Original Article
ISSN (Online): 2456-8651

International Journal of Engineering Science Technologies
March-April 2023 7(2), 1–11

IDENTIFICATION OF THE DISCRIMINATING MOLECULAR PROFILES OF FIVE MAIN RUBBER TREE (HEVEA BRASILIENSIS) GENOTYPES RECOMMENDED IN IVORY COAST

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Received 15 January 2023
Accepted 13 February 2023
Published 02 March 2023

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DOI 10.29121/IJOEST.v7.i2.2023.463

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ABSTRACT

This study was undertaken to identify the microsatellite markers characteristic of rubber tree genotypes, producers of natural rubber. Twelve pairs of microsatellite primers obtained from expressed sequence tags (EST), available in the GENBANK genomic bank, were used to screen a sample of 40 individuals, that is, 8 per genotype. These microsatellite loci are di, tri or tetranucleotides, patterned perfect, imperfect, compound or complex. This study on the GT1, PB217, IRCA41, IRCA230 and IRCA331 genotypes, recommended in Ivory Coast, showed that it is possible to characterize them by using three microsatellite loci, such as Hb36, Hb43 and Hb110. The abundance of repeats observed in the genome of rubber tree clones was similar to those reported in other works. These can be exploited and used in rubber tree clonal improvement programs.

Keywords: Hevea Brasiliensis, Discriminating Microsatellite Sequences, Ivory Coast

1. INTRODUCTION

Microsatellite, also known as simple sequence repeat (SSR) markers, are extremely precise. They have been classified as some of the most reliable molecular markers due to their abundance and variability in the genome, because they are randomly distributed Powell et al. (1996). Microsatellites possess polymorphisms, or variations in the length of their repeats, which can be studied using PCR and gel electrophoresis with admirable ease. Interpreting microsatellites is straightforward and their genetic analysis is now achievable thanks to the primer sequences published by Saghai et al. (1994). SSR markers have been applied in plants for constructing genetic maps, evaluating existing genetics within cultivars and performing pedigree studies - as evidenced by Taramino and Tingey (1996), Doldi et al. (1997). and Sefc et al. (1998). Recently, microsatellites of many species such as apple, grapevine and peach have been isolated and characterized - as seen in Sefc et al. (1999), Testolin et al. (2000), Liebhard et al. (2002) and Bindu et al. (2004).

The rubber tree is a forest tree with many uses, especially in medicine and the tire industry according to Compagnon (1986), supplying 98% of Ivory Coast's rubber Bindu et al. (2004). However, its lengthy cultivation cycle makes genetic analysis challenging due to its perennial nature. Thankfully, the discovery of PCR technology in the 1990s boosted research by providing markers such as RAPD, AFLP, microsatellites and SNPs Mullis et al. (1986). Studies on rubber trees have employed these markers to determine variability among cultivated clones and wild accessions. RAPD markers were used to identify clonal diversity, mitochondrial DNA RFLP was used to determine phylogenetic relationships, and resistance genes were located with RAPD techniques Besse et al. (1994), Shoucai et al. (1994), Luo et al. (1995), Varghese et al. (1997), Venkatachalam et al. (2004). For the first time, microsatellites were detected in the rubber tree genome by searching the database of its gene sequences according to Low et al. (1996). Besse et al. (1993) reported on using human minisatellite probes for DNA fingerprinting and Atan et al. (1996) described constructing an enriched microsatellite. Lespinasse et al. (2000) additionally outlined the construction of a rubber tree genetic linkage map, using various molecular markers.

In Ivory Coast, few publications have been made on rubber tree microsatellites. With Lespinasse et al. (2000) and Lekawipat et al. (2003), being two notable examples, it was deemed essential to determine the microsatellite profiles of the predominant rubber tree clones. Consequently, this paper covers the characterization of the electrophoretic profiles of five hevea genotypes that are commonly recommended in Ivory Coast.

