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Allelic Diversity Revealed Through SSR Polymorphisms at the Locus Encoding HMG-CoA Reductase in Rubber (*Hevea brasiliensis*)

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Abstract

This study was carried out to define the extent of allelic variation of 3-hydroxy-3-methylglutaryl-CoA reductase gene (*HMGR*) in wild *Hevea* accessions, based on SSR polymorphisms existing at their 3'-untranslated

regions (UTRs). Existence of two microsatellite alleles and their repeat compositions was demonstrated earlier in cultivated rubber clones. Both alleles contained perfect poly (AG)_n repeats interrupted by a short sequence of 12 nucleotides and allelic variation at this microsatellite locus was the result of repeat length polymorphisms. In wild populations of rubber, nine microsatellite alleles ('A' to 'I') were identified at the *HMGR* locus revealing a wide allelic diversity compared to cultivated clones. Out

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of nine, four alleles ('B', 'C', 'D' and 'G') were present in higher frequencies than the others. In total, 15 allelic combinations were noticed for *HMGR* among wild accessions and four of them were unique. Twenty-five out of 60 wild accessions were found to be homozygous for the above four alleles ('BB', 'CC', 'DD' and 'GG') and the rest were heterozygous, characterized by 11 different allelic combinations. Repeat-length polymorphisms were noticed in these four alleles prevailing among wild *Hevea* accessions. Genetic relatedness of Mato Grosso accessions with cultivated *Hevea* clones, as revealed through this study, is in agreement with earlier reports on phylogenetic studies using molecular markers. This work is a significant step towards understanding the functional variability of *HMGR* for latex production in *Hevea brasiliensis*.

Key words: allelic diversity, *Hevea brasiliensis*, HMG-CoA reductase, simple sequence repeats (SSRs).

Introduction

Hevea brasiliensis (Wild. Ex. Adr. de. Juss. Muell. Arg.) is an important tree crop producing latex of commercial utility. Cultivated *Hevea* clones of the South East Asian rubber growing countries had their origin from 'Wickham clones' (WYCHERLEY, 1968; DEAN, 1987). Since the cultivated clones are characterized with a low genetic variability, an international effort was taken to enrich the available genetic variability of *Hevea* through the introduction of wild germplasm into the breeding pool. The International Rubber Research and Development Board (IRRDB) and the Brazilian government organized a major collection expedition to the Amazon rain forest in 1981. The team collected 64,736 seeds and bud wood from 194 high yielding mother trees from the state of Acre, Mato Grosso and Rondonia of Brazil (ONG et al., 1983; MOHD. NOOR and IBRAHIM, 1986). India received her share of *Hevea* germplasm during the period 1984–1990 for their conservation, evaluation and utilization in breeding programme.

Conventional genetic analysis in *Hevea* is very difficult because of its perennial nature, long breeding and selection cycles and difficulties in raising 'F₂ progenies'. Therefore, the application of molecular markers has been realized for such genetic analysis in rubber (BESSE et al., 1994; SHOUCAI et al., 1994; LUO et al., 1995; LOW et al., 1996; VARGHESE et al., 1997; LESPINASSE et al., 2000; ROY et al., 2004; SAHA et al., 2005).

Microsatellite markers, based on variation in the number of simple sequence repeats (SSRs) have become the marker of choice due to their ease of use and co-dominant nature (POWELL et al., 1996). Recently many reports have demonstrated that large numbers of SSRs are located in transcribed regions of genomes (MORGANTE et al., 2002). The use of ESTs or cDNA based SSRs has been reported for several plant species (CHO et al., 2000; SCOTT et al., 2000; THIEL et al., 2003). The presence of SSRs in transcripts of known genes suggests that they might have a role in gene expression or function. Substantial data indicates that SSR expansions and/or contractions in protein-coding regions can lead to a gain or loss of gene function via frame-shift mutation or expanded toxic mRNA. SSR variations in 5'-UTRs could regulate gene expression by affecting transcription

and translation. The SSR expansions in the 3'-UTRs cause transcription slippage and produce expanded mRNA, which can be accumulated as nuclear foci, and which can disrupt splicing and possibly disrupt other cellular function (LI et al., 2004).

