# Analysis of genetic variation in clones of rubber (*Hevea brasiliensis*) from Asian, South and Central American origin using RAPDs markers

# Análisis de la variación genética en clones de caucho (*Hevea brasiliensis*) de Asia, Suramérica y Centroamérica usando marcadores RAPD

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**ABSTRACT**
Rubber (*Hevea brasiliensis*) represents a potential species for reforestation and commercial exploitation programmes in tropical countries such as Colombia. The genetic variability of a rubber collection kept at the Paraguaicito Experimental Station in Buenavista in the Quindio department of Colombia was studied to improve knowledge regarding this species and make better use of the trees available. A total of 25 clones, six from South-America, 17 from Asia and two from Central-America were selected and analysed using RAPDs. DNA samples isolated from the trees were screened with 102 primers, 23 of which revealed polymorphism. Although a high degree of similarity was found, clustering analysis of the data led to differentiating the rubber trees in terms of their geographical origin. Furthermore, genetic relationships were found amongst the clones which could help in selecting parents for use in breeding programmes and designing strategies for conserving clones having desirable agronomic traits.

**Key words:** cultivar identification, genetic distance, genetic diversity, *Hevea brasiliensis*, RAPD marker.

**RESUMEN**
El caucho natural (*Hevea brasiliensis)* representa a especies potenciales para reforestación y programas de explotación comercial en ciudades tropicales como Colombia. La variabilidad genética de una colección de caucho que se encuentra en la Estación experimental de Paraguaycito en Buenavista, departamento del Quindío en Colombia fue estudiada para aumentar el conocimiento en cuanto a las especies y realizar un mejor uso de los árboles disponibles. Un total de 25 clones, seis de Sur América, 17 de Asia y 2 de América Central fueron seleccionados y analizados usando RAPDs. Las muestras aisladas de ADN de los árboles fueron con 102 primers, 23 de los cuales mostraron polimorfismos. Aunque se encontró un alto grado de similaridad, los análisis grupales de datos llevaron a diferenciar los árboles de de caucho en términos de su origen geográfico. Por lo tanto, las relaciones genéticas que se encontraron entre los clones podrían ayudar a seleccionar parentales para uso en programas de reproducción y diseño de estrategias para la conservación de los clones que tengan características agronómicas deseables.

**Palabras clave:** identificación de cultivos, distancia genética, diversidad genética, *Hevea brasiliensis*, marcadores RAPD.

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## INTRODUCTION

Rubber (*Hevea brasiliensis*) represents a potential species for agricultural production and reforestation programmes because it protects water resources and provides the community with long-term profitability (Rincón, 1996). However, the appropriate use of clonally propagated trees is hindered by a lack of knowledge regarding their genetic variability. Cultivated rubber's genetic basis is very constricted since it has been derived from a limited number of trees which were taken to England from the Amazonian rain-forests by Sir Henry Wickham in 1876 (Carron et ál., 1989). Rubber trees were introduced to Asia, Africa and back to the Americas from this original collection. The trees were probably underwent different breeding strategies in these locations, involving selecting intra- and inter-specific cross-derived offspring.

Several methods have been used for studying rubber's genetic variability, including isoenzyme analysis and other molecular techniques such as RFLPs, RAPDs and micro-satellites (Besse et ál., 1993, 1994; Seguin et ál., 1995). These studies have revealed a degree of clustering according to geographical origin in wild and clonally selected populations, the latter offering the advantage of not being affected by environmental factors and leading to determining the degree of divergence amongst different trees. RAPDs markers have been used for determining rubber's genetic variability and (although rather high variability was found) the markers used did not lead to discriminating the trees' geographical origin (Staub et ál., 1996; Varghese et ál., 1997).

The molecular characterisation of 25 rubber clones from Asia, South- and Central-America origins was carried out in this study using several RAPDs markers. This approach led to evaluating the potential of the trees to be used in programmes aimed at introducing the species into the central Colombian coffee-growing area.

## MATERIALS AND METHODS

### Plant Materials

Samples of vegetatively propagated clones were collected from Paraguaicito Experimental Station nursery stock (CENICAFE), Buenavista, in the Quindio department of Colombia. Young leaves were collected from one-year-old trees from 25 South-American (6), Asian (17) and Central-American (2) clones (Table 1).