2. MATERIAL AND METHODS

2.1. MATERIAL AND STUDY LOCATION

The leaves of five recommended Hevea brasiliensis genotypes from Ivory Coast were used, specifically taken from young plants with 3 to 4 storeys of leaves. The clones included in the study were GT1, PB217, IRCA41, IRCA230 and IRCA331. Each of these cultured genotypes was cloned by grafting to install almost all of the JBGs (Budwood Gardens).

In the budwood garden of Songon (a locality situated in southern Ivory Coast), a study was conducted. The geographical coordinates of this site are 5°00 north latitude, and 3°00 west longitude. The climate here is humid subtropical, with four distinct seasons. It has a bimodal rainfall pattern, featuring two periods of rain (April

through July and October through November) and two dry times (December to February or March and August to September). The yearly precipitation average usually falls between 1700 to 1800 mm. Kéli et al. (1992), found the average monthly temperature in these areas to be between 25.5 and 27°C. The terrain is generally composed of plains ranging from 0 to 100 m in altitude, with sporadic hills reaching up to 200-300 m high. These regions are characterized by deep, loosened, and well-drained Ferralsoils which are low in exchangeable bases and have a slightly acidic PH (6 ≤ pH ≤ 7.5), according to Obouayeba (2005) and Assiri et al. (2015).

2.2. METHODS

- **Sampling**

Leaves were carefully collected from 8 healthy individuals of each genotype and placed in labeled envelopes. This sample was kept for the duration of the study.

- **DNA extraction**

For each individual, we crushed 150mg of fresh leaf blades with liquid nitrogen at -196°C and then utilized a buffer from the "ZR Plant/Seed DNAMiniPrepTM", an extraction kit (ZYMO RESEARCH, USA) to incubate the material for genomic DNA extraction. We isolated the DNA by running it through multiple centrifugal processes in distinct solutions, and then refined it in a filtration column based on the instructions given by the kit provider. To conclude, we diluted the isolated DNA with 50 µl of freshwater and stored it at -30°C.

- **DNA quantification**

The extracted DNAs were quantified using a UV Vis 2000 nanodrop (Fisher Thermoscientific, USA) from a volume of 1 µl per sample of crude DNA extract. This volume was used to measure the optical density (OD) at 260 nm (the DNA concentration in ng/µl), 280 nm (the amount of protein contained in the DNA suspension), and the ratio (OD260/OD280) of the extracts. This ratio between these two measurements of OD: OD260/OD280 is a way to assess the quality or purity of our DNA extract. For a ratio around 2, the DNA extract is qualified as better or pure and its use in several amplification techniques is likely to give good results.

- **PCR amplification of microsatellites**

Twelve pairs of SSR primers (Hb31, Hb32, Hb33, Hb36, Hb43, Hb45, Hb53, Hb55, Hb64, Hb68, Hb78, Hb110) had been used to enlarge the DNA fragments via PCR. The use of a geneamp gadget 9700 thermal cycler (implemented biosystem, usa) shown in Table 1. These SSR markers have been chosen from the genbank databank, available at https://www.Ncbi.Nlm.Nih.Gov. The amplification became completed in a 10 µl medium composed of mix dream taq green PCR master buffer (1X)'', taq DNA polymerase (1 unit), DNTP 0.4 mM each, 4 mM of mgcl2, 0.1 µM of every primer and 10 ng of DNA extract. The PCR use the primers with fluorochromes IRD700 and IRD800 that are used for visualisation on a display after laser analyzing.

- **Migration and revelation of amplification products**

The li-cor 4300 DNA analyzer changed into used for each 6.5% lengthy ranger type polymer gel electrophoresis and amplification product revelation. The sizes (in base pairs) of the alleles of found out microsatellite markers had been decided by means of relating to the bands of a size marker (IRD700 and IRD800).

- **Allele coding**

The sizes (in wide variety of base pairs) of the alleles of found out microsatellite markers have been decided with the aid of relating to the bands of a length marker (IRD700 and IRD800). The sizes (in base pairs) of the alleles of microsatellite markers had been reported relatively to the bands of a size marker (IRD700 and IRD800).

