Research Article

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RAPD analysis of genetic distance among *Hevea* clones differing in vigour

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Abstract

Random amplified polymorphic DNA (RAPD) fragments were used to assess the genetic relationship among *Hevea* clones differing in growth vigour (high/low girth). DNA samples from *Hevea* polyclonal seedlings raised from cultivated clones and wild germplasm accessions were analyzed. Ten 10-mer primers selected from a set of 80 random oligonucleotide primers, depending on the ability to amplify genomic DNA were used to detect RAPD variations and genetic distance computation. Genetic distance of the trees between the low and high girth categories was greater than the trees within a girth category in the case of wild accessions. In the case of polyclonal seedlings there was not much change in the genetic distance between or within the two vigour categories obtained from the progenies of modern cultivated clones. The results suggest a more shared evolutionary heritage for the polyclonal seedlings than the wild germplasm accessions.

Key words : *Hevea*, RAPD markers, polyclonal seedlings, wild germplasm, genetic distance, evolutionary heritage

Introduction

Extension of natural rubber cultivation to marginal and sub-optimal agro climatic zones is being done in several rubber growing countries for socio-economic and commercial reasons. In India, rubber cultivation has been extended to agro-climatically less suitable areas like the North Konkan, parts of Orissa, Madhya Pradesh and North Eastern states where the trees face different kinds of environmental stresses such as drought and cold (Jacob *et al.*, 1999). If we grow relatively drought cold resistant clones in these areas, they can withstand these stresses to a great extent. Polyclonal seedlings were introduced in these areas along with clonal budded plants to monitor/ assess their performance in such areas. Unlike the bud-grafted plants, a certain amount of genetic variability is expected among seedlings since *Hevea* is a predominantly open-pollinated tree species.

An understanding of the extent of genetic diversity among different *Hevea* clones is essential for the selection of clones in breeding programmes. Molecular approaches hold great potential for plant breeding as they promise to shorten the time taken to produce crop varieties with desirable characters. Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), microsatellites or simple sequence repeats (SSRs) and Amplified Fragment Length Polymorphism (AFLP) are the techniques used in the selection for desirable characters using molecular markers. The techniques differ in their underlying principle and generate various amounts of information (Das *et al.*, 1999). RAPD technique has been used to determine the genetic relationship in some perennial species (Belaj *et al.*, 2002; Sudupak *et al.*, 2002).

Several molecular markers have been developed to detect genetic variations of *Hevea* clones (Besse *et al.*, 1994; Lou and Boutry, 1995; Low *et al.*; 1995; Lespinasse *et al.*, 2000; Saha *et al.*, 2004). Studies conducted by Varghese *et al.*, (1997) and Venkatachalam *et al.*, (2002) concluded that RAPD analyses are useful in the determination of genetic relationships and clone identification in *Hevea*. Shoucai *et al.* (1994) used RAPD to identify mildew resistant genes in *Hevea*. The RAPD technique is simple, requires relatively very small

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amounts of DNA and involves no radioactivity. The application of RAPD does not need any prior knowledge of genomic nucleotide sequences (Williams *et al.*, 1990). In this study, we report the genetic relationships between the high and low girth trees of the *Hevea* polyclonal seedlings raised from cultivated clones and high and low girth trees of *Hevea* wild germplasm using RAPD analyses. This study was conceived to test whether the high and low girth categories have had a more common and shared evolution within themselves than between the two vigour categories.

Materials and Methods

Plant material

The study was done in 16 polyclonal seedlings (8 high and 8 low girth) from RRS, Dapchari and 18 wild accessions (9 high and 9 low girth) from RRII nursery at CES, Chethackal. Fully expanded and disease-free leaves were collected from each category.