Analysis of genetic variability based on markers that target functional loci provides opportunities to study functional diversity and to identify corresponding genes controlling agronomic traits of complex inheritance. In rubber, the gene *HMGR* encoding HMG-CoA reductase, involved in latex production, has been detected with dinucleotide repeats (AG)_n at the 3'-UTR of mRNA. This repeat sequence was earlier used as SSR marker for genetic relationship studies in rubber (LESPINASSE et al., 2000; LEKAWIPAT et al., 2003). Little information is available on the allelic variation of the *HMGR*. In our earlier study (SAHA et al., 2005), the existence of two microsatellite alleles and their repeat compositions was demonstrated through sequencing of the *HMGR* in 15 cultivated clones of rubber including 13 primary Wickham clones. Both alleles contained perfect poly (AG)_n repeats interrupted by a short sequence of 12 nucleotides and allelic variation at this microsatellite locus was the result of repeat length polymorphisms. The existence of repeat sequences in the 3'-UTR of *HMGR* in *Hevea* is very unique as revealed through the database search, which presumably may have regulation over the production of HMG-CoA reductase thereby affecting latex yield. Therefore it was felt necessary to identify the allelic variation of the locus *HMGR* existing in the wild *Hevea* gene pool. In this study we demonstrate allelic diversity existing at this locus among wild *Hevea* germplasm originating from Acre, Rondonia and Mato Grosso provinces of Brazil, the primary center of origin of rubber and establish genetic relationship among them based on *HMGR* allelic compositions.

Materials and Methods

Plant material and DNA extraction

Sixty wild accessions originating from three different provinces of Brazil namely, Acre, Rondonia and Mato Grosso (*Figure 1*) were used as the experimental material (*Table 1*) along with three cultivated popular clones: RRII 105, RRIC 52 and RRIM 600. Total genomic DNA was extracted from 0.5 g of young leaves of each clone following the CTAB (cetyl trimethyl ammonium bromide) protocol of DOYLE and DOYLE (1990).

Amplification of HMG-CoA reductase gene harboring the simple sequence repeats

Two stretches of (AG)_n repeats were identified at the 3'-UTR of the cDNA of HMG-CoA reductase from *H. brasiliensis* through sequences published in GenBank (AF429388). A pair of flanking primers, close to the repeats, was synthesized (5' GAATCTGGTC-CCAGCAATGT 3' and 5'GAAGAAGGAGAGGCAAAG-CA 3') in order to amplify a fragment that facilitates its detection through polyacrylamide gel electrophoresis (PAGE). The forward primer was end labelled with ³³P γATP using 5' end labeling kit (Promega, USA). The PCR reaction was carried out in a 10 µl final volume

containing 20 ng of genomic DNA, 0.2 μ M each of the forward and reverse primers, 200 μ M dNTPs and 0.7 units of AmpliTaq Gold polymerase along with the buffer supplied by Applied Biosystems. The temperature cycle profile involved an initial denaturation step of 5 minutes at 95°C followed by a touch down PCR program. Temperature profiles of the touch down PCR for 7

cycles were as follows: 94°C for 30 seconds, 63°C for 1 minute, Δ 1°C for 7 cycles, 72°C for 1 minute. This was followed by a normal cycling of 94°C for 30 seconds, 56°C for 1 minute, 72°C for 1 minute for 23 cycles and a final extension at 72°C for 10 minutes. The touch down protocol was used to eliminate stuttering and artifact bands. Once the PCR was completed, the reactions were stopped immediately by the addition of 10 μ l formamide loading buffer (0.1% each of bromophenol blue and xylene cyanol FF, 10 mM EDTA (pH 7.5) and 98% deionized formamide) and stored at -20°C. Amplification products were run on a 6% denaturing polyacrylamide gel containing 7 M urea using 0.6x TBE buffer at a constant power of 55 W. The gels were then dried and autoradiographed on X-ray film using standard procedures.

Polymorphism evaluation and data analysis

The number of alleles detected by the microsatellite was estimated for each clone/genotype. Expected heterozygosity (H_e) was estimated as: $H = 1 - \sum p_i^2$ (NEI, 1973), where p_i is the frequency of the i^{th} allele in the examined genotypes. Observed heterozygosity (H_o) was determined as the ratio between the number of heterozygous genotypes and the total number of genotypes analyzed. Population genetic parameters namely, effective number of alleles (n_e), inbreeding coefficient (F_{IS}) and genetic differentiation (F_{ST}) was performed using the software POPGENE, version 1.32 (YEH and BOYLE, 1997; YEH et al., 2001).