- **Statistical analyses**

Genetix software program model 4.03, F-Stat version 2.9.3 and Genalex model 6.5 Peakall and Smouse (2012), Darwin model 6.5 Perrier and Jacquemoud (2006) and shape model 2.3.2 Pritchard et al. (2000) were used to manner the information contained in the generated genetic data matrix. The ones software program application made it possible to appearance the polymorphism of the microsatellites, the allelic richness, the intra- and inter-populace variability, in addition to the genetic shape of the population. The pics of the gels stored at the sequencer had been analyzed the use of Xn View v2.2, Jelly v0.1 (rami, unpublished) and Excel v2019. The shape software probabilistically assigns clone samples to genetically groups and predicted proportions for every clone pattern in a populace.

Table 1

Table 1 List of Primers Tested and Summary Table of The Sizes in Base Pairs (Bp) of SSR Loci, Developed by A Library Enriched in Di or Tri-Nucleotides (A), F: Forward, R: Reverse, (B) Perfect Pattern, (C) Imperfect Pattern, (D) Compound Pattern, (E) Complex Pattern.

Locus	Sequence (5'- 3')	Predicted sizes (bp)	Repeat patterns
HB 31	F : CCACTGTTTGTGTCATTTGGA	174	$(GGAA)_3$[b]
	R : TGGACAAGCAAAACAGCCTA		
HB 32	F : TCCCATTCCATTCCATTCA	238	$(CT)_6$ CC $(CT)_6$[c]
	R : AAACCGATAGCAAGCACCTC		
HB 33	F : GATAATTGCTTCCTGGTCA	188	$(ATG)_3(ATGA)_5$[d]
	R : AATGCCTTCTTTCCCTAAC		
HB 36	F : AGTGGCCAAGAAAGAATAAAA	230	$(AG)_{18}$[b]
	R : TACTACCCATCCACCAACCTAA		
HB 43	F : TTGTCTCCCCTTAATTCTGCTCTT	214	$(TC)_{18}$[b]
	R : GTGATCTGCCCATAACTACTCCAT		
HB 45	F : GTCAGAAGCAACCCACAAAC	170	$(AG)_{16}$[b]
	R : ATGCTTACCCAAAAATCAATG		
HB 53	F : CCAGCTTGAGGGAGAGTGTT	216	$(TG)_{11}$[b]
	R : TAGCAAGGAGGGAGAAAAGAG		
HB 55	F : AGATGGGCATGTTTTCGTTGTA	171	$(GA)_{15}$[b]
	R : TTTTTGCCCCTCCCTTATCA		
HB 64	F : AATCCACCCAGCCTTACAG	174	$(AG)_{17}$[b]
	R : AATTGGTGAGCTTCGTTTTT		
HB 68	F : AAAGAATATTGCAAAAACAGG	148	$(CT)_{15}$[b]
	R : GTTATTTTCCCCATCCTTTGA		
HB 78	F : CCATAGGGAAAGAAAAGAAAAA	200	$(TC)_9$[b]
	R : TCAACCTGTCCAAAAGAGAAG		

HB 110	F : ATGCAGCGATGTAGATAAAAGA	285	$(GC)_5(CA)_5...(GA)_{14}$[e]
	R : TCAAGATGTAAGCACCAGAACT		

3. RESULTS AND DISCUSSION

Table 2 shows the determination of microsatellite markers that better discriminate the rubber tree clones recommended in Ivory Coast. Out of twelve primer pairs used in this study, only 6 got amplified. Amongst those amplified microsatellite markers, three, or 50%, proved to be polymorphic and discriminating. These covered markers Hb43, hb36 and Hb110. This small number of discriminating SSR primers generated amplification products on forty individuals of the five rubber tree genotypes analyzed. Comparable effects had been stated by means of Creste et al. (2004), who analyzed the genetic diversity of 58 Musa genotypes using 33 SSR markers. A total of 15 primers, or 45% generated amplification products. These products are deemed to be characteristic of the identified genotypes. These works are similar to those of Koffi et al. (2019), where only a small fraction of the SSR primers tested, that is, 12 out of 30, or 40% generated amplification products on the 42 plantain genotypes analyzed. The microsatellite markers used were mainly obtained from various diploid genotypes (Calcutta-4 and diploid FHIA SH 3362, all belonging to Musa accuminata with AA genome) and East African highland banana cultivars (AAB), all of which are genetically diverse Mbanjo et al. (2013). The ideal perspective of this work is the broadening of the objectives aiming at having genetic elements or even more universal microsatellite markers, which offer the possibility of observing a wide genetic variability.