Preparation of genomic DNA

A modified method described by Porebski *et al.* (1997) with modification (Molly Thomas *et al.*, 2001) was used for the extraction of DNA. The leaf samples were ground to a fine powder using liquid nitrogen. The powder was mixed with 5 ml pre-warmed (60°C) extraction buffer (100mM Tris, 1.4 M NaCl, 20mM EDTA, 2% CTAB at pH 8.0, containing 0.3% β-mercaptoethanol, which was added to the buffer immediately before use), and 50 mg polyvinylpyrrolidone. After incubation at 60°C for 30 min., the solution was extracted with one volume of chloroform: isoamyl alcohol (24:1). Phases were separated by centrifugation at 3000 rpm for 20 min. at room temperature and the top phase was extracted again using chloroform: isoamyl alcohol mixture. The DNA was precipitated by addition of 0.5 volumes of sodium chloride and ice cold ethanol. The tubes were spun at 3000 rpm for 6 min. and the pellet was washed with ice cold 70% (v/v) ethanol. The DNA pellet was dried at room temperature and dissolved in 300 µl of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The DNA solution was subjected to RNase A and Proteinase K treatment and washed with neutral phenol. The DNA was precipitated by adding 1/10 volume of 2M sodium acetate and 2 volumes of absolute ethanol. The tubes were centrifuged at 12000 rpm for 10 min. and the pellet was washed with ice cold 70% (v/v) ethanol. The pellet was dried at room temperature and then dissolved in 200 µl TE.

DNA amplification by PCR

PCR amplifications were carried out using ten Operon arbitrary decamer primers that gave informative amplification with *Hevea* DNA. PCR was performed in a total reaction volume of 25 µl and contained 10 picomoles primer and 50 ng template DNA, 1X polymerase buffer (Promega, USA), 2 mM dNTPs and 0.7 unit of Taq DNA polymerase (Promega, USA). Samples were initially heated to 94°C for 3 min. followed by 40 cycles of denaturation at 94°C for 30 sec. annealing at 36°C for 1 min., polymerization at 72°C for 2 min. and a final extension at 72°C for 7 min. PCR amplicons were then run in a 1.5% agarose gel, stained with ethidium bromide and visualized using a UV light source.

RAPD data analysis

Data were scored as discrete variables using 1 tc indicate the presence and 0 the absence of a band. Only bands reproducible in repeated amplifications were considered for estimation of genetic distances. Genetic distance between the clones was calculated using the modified Jaccard index (Jackson *et al.*,1989). $Gd_{ij} = (B_{ij}/M_{ij})*100$; where, Gd_{ij} is the genetic distance between the samples I and j; B_{ij} is the number of polymorphic bands between I and j and M_{ij} is the total number of band positions in I and j.

Results and Discussion

In the present study, we used 10 random primers, which were found to be informative from previous RAPD analyses in *Hevea* involving 80 arbitrary primers (Saha, unpublished data). All the DNA samples got amplified by these primers, yielding reproducible RAPD profiles (Figs. 1 and 2). The amplified fragments ranged in size

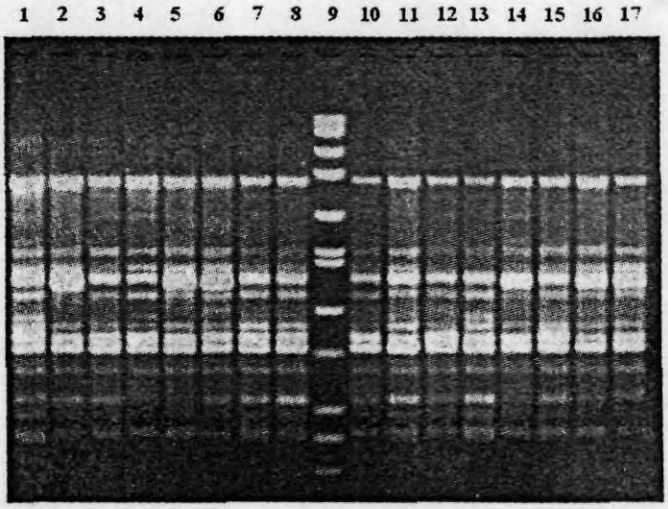

Fig.1. RAPD profile of *Hevea* polyclonal seedlings generated by the Operon primer OPD-08. Lane 1-8, high girth trees, Lane 9, Size marker, Lane 10-17, low girth trees.

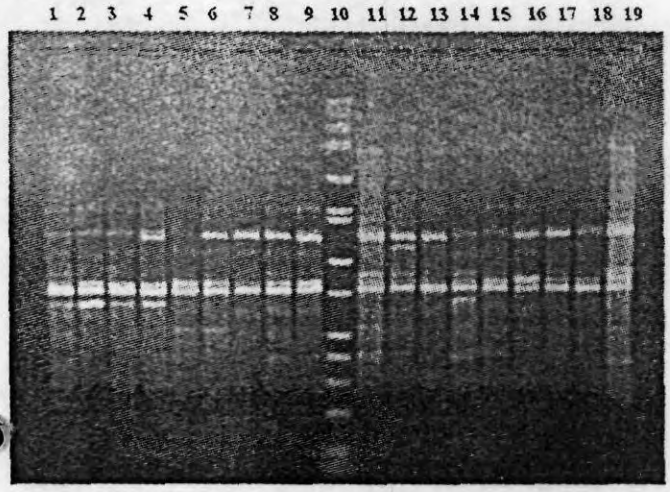

Fig.2. RAPD profile of *Hevea* wild germplasm generated by the Operon primer OPD-08. Lane 1-9, low girth trees, Lane 10, Size marker, Lane 11-19, high girth trees.