Amplification products (alleles) were scored on the basis of their presence or absence in the gel. Pair-wise comparisons based on both unique and shared amplifi-



Figure 1. – Geographical location of the three provinces Acre, Rondonia and Mato Grosso in Brazil from where wild *Hevea* accessions originated. Accessions used in this study are from the indicated (*) regions of the three provinces.

Table 1. – List of wild *Hevea* accessions from three different provinces of Brazil with their allelic compositions of *HMGR*.

| Code | ACRE accession | Allele composition of <i>HMGR</i> | Code | RONDONIA accession | Allele composition of <i>HMGR</i> | Code | MATO GROSSO accession | Allele composition of <i>HMGR</i> |
|------|-------------------|---|------|-----------------------|---|------|--------------------------|---|
| A1 | AC/S/8 35/88 | BC | R1 | RO/CM/10 44/24 | DG | M1 | MT/C/2 10/169 | DD |
| A2 | AC/S/12 42/223 | CC | R2 | RO/CM/10 44/43 | CD | M2 | MT/IT/12 26/75 | DG |
| A3 | AC/S/12 42/248 | CC | R3 | RO/J/6 32/77 | BB | M3 | MT/IT/13 29/83 | DG |
| A4 | AC/S/10 37/67 | CG | R4 | RO/J/6 32/42 | BC | M4 | MT/C/1 18/9 | EG |
| A5 | AC/S/12 42/120 | CG | R5 | RO/A/7 25/329 | CC | M5 | MT/IT/16 34/208 | DD |
| A6 | AC/F/7 38/197 | CC | R6 | RO/JP/3 22/64 | CD | M6 | MT/C/1 18/117 | GG |
| A7 | AC/S/12 42/348 | CD | R7 | RO/OP/4 20/15 | CC | M7 | MT/IT/15 28/70 | DH |
| A8 | AC/S/12 42/365 | BB | R8 | RO/JP/3 22/465 | CC | M8 | MT/C/1 18/107 | DG |
| A9 | AC/S/10 37/336 | AG | R9 | RO/OP/4 20/119 | CF | M9 | MT/C/2 10/169 | CD |
| A10 | AC/S/8 35/239 | BC | R10 | RO/C/8 24/458 | CI | M10 | MT/IT/17 27/1 | GG |
| A11 | AC/S/10 37/161 | GG | R11 | RO/PB/1 2/32 | CC | M11 | MT/C/1 18/90 | DG |
| A12 | AC/B/19 56/239 | BB | R12 | RO/OP/4 20/147 | CC | M12 | MT/C/1 18/91 | CG |
| A13 | AC/S/11 41/356 | BG | R13 | RO/J/5 33/93 | CF | M13 | MT/C/1 18/80 | DG |
| A14 | AC/S/9 39/10 | CD | R14 | RO/OP/4 20/97 | BC | M14 | MT/C/2 10/36 | GG |
| A15 | AC/S/9 39/15 | BC | R15 | RO/C/8 24/177 | CI | M15 | MT/IT/16 34/174 | DG |
| A16 | AC/S/9 39/22 | BB | R16 | RO/CM/10 44/105 | CF | M16 | MT/IT/14 30/13 | DD |
| A17 | AC/S/12 42/23 | CD | R17 | RO/JP/3 22/483 | CC | M17 | MT/IT/14 30/67 | GG |
| A18 | AC/S/9 39/50 | BC | R18 | RO/C/9 23/152 | CC | M18 | MT/IT/15 28/91 | DH |
| A19 | AC/S/9 39/60 | BD | | | | | | |
| A20 | AC/F/5 21/278 | BB | | | | | | |
| A21 | AC/S/8 35/291 | BC | | | | | | |
| A22 | AC/S/10 37/86 | CG | | | | | | |
| A23 | AC/S/10 37/142 | BB | | | | | | |
| A24 | AC/S/8 35/430 | GG | | | | | | |