Table 2

Table 2 Microsatellite Markers, their Pattern, their Sequence, and their Hybridization Temperature

Locus	Sequence (5'- 3')	Length of determined alleles (bp)	Ta (°C)
HB 43	F: TTGTCTCCCCTTAATTCTGCTCTT	232 / 238	58
	R: GTGATCTGCCCATAACTACTCCAT		
HB 36	F: AGTGGCCAAGAAAGAATAAAA	240 / 253	58
	R: TACTACCCATCCACCAACCTAA		
HB 110	F: ATGCAGCGATGTAGATAAAAGA	287 / 295	58
	R: TCAAGATGTAAGCACCAGAACT		

These results are in disagreement with those of Noyer et al. (2005), whose studies were typically based on the analysis of 30 plantain genotypes, constituting a representative sample of the phenotypic diversity of the CARBAP collection using SSR and AFLP markers. Only one locus, out of 9 SSR loci used, was found to be polymorphic and also only one level of polymorphism was observed with marker AFLP on 633 bands provided by 8 primer pairs.

A polymorphism with a complete of 6 alleles became identified within the 5 populations of rubber tree clones for all 3 microsatellite markers used. The wide variety of alleles becomes two for the 3 loci Hb43, Hb36 and Hb110 with a mean of two alleles according to locus shown in table 3. The 3 loci Hb36, Hb43 and Hb110

had been used in mixture to reap the everyday profiles of the 5 clones cultivated in Ivory Coast shown in Table 4.

Table 3

Table 3 Range of Alleles According To SSR Locus and Consistent With Clone

Locus	GT1	PB217	IRCA41	IRCA230	IRCA331	Total
HB43	1	2	2	2	1	2
HB36	2	1	1	2	1	2
HB110	1	2	1	1	1	2
Total	4	5	4	5	3	6
%	66.7	83.3	66.7	83.3	50	
Average number of alleles per clone and per locus	1.33	1.66	1.33	1.66	1	2

The level of polymorphism being all the more important than the individual discrimination of the genotypes considered, it is therefore important to have a high level of polymorphism in order to facilitate the distinction between individuals Koffi et al. (2019). However, the molecular analysis carried out by this author, using 59 SNP markers, made it possible to identify plantain genotypes with great precision. Consequences from numerous cultivars with the identical inflorescence kinds confirmed 100% concordance, demonstrating that the kaspar technique is a reliable platform for producing plantain DNA fingerprints with high accuracy. The 59 SNP markers kept at the end of this work therefore constitute a resource of profitable and appropriate markers for the characterization of plantain germplasm.

Table 4

Table 4 Genotypes of the Five Rubber Tree Clones Cultivated in Ivory Coast, Obtained Via Mixture of Loci Hb43, Hb36 and Hb110

Clones	HB43	HB36	Hb110
GT1	232 / 232	240 / 253	295 / 295
PB217	232 / 238	240 / 240	287 / 295
IRCA41	232 / 238	240 / 240	295 / 295
IRCA230	232 / 238	240 / 253	295 / 295
IRCA331	232 / 232	240 / 240	295 / 295