### DNA extraction

DNA was extracted following the methodology described by Varghese et ál., (1997) with some modifications. Young leaves from each previously stored sample were macerated to a fine powder in liquid nitrogen; 50-100 mg of macerated tissue was then mixed with 4 vol 1X CTAB extraction buffer (2% hexadecyltrimethyl-ammonium bromide (CTAB), 20 mM EDTA, 100 mM Tris HCl pH 8.0, 1.4 M NaCl). Samples were placed in a water bath at 65 ºC for 30 min followed by two chlorophorm/isoamil (24:1) extractions. DNA was precipitated by adding 1 vol cold isopropanol with gentle mixing until DNA strands were observed. The samples were then centrifuged at 13,000 rpm and the precipitate was washed with cold 70% ethanol at room temperature. The resulting DNA pellet was then dried and suspended in 50-100 μL TE buffer (1 mM Tris HCl and 0.1 mM EDTA, pH 8.0). DNA concentration was determined by BioRad VersaFluor Fluoremeter and then diluted to final 10 ng/μL concentration.

### RAPD assay

DNA amplification conditions using RAPD primers were similar to those described by Varghese et ál., (1997). However, the primers used were from Operon Technologies series OPA, OPAN, OPBA, OPE, OPM plus OPK-19 and OPD-17 previously reported by Seguin, et ál., (1995). A total of 102 primers were tested for choosing those giving polymorphic patterns. Each 25 μL reaction mixture consisted of 0.1 mM dNTPs (Promega, Madison, WI, USA), 1.9 mM $MgCl_2$, 1X buffer, 0.2 μM primer, 1.4U *Taq* DNA polymerase (Promega®, Madison, WI, USA) and 50 ng genomic DNA. Amplifications were performed on an MJ Research Inc. Thermal Cycler PTC-100, with an initial cycle of 30s at 94 ºC, 45 cycles of 1 min at 92 ºC, 1 min at 35 ºC and 2 min at 72 ºC, followed by a final cycle of 5 min at 72 ºC. Amplification products were then separated by electrophoresis at 100V (0.05 Amp) for 3h on 1.4% agarose gels in 1X TBE buffer with 3 mg/mL ethidium

**Table 1.** Identification and geographical origin of the 25 *Hevea brasiliensis* clones used and analysed in this study

| Id | Clone | Geographical Origin |
|---|---|---|
| 1 | IAN- 713 | Brazil (they came to Paraguaicito from Caquetá) |
| 2 | IAN- 717 | Brazil |
| 3 | IAN- 2878 | Brazil |
| 4 | IAN- 3087 | Brazil |
| 5 | IAN- 710 | Brazil |
| 6 | IAN-873 | Brazil |
| 7 | RRIC- 121 | Sri Lanka (they came to Paraguaicito from Guadalupe Island in the Antillas) |
| 8 | RRIC- 130 | Sri Lanka |
| 9 | RRIC- 132 | Sri Lanka |
| 10 | RRIC- 42 | Sri Lanka |
| 11 | RRIC-102 | Sri Lanka |
| 12 | PR- 255 | Indonesia |
| 13 | PR- 261 | Indonesia |
| 14 | 93114 | Indonesia |
| 15 | RRIM- 228 | Malaysia |
| 16 | RRIM 600 | Malaysia |
| 17 | RRIM- 703 | Malaysia |
| 18 | PB 28/59 | Malaysia |
| 19 | PB- 254 | Malaysia |
| 20 | PB- 235 | Malaysia |
| 21 | IR- 22 | Vietnam |
| 22 | IR- 42 | Vietnam |
| 23 | AVROS- 2037 | Sumatra |
| 24 | GT-1 | Guatemala |
| 25 | GU- 198 | Guatemala |

bromide. A 1 kb DNA ladder (Promega, Madison, WI, USA) was used as molecular weight standard. The gels were photographed under ultraviolet light using a Kodak Digital Science® camera system and Adobe Photo de Luxe software.

### Statistical analysis

Data was scored as discrete variables using 1 to indicate the presence and 0 the absence of a band. All amplifications were repeated at least three times and only bands which were 100% reproducible in repeated amplifications were considered for estimating genetic distances. Genetic distance was estimated by using Dice's coefficient of similarity (Dice, 1945). A pair-wise distance matrix between genotypes was the basis for cluster analysis by un-weighted pair-group mathematical average (UPGMA) using numerical taxonomy and multivariate analysis system (NTSYS) software, version 2.0 (Rohlf, 1998).