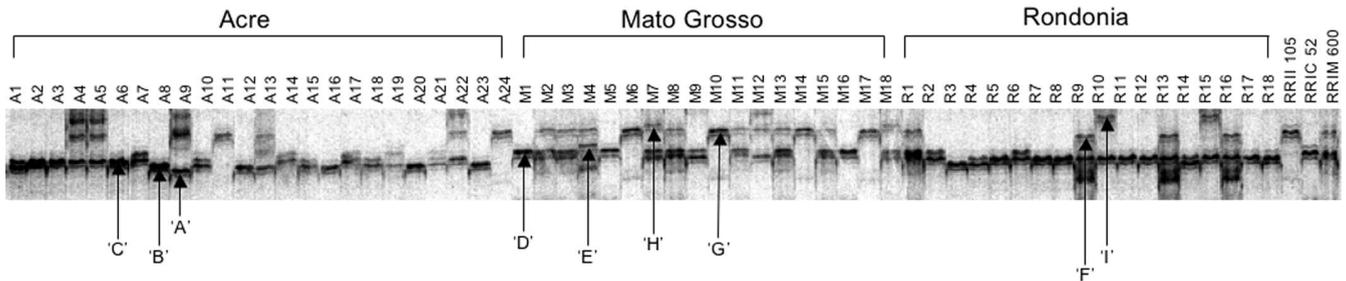


Figure 2. – Autoradiogram showing the allelic variation at the locus *HMGR* among wild *Hevea* germplasm originating from Acre, Rondonia and Mato Grosso provinces of Brazil. Nine microsatellite alleles ('A' to 'I') were identified at this locus revealing wide allelic diversity among the wild population of rubber.

cation products, were employed to calculate genetic distance (LINK et al., 1995). The data was subsequently used for cluster analysis using UPGMA to construct a dendrogram. All calculations were made using the Treecon programme (VAN DE PEER and DE WACHTER, 1994).

Sequencing of the alleles for repeat length polymorphisms

Allelic bands were excised from the PAGE after aligning the autoradiogram with the blotted gel. DNA was extracted from the gel slices and reamplified with the respective primer-pairs. PCR products were purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) and sequenced at Macrogen Inc., Korea to identify sequence variability in the repeat length as well as in the flanking zones.

Results

Allelic diversity

The gene *HMGR*, a key enzyme involved in latex production in *Hevea* contains (AG)_n repeats, clustered away

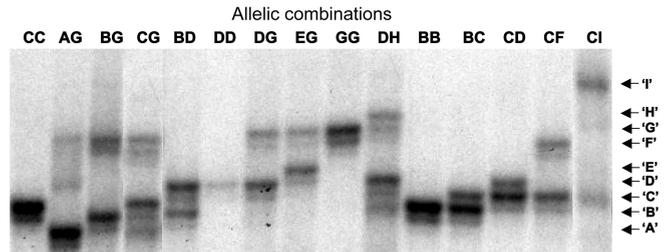


Figure 3. – Autoradiogram showing clear discrimination of nine alleles ('A' to 'I') in 15 different combinations identified among wild *Hevea* accessions. Homozygosity was detected for 'B', 'C', 'D' and 'G' alleles.

from the coding sequences in the 3' untranslated region. Earlier we reported the existence of two alleles in popular *Hevea* clones based on repeat length polymorphisms. The same primer-pair was used to amplify the alleles in wild *Hevea* accessions. Nine microsatellite alleles, designated as 'A' to 'I' were identified at the *HMGR* locus revealing a wide allelic diversity in wild populations than the cultivated clones of *Hevea brasiliensis* (Figure 2). Nine alleles existing in 15 different combinations

Table 2. – *HMGR* alleles and their frequency of occurrence in wild *Hevea* accessions from three provinces of Brazil.

| Allele | Allele frequency in wild <i>Hevea</i> accessions from three provinces of Brazil | | | Overall allele frequency in wild <i>Hevea</i> accessions |
|---|---|----------|-------------|--|
| | ACRE | RONDONIA | MATO GROSSO | |
| A | 0.021 | - | - | 0.008 |
| B | 0.354 | 0.111 | - | 0.175 |
| C | 0.354 | 0.639 | 0.056 | 0.350 |
| D | 0.083 | 0.083 | 0.417 | 0.183 |
| E | - | - | 0.028 | 0.008 |
| F | - | 0.083 | - | 0.025 |
| G | 0.188 | 0.028 | 0.444 | 0.217 |
| H | - | - | 0.056 | 0.017 |
| I | - | 0.056 | - | 0.017 |
| Expected heterozygosity (<i>H_e</i>)* | 0.707 | 0.562 | 0.622 | 0.765 |
| Observed heterozygosity (<i>H_o</i>) | 0.583 | 0.556 | 0.611 | 0.583 |
| Effective number of allele (<i>n_e</i>) | 3.408 | 2.282 | 2.645 | 4.255 |
| Inbreeding coefficient (<i>F_{IS}</i>) | 0.174 | 0.011 | 0.018 | 0.074 |
| Genetic differentiation (<i>F_{ST}</i>) | -- | -- | -- | 0.177 |