This part deal with the analysis of the electrophoretic profiles generated by the 3 polymorphic and discriminating markers (Hb43, Hb36, Hb110) of the 5 rubber tree genotypes. The limited number of amplified primers could be due to the sufficient difference in the anchoring point of the primers, of the sequences flanking the microsatellite loci. Indeed, these microsatellite markers used were mainly obtained from various rubber tree genotypes. Some of these genotypes could not be discriminated by so-called monomorphic markers, not differentiating between a homozygous individual for the dominant allele and a heterozygous individual Brown et al. (1996). In contrast, polymorphic markers easily distinguish between individuals because of their stability, specificity and their uniform distribution of chromosomes Burow et al. (2012). The 3 markers (Hb36, Hb43, and Hb110) kept

generated clear and easily interpretable profiles Le Guen et al. (2011). They are able to therefore be recommended for the molecular characterization of clones cultivated in Ivory Coast. But, considering the minimum or even insufficient quantity of these selected markers, one should resort to more universal microsatellite markers for the molecular characterization of the genus Hevea, in order to explore a great genetic variability of the clones cultivated in Ivory Coast, in step with the work of Mbanjo et al. (2013).

Clone GT1. This clone is homozygous for markers Hb110 and Hb43 with a band of 295 bp and a band of 232 bp, respectively. GT1 is heterozygous for marker Hb36 with bands of 240 and 253 bp shown on Figure 1.

Figure 1

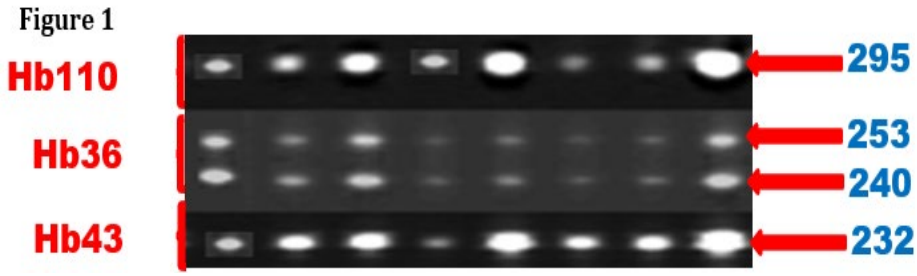

Figure 1 Electrophoretic Profile at Microsatellite Loci Hb43, Hb36, Hb110 In 8 Individuals of Clone GT1

Clone PB217. PB217 is homozygous for marker Hb36 with a band of 240 bp and heterozygous for markers Hb110 and Hb43, with two bands of (295 bp, 287 bp) and (238 bp, 232 bp), respectively shown on Figure 2.

Figure 2

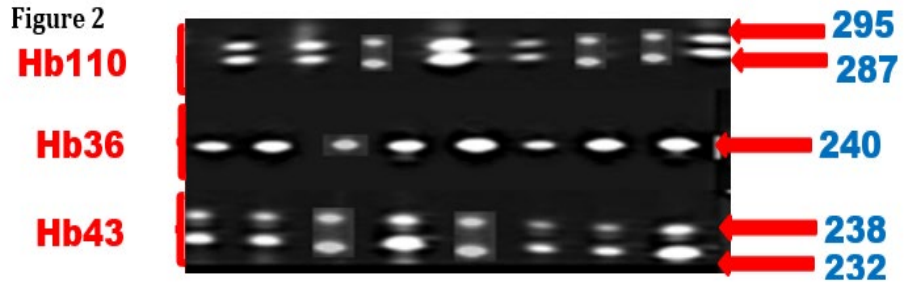

Figure 2 Electrophoretic Profile at Microsatellite Loci Hb43, Hb36, Hb110 In 8 Individuals of Clone PB217

Clone IRCA41. IRCA41 is represented through homozygous samples for markers Hb110 and Hb36 with a band of 295 Bp and a band of 240 bp, respectively. They're additionally heterozygous for marker Hb43 with bands of 238 and 232 bp shown on Figure 3.