## RESULTS AND DISCUSSION

A total of 102 primers were tested using a mixture of DNA from three clones from different geographical origins (IAN-713, RRIC-121 and GT-1). From these, 65 (23%) presented polymorphism amongst the clones and 42 (65%) produced monomorphic fragments. Figure 1 shows amplification products with primers. The number of polymorphic DNA fragments for each evaluated primer ranged from 1 to 12, having an average of 6 bands per primer.

Amplified product size varied from 350 bp (primers BA-7 and AN-17) to 3,500 bp (primer AN-15), agreeing with the range reported by Nurhaimi-Haris et ál., (1998) who found fragments ranging from 300 to 2,000 bp. A 1,250 bp fragment was frequently present on Asian clones (PR-261 and RRIC–121) but absent on South-American and Central-American ones; this marker was thus able to differentiate American clones.

Analysing genetic similarity revealed clones clustering into two well-differentiated groups, A and B (Figure 2) differing by 43% from the markers. Other studies have shown small genetic differences ranging from 20% to 30% (Seguin et ál., 1995; Nurhaimi-Haris et ál., 1998). This indicates that (as expected) there was an important genetic relationship amongst the individuals from this collection but that there is still a source of genetic variation which can be used in breeding programmes. The analysis also revealed an evident geographical correlation, since all South-American clones were in group A together with a few Asian clones (RRIC-130, RRIC-121, RRIC-132 and PB–254). This differs from that found by several authors (Varghese et ál., (1997); Seguin et ál., (1995); Besse et ál., (1993) and Nurhaimi-Haris et ál., (1998)) where no correlation was found between genetic diversity and geographical

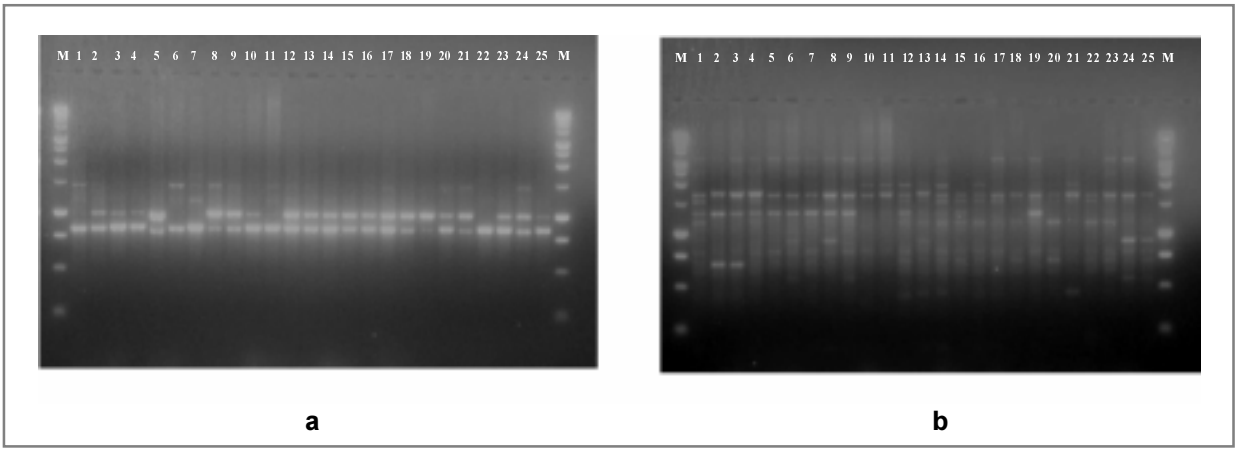


**Figure 1.** Agarose gel electrophoresis of the amplified products from 25 *Hevea* clones using the OPBA9 (a) and OPAN15 (b) primers. M corresponds to the 1 Kb ladder (Promega). Lanes 1-25 correspond to the amplification products from the different rubber clones in the same order as indicated in Table 1. Samples were run on 1.4% agarose gel following PCR reactions.