'-' = absence of allele; * Nei's expected heterozygosity (1973).

revealed the existence of 15 genotypes in respect to *HMGR* locus (Figure 3). Homozygosity was detected only for four alleles 'B', 'C', 'D' and 'G' among these wild accessions. Out of 60 accessions, 25 were found to be homozygous (Table 1). Two popular clones out of the three included in this study, RRII 105 and RRIC 52, were homozygous for the alleles 'G' and 'D'; and the clone RRIM 600 was heterozygous having 'DG' allelic combination. Three unique allelic combinations 'AG', 'BG' and 'BD' were noticed in wild accessions AC/S/10 37/336, AC/S/11 41/356 and AC/S/9 39/60 respectively, originating from the same district 'Sena Madureira' of Acre province. Another unique combination 'EG' was found in a Mato Grosso accession MT/C/1 18/9. Allelic combination 'CI' present only in two Rondonian accessions RO/C/8 24/177 and RO/C/8 24/458, and 'DH' only in two Mato Grosso accessions MT/IT/15 28/70 and MT/IT/15 28/91 were considered to be the rare allelic combinations/genotypes. These two genotypes 'CI' and 'DH' were found to be restricted to a particular location of the district 'Calama' of Rondonia and 'Itauba' of Mato Grosso respectively. Among the nine alleles, five alleles 'A', 'B', 'C', 'D' and 'G' were distributed among Acre accessions; six alleles 'B', 'C', 'D', 'F', 'G' and 'I' in Rondonian accessions and five alleles 'C', 'D', 'E', 'G' and 'H' in Mato Grosso accessions (Table 2). Allele 'A' was found to be

restricted only in Acre accessions. Similarly two alleles 'F' and 'I' were unique in Rondonian accessions and allele 'H' was present only in Mato Grosso accessions.

Allele frequency was calculated for each of the population Acre, Rondonia and Mato Grosso. Highest frequencies of the alleles 'B' and 'C' (0.354) were detected among Acre accessions, whereas allele 'C' (0.639) and allele 'G' (0.444) were present in high frequency among Rondonian and Mato Grosso accessions respectively. Presence of few alleles in high frequency within a population, tend to display low heterozygosity values as detected in Rondonian accessions, where only the allele 'C' out of six alleles showed high frequency (0.639) distribution. However, the overall allele frequencies revealed a higher distribution of 'C' allele (0.35) among wild *Hevea* accessions. Effective number of alleles (n_e), which expresses allele frequency distribution at the locus *HMGR* within the population, ranged between 2.282 to 3.408 in Rondonian and Acre populations respectively.

In all three populations Acre, Rondonia and Mato Grosso, the observed heterozygosity (H_o) was lower than those expected (H_e) in a random mating population leading to positive estimates of inbreeding coefficients (F_{IS}), which measures the heterozygote deficit within population based on geographic regions (Table 2). The popula-

