRATTAPAGLIA, D. Genotyping systems for Eucalyptus based on tetra-, penta-, and hexanucleotide repeat EST microsatellites and their use for individual fingerprinting and assignment tests. **Tree Genetics & Genomes**, Berlin, v. 7, n. 1, p. 63-77, 2011.

GONÇALVES, P. S.; SCALOPPI JÚNIOR, E. J.; MARTINS, M. A.; MORENO, R. M. B.; BRANCO, R. B. F., GONÇALVES, E. C. P. Assessment of growth and yield performance of rubber tree clones of the IAC 500 series. **Pesquisa Agropecuária Brasileira**, Brasília, v. 46, n. 12, p. 1643-1649, 2011.

GOUDET, J. FSTAT **Version 2.9. 3.2 for Windows:** a computer program to calculate F-statistics. 2002. Disponível em: < http://www2.unil.ch/popgen/softwares/fstat >. Acesso em: 02 Jun. 2017

HARDY, O. J.; VEKEMANS, X. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. **Molecular Ecology Notes**, Oxford, v. 2, n. 4, p. 618-620, 2002.

HEDRICK, P. W. A standardized genetic differentiation measure. **Evolution**, v. 59, n. 8, p. 1633-1638, 2005.

HOMMA, A. K. O. Extrativismo vegetal ou plantio: qual a opção para a Amazônia? **Estudos Avançados**, São Paulo, v. 26, n. 74, p. 167-186, 2012.

KAGEYAMA, P. Y.; OLIVEIRA, R. S. D.; FERRAZ, P. A.; FURTADO, E. L.; SOUZA, A. D. D.; SEBBENN, A. M. Ganhos na seleção para a produtividade de látex em população natural de *Hevea brasiliensis* na Reserva Chico Mendes: estudo de caso da IAPs (Ilhas de Alta Produtividade). **Scientia Florestalis**, Piracicaba, v. 61, n. 1, p. 79-85, 2002.

LE GUEN, V.; DOARÉ, F.; WEBER, C.; SEGUIN, M. Genetic structure of Amazonian populations of *Hevea brasiliensis* is shaped by hydrographical network and isolation by distance. **Tree Genetics & Genomes**, Berlin, v. 5, n. 4, p. 673-683, 2009.

LOISELLE, B. A.; SORK, V. L.; NASON, J.; GRAHAM, C. Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). **American Journal of Botany**, p. 1420-1425, 1995.

NEI, M. Analysis of gene diversity in subdivided populations. **Proceedings of the National Academy of Sciences**, v. 70, n. 12, p. 3321-3323, 1973.

OLIVEIRA, A. L. B.; GOUVÊA, L. R. L.; VERARDI, C. K.; SILVA, G. A. P.; GONÇALVES, P. S. Genetic variability and predicted genetic gains for yield and laticifer system traits of rubber tree families. **Euphytica**, v. 203, n. 2, p. 285-293, 2015.

PERSEGUINI, J. M. K. C.; ROMÃO, L. R. D. C.; BRIÑEZ, B.; SCALOPPI JUNIOR, E. J.; GONÇALVES, P. S.; BENCHIMOL, L. L. Genetic diversity of cultivated accessions and wild species of rubber tree using EST-SSR markers. **Pesquisa Agropecuária Brasileira**, Brasília, v. 47, n. 8, p. 1087-1094, 2012.

PETHIN, D.; NAKKANONG, K.; NUALSRI, C. Performance and genetic assessment of rubber tree clones in Southern Thailand. **Scientia Agricola**, Piracicaba, v. 72, n. 4, p. 306-313, 2015.

PHUMICHAI, C; PHUMICHAI, T; WONGKAEW, A. Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild Hevea rubber. **Plant Molecular Biology Reporter**, v. 33, n. 5, p. 1486-1498, 2015.

RIGHI, C. A.; LUNZ, A. M. P.; BERNARDES, M. S.; PEREIRA, C. R.; TERAMOTO, E. R.; FAVARIN, J. L. Coffee water use in agroforestry system with rubber trees. **Revista Árvore**, Viçosa, v. 32, n. 5, p. 781-792, 2008.

SANTOS, H. G.; JACOMINE, P. K. T.; ANJOS, L. H. C.; OLIVEIRA, V. A.; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A.; ARAUJO FILHO, J. C.; OLIVEIRA, J. B.; CUNHA, T. J. F. **Sistema brasileiro de classificação de solos**. 5. ed. Brasília: Embrapa, 2018.

SOUZA, A. M. D.; GOUVÊA, L. R. L.; OLIVEIRA, A. L. B.; SILVA, G. A. P.; GONÇALVES, P. S. Estimates of genetic parameters for the rubber yield and secondary traits in rubber tree. **Industrial Crops and Products**, v. 98, p. 19-24, 2017.

SOUZA, L. M.; LE GUEN, V.; CERQUEIRA-SILVA, C. B. M.; SILVA, C. C.; MANTELLO, C. C.; CONSON, A. R. O.; VIANNA, J. P. G.; ZUCCHI, M. I.; SCALOPPI JR, E. J.; FIALHO, J. F.; MORAES, M. L. T.; GONÇALVES, P. S.; SOUZA, A. P. Genetic diversity strategy for the management and use of rubber genetic resources: more than 1,000 wild and cultivated accessions in a 100-genotype core collection. **PloS one**, v. 10, n. 7, p. e0134607, 2015.

SOUZA, L. M.; MANTELLO, C. C.; SANTOS, M. O.; GONÇALVES, P. S.; SOUZA, A. P. Microsatellites from rubber tree (*Hevea brasiliensis*) for genetic diversity analysis and cross-amplification in six Hevea wild species. **Conservation Genetics Resources**, v. 1, n. 1, p. 75, 2009.

UNESP - UNIVERSIDADE ESTADUAL PAULISTA. Departamento de Fitossanidade, Engenharia Rural e Solos/ Área de Hidráulica e Irrigação. **Dados climáticos de Ilha Solteira.** 2017. Disponível em: < http://clima.feis. unesp.br/dados_diarios.php. Accessed 09 May 2017 >. Acesso em: 10 nov. 2018

VIRGENS FILHO, A. C. Sistemas agroflorestais com seringueira. In: Alvarenga, A.P.; Carmo, C. A. F. S. (Eds.). **Seringueira**. Viçosa: Epamig, 2014. cap.16, p. 793-840.

WEIR, B. S. **Genetic data analysis II. Methods for discrete population genetic data**. North Caroline State University, Sinauer Associates Inc. Pub., Suderland, Massachusets, 1996. 445 p.