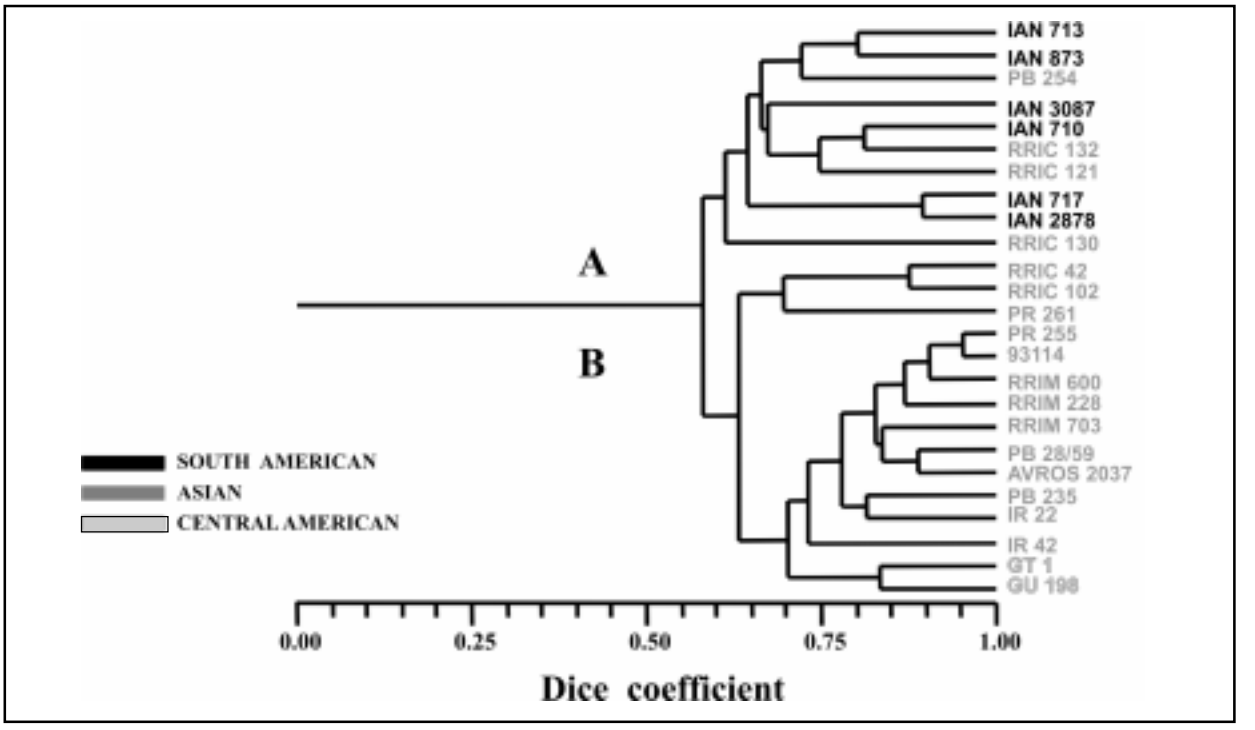


**Figure 2.** Dendogram of 25 *Hevea* clones based on RAPD analysis. Genetic distance were estimated according to Dice coefficient of similarity and clustering was done using the UPGMA method.

origin. The difference observed here may correspond to the fact that a different set of primers was used in this study.

Group B included most of the Asian clones plus two Central-American ones (GT-1 and GU–198). This tendency was also observed when the markers from individual primers were analysed independently (data not shown), indicating that the Central American clones genetic basis was more closely related to the Asian ones, sharing 70% of their markers. This may indicate that Guatemala germplasm was directly introduced from Asian clones.

Figure 2 also indicates that the Asian PB-254 clone forms a subgroup with South-American IAN-713 and IAN-873 clones, being different from the subgroup to which all the other Asian clones belonged. These findings could be useful for breeders since it could be a good idea to cross clone PB-254 with the Asian clones in the other subgroup to increase their genetic base, especially for programmes aiming to improve quantitative agronomic characteristics, such as latex production.

South-American clones IAN-713 and IAN- 873 belonged to same subgroup and may not be good candidates for being crossed in a breeding programme, given their more than 75% Similarity. Clone IAN-710 and clones RRIC-121 and RRIC-132 shared 73% similarity and clone RRIC-130 shared 62% similarity with all South-American clones, being more closely related to clones IAN-717 and IAN-2878.

The present study has shown that the RAPD markers used were able to differentiate South-American *Hevea* clones from most of the others which have been reintroduced from Asia. Analysing the markers obtained led to visualising relationships amongst the clones which may be useful in breeding programmes. This fact underlines the importance of carrying out genetic similarity studies since they may give a better idea of the genetic relationships of plant materials used for genetic improvement.

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