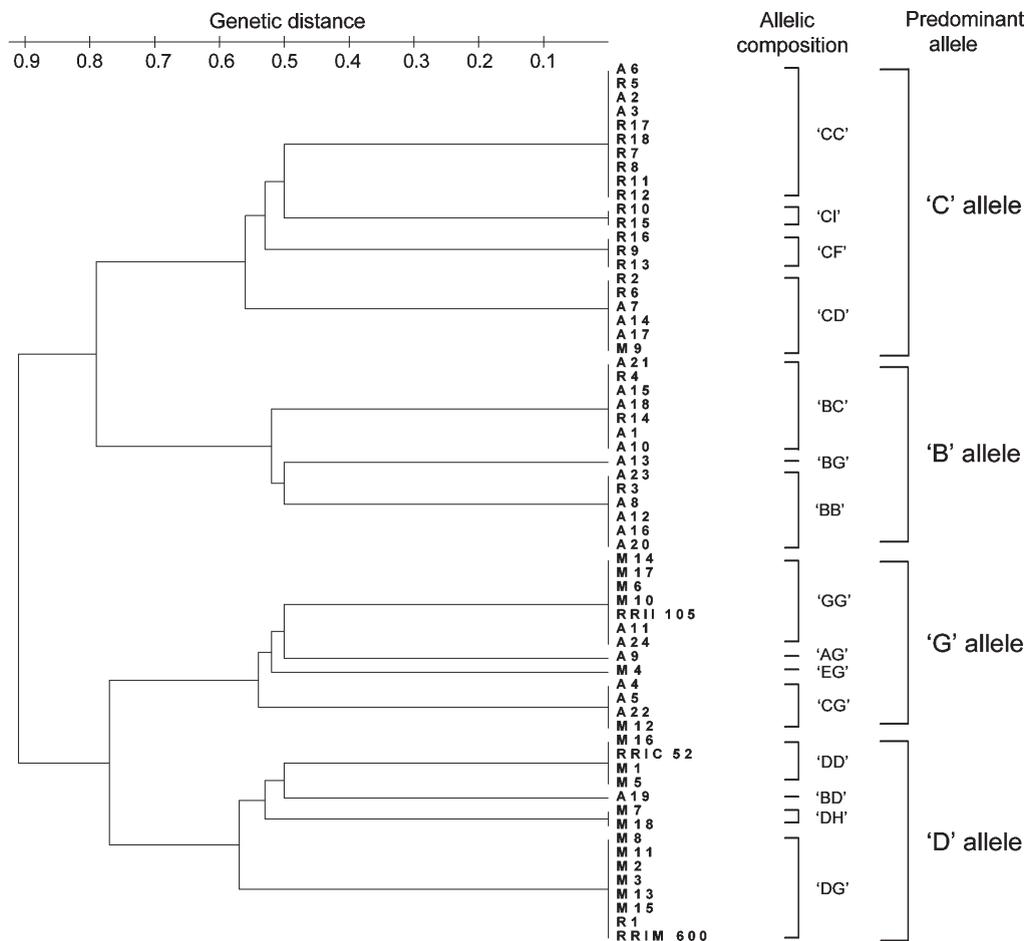


Figure 4. – Dendrogram generated from the UPGMA cluster analysis of the allelic data of *HMGR* showing interrelationships among 60 wild accessions and three popular clones of *Hevea*. All these *Hevea* accessions were clustered into 15 allelic groups. Predominant *HMGR* alleles belonging to each group are also indicated.

tion of Acre showed the highest inbreeding coefficient ($F_{IS} = 0.174$), while the lowest inbreeding coefficient was observed in Rondonian population ($F_{IS} = 0.011$) for the locus *HMGR*. Genetic differentiation among the populations originating from three provinces was assessed by their F_{ST} estimates. The F_{ST} value (0.177) indicated a relatively high degree of genetic differentiation among these three populations.

Genetic relationships of wild accessions

A dendrogram generated from the UPGMA cluster analysis of the allelic data of *HMGR* distinguished 60 wild accessions and three popular clones into two main clusters at an average distance coefficient of 0.85 (Figure 4). Wild *Hevea* accessions, mainly from Acre and Rondonia provinces formed one cluster and majority of the Mato Grosso accessions along with the three popular clones formed another cluster. The clusters of Acre and Rondonian accessions were grouped into two at an average distance coefficient of 0.6 based on the presence of alleles 'B' and 'C' in combination with other alleles. Similarly, there were two sub-clusters based on the prevalence of the alleles 'D' and 'G' under the main cluster comprising of the Mato Grosso accessions along with three popular clones. It was evident from the dendrogram that all the wild accessions including popular clones could be grouped into 15 genotypes based on allelic differentiation at the *HMGR* locus (Figure 4). Sequencing of the four alleles 'B', 'C', 'D' and 'G', prevail-

ing among wild *Hevea* accession, clearly showed repeat-length polymorphisms in both stretches of AG repeats flanking a conserved region of 12 nucleotides (GAAGGGAGGGAT) (Figure 5). Only the alleles, designated as 'D' and 'G' of the locus *HMGR* in wild *Hevea* accession were found to be present in cultivated clones.