Figure 3

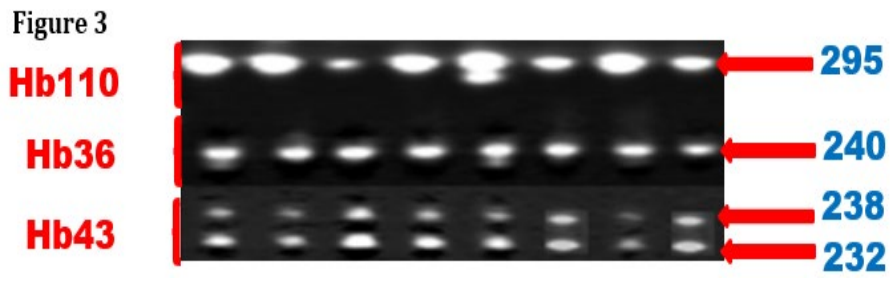

Figure 3 Electrophoretic Profile at Microsatellite Loci Hb43, Hb36, Hb110 In 8 Individuals of Clone IRCA41

Clone IRCA230. This clone is homozygous for marker Hb110 with a band of 295 bp and heterozygous for markers Hb36 and Hb43 with the observation of two bands of (253 bp, 2240 bp) and (238 bp, 232 bp), respectively shown on figure 4.

Figure 4

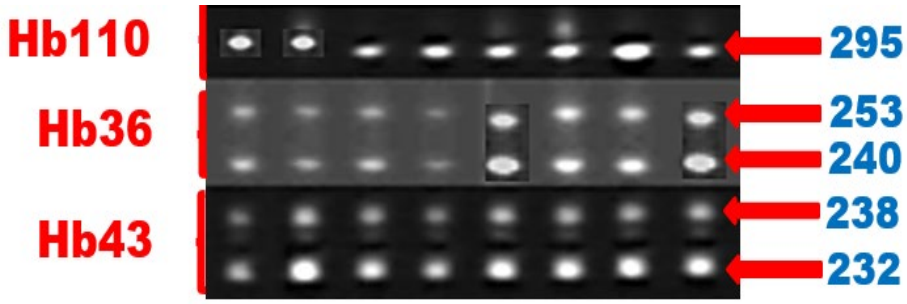

Figure 4 Electrophoretic Profile at Microsatellite Loci Hb43, Hb36, Hb110 In 8 Individuals of Clone IRCA230

Clone IRCA331. IRCA331 is homozygous for all markers Hb110, Hb36 and Hb43 with a band of 295 bp and a band of 240 bp and 232 bp, respectively shown on figure 5.

Figure 5

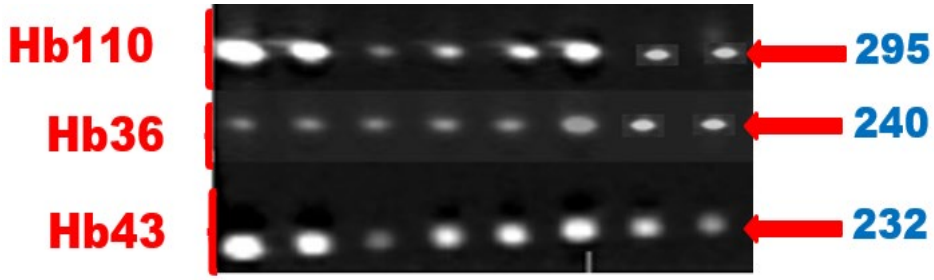

Figure 5 Electrophoretic Profile at Microsatellite Loci Hb43, Hb36, Hb110 In 8 Individuals of Clone IRCA331

4. CONCLUSION

The identification of the profiles of the five rubber three genotypes, was totally based on three discriminating microsatellite markers (Hb36, Hb43 and Hb110). The alleles and allele frequencies at the discriminating microsatellite loci vary

considerably between the clones. These markers best discriminate the 5 rubber tree genotypes. The determined electrophoretic profiles, based on the microsatellite sequences can be recommended for further characterization studies since they specifically characterize the five main rubber tree clones recommended in Ivory Coast.

CONFLICT OF INTERESTS

None.

ACKNOWLEDGMENTS

We would like to thank the Interprofessional Fund for Agricultural Research and Advisory (FIRCA) for financing the project on which we worked and all the coordinators for their valuable contributions to this work. We would also like to thank, the University Péléforo Gon Coulibaly for the close scientific collaboration and also all the anonymous readers of this article.

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