from 0.35 kb to 3.5 kb. The number of polymorphic bands per primer ranged from 1 to 10 (Table 1). Wild accessions showed higher polymorphism than the polyclonal seedlings used for each primer (Table 1) suggesting increased diversity existing among the germplasm lines compared to the seedlings as one would expect. It has been demonstrated that wild accessions were more polymorphic than cultivated Wickham clones in other studies also using molecular markers (Chevallier, 1988; Besse *et al.*, 1993; Lekawipat *et al.*, 2003). However, reports on genetic relationships based on differences in growth vigour (girth), are not available.

Table 1. RAPD primers and number of amplification products in *Hevea* clones (values with different alphabets differ at $p < 0.05$)

Primer code	Number of bands			
	Polyclonal Seedlings		Wild Germplasm	
	Total	Polymorphic	Total	Polymorphic
OPG-17	9	5	14	10
OPG-10	12	5	11	7
OPJ-20	12	1	12	6
OPI-06	7	2	14	9
OPH-03	11	5	9	6
OPJ-19	9	2	9	7
OPA-01	6	4	7	4
OPG-03	11	4	10	5
OPE-03	9	5	11	8
OPD-08	13	3	13	8
Total	99[a]	36[b]	110[a]	70[c]

Results of genetic distance computation are given in Table 2. The genetic distance between the low and high girth category was 17% in the case of polyclonal seedlings and 43% in the wild accessions. The genetic distance among the genotypes of low girth category was 19% and that of high girth category was 16% in the case of polyclonal seedlings whereas it was 35% and 34% respectively in the case of low and high girth wild accessions. Genetic distance of the trees between the two girth categories was greater than the trees within a girth category in the case of wild accessions. In the case of polyclonal seedlings from Dapchari, there was not much difference in the genetic distance between the two vigour categories.

Table 2. Genetic distance between and among low and high girth genotypes of *Hevea* (values with different alphabets differ at $p < 0.05$)

Genotypes	Genetic distance (%)		
	Low x High	Low	High
Polyclonal seedlings	17[a] ± 3.3	19[a] ± 2.7	16[a] ± 3.7
Wild germplasm	43[b] ± 2.9	35[c] ± 2.1	34[c] ± 1.6

Morphological and agronomic characters are commonly used for identifying *Hevea* clones. Although these characters are helpful in determining the phenotypic variability among clones, they are highly sensitive to environmental effects (Seguin *et al.*, 1996). Many genetic diversity studies in plants are available based on RAPD markers and the results are unaffected by the environment. Studies conducted in *Hevea* showed that RAPD marker techniques are feasible in *Hevea*. They are an effective method for clone identification and for the analysis of genetic relationships among clones.

In the present study, when a comparison was made between the wild accessions and seedlings, the former were found to be more variable than the latter. The seedlings that were raised from cultivated Wickham clones had come from a very narrow genetic base and this may be the reason for the lesser variability in seedlings unlike the germplasm lines that came from a much broader genetic base from the wild. Since *Hevea* is a predominantly open-pollinated tree species, certain amount of genetic variability is expected among the plants raised from the seeds. This is true also when the comparison is made among different clones using different molecular techniques (Chevallier, 1988; Besse *et al.*, 1993; Low *et al.*, 1996; Varghese *et al.*, 1997; Venkatachalam *et al.*, 2002). But when the analyses were done based on differences in growth vigour (high/low girth), the genetic distance between the two girth categories was similar to that within a girth category in the case of seedlings. On the other hand, the genetic distance between the two girth categories was greater than the trees within either girth category in the case of wild accessions. Our results give molecular evidence that the seedlings used here from the Wickham base were more closely related than the wild germplasm lines studied. The genetic distance between the low and high vigour seedlings were not significantly different

suggesting their common evolutionary heritage from the Wickham base. On the other hand, the statistically significant high genetic distance between the high and low vigour germplasm lines suggests their less shared or more distant evolutionary origins.

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