Discussion

SSR markers derived from transcribed regions of the genome are useful in marker-assisted selection, comparative genetic analysis and for exploiting genetic resources by providing a more direct estimate of functional diversity based on allelic combinations. Although in general, gene-derived SSRs are having low polymorphism information content than other genomic SSRs, they are reliable means of assessing functional diversity relating to complex inherited traits (NERAJA et al., 2005). SSR markers developed from repeat regions, present in either the 5'-UTRs or 3'-UTRs, was most likely to detect polymorphisms compared to those existing in coding regions (LEWERS et al., 2005).

Identification of SSR markers in gene sequences or in ESTs from the database appears to be an alternative economical method compared to the conventional method of screening genomic libraries provided there are sufficient entries in the database. In *Hevea*, there are four gene sequences in the database i.e., HMG-CoA reductase (*HMGR*), Mn-SOD, thioredoxin h and β -1,3-

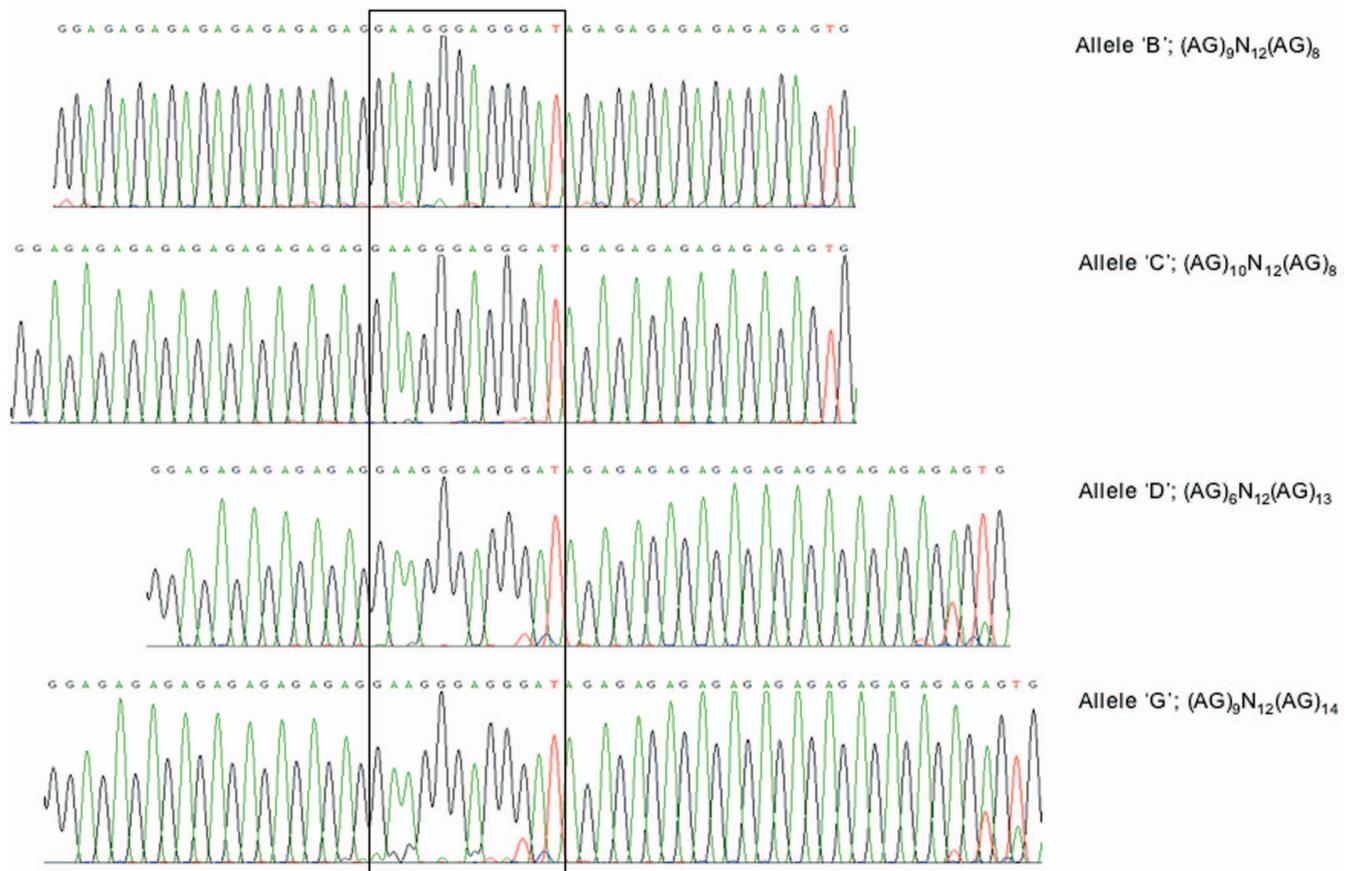


Figure 5. – Chromatograms showing four predominant microsatellite alleles ('B', 'C', 'D' and 'G') of the locus *HMGR* existing among wild *Hevea* germplasm. Allelic variation is due to the repeat length polymorphisms. Twelve nucleotides (GAAGGGAGGGAT) long conserved regions are shown within the box.

glucanase, detected with repeat sequences at the untranslated regions of mRNA or in introns in genomic sequences. Dinucleotide (CT)_n repeats, detected in the intron of Mn-SOD has been used as markers for genetic relationship studies (LESPINASSE et al., 2000; LEKAWIPAT et al., 2003). However, marker could not be developed from the thioredoxin h sequence having trinucleotide repeats (AAG)_n at its 5'-UTR due to insufficient flanking regions for primer designing.

Studies aimed at defining the extent of allelic variation of *HMGR* in wild *Hevea* accessions revealed the presence of nine alleles based on SSR polymorphisms at their 3'-UTRs. This is essential for understanding the functional variability of *HMGR* in *Hevea* as the presence of nucleotide variation at the 3'-UTR of mRNA is known to influence both the stability and rate of translation of the respective gene (CHAN and YU, 1998). In our earlier studies with 15 cultivated clones, it was confirmed through sequencing of the amplified partial *HMGR* fragments (500 bp including 3'-UTR) that the allelic variation was only due to repeat length polymorphisms at their 3'-UTR. In the present study, sequencing of nine alleles generated in wild accessions also revealed the same as reported in cultivated rubber clones (SAHA et al., 2005). Repeat length variation was noticed in both stretches of dinucleotide (AG)_n repeats. Cluster analysis of the allelic data of *HMGR* showed that all Mato Grosso accessions except one, formed a major cluster along with three cultivated clones used in this study, characterized by the predominance of 'D' and 'G' alleles. Genetic relatedness of Mato Grosso accessions with cultivated *Hevea* clones, revealed through this study, supported earlier views on phylogenetic relationships using molecular markers *viz.*, isozymes (CHEVALLIER, 1988), RFLPs (BESSE et al., 1994), RAPDs and chloroplast microsatellites (SAHA et al., unpublished).

Microsatellite allelic variation at the UTRs of a gene (as detected in *HMGR* sequence in *Hevea*) may be involved in the regulation of gene expression either at the transcriptional or at translational level. The waxy gene in rice, for instance, contained (CT)_n repeats in the 5'-UTR, where length polymorphism was found to be associated with amylose content (AYERS et al., 1997; BAO et al., 2002). SCHUPPERT et al. (2006) identified polymorphic (AT)_n and (GT)_n repeats in the 3'UTR of *FAD2-1*, a seed-specific oleoyl-phosphatidyl choline desaturase in developing seeds of sunflower. Consequently, SSR markers were developed for *FAD2-1* and used in marker-assisted selection of *Ol* mutant in mid- and high-oleic sunflower breeding programs. However, an association of microsatellite variation with functional diversity of *HMGR* in rubber has not been attempted so far. Hence, quantification of transcripts to correlate with allelic variation in cultivated clones has been our primary interest for understanding the mechanism regulating the gene encoding HMG-CoA reductase in *Hevea*.

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Interspecific Differences in Postharvest Quality on Mexican Christmas Trees

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Abstract

There are no comparative studies in postharvest quality of Mexican Christmas trees. The objective of this

study was to identify the best postharvest performing Mexican cultivated species. The experiment was done in the 2004-2005 season with six replications (trees) of *Abies religiosa*, *Cupressus lindleyi*, *Pinus ayacahuite*, and *Pseudotsuga menziesii*; from two provenances (Tlaxcala and Veracruz) for the last two species. Cultural management was similar. Each tree was placed under dry conditions according to a completely randomized design. Secondary branches, twig diameter and density, initial and final weight, biomass allocation, areas and volumes, total and twig moisture content, foliage density, color, chlorophyll a/b ratio, CO₂ and ethylene production were evaluated. Analyses of variance, comparisons of means, correlation, and simple regression were performed. The four studied species displayed undesirable characteristics. Genetic improvement is required.

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