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paula@cpaa.embrapa.br

Carvalho[1].:NDM; Soares[2]. SKB; Assunção[3]. EN; Angelo[4]. PCS; Sastolfi-Filho[5]. S; Atroch[6]. AL
[1]Embrapa Western Amazon PIBIC/CNPq; [2]Embrapa Western Amazon PAIC/FAPEAM fellowships; [3]DNA Technology Laboratory technologist and [5]Dr., Federal University of Amazonas, [4]Dr.; [6]M.Sc., Researchers, Embrapa Western Amazon, Rod. AM 010, km 29, Manaus-AM, CEP 69011-970.

Dinucleotide repeats in guarana fruits and SEEDS ESTs

The guarana plant (*P. cupana* var. *sorbilis*) is an Amazon rainforest Sapindaceae that is commercially cultivated only in Brazil. Annual production is about 4,000 tons, of which 70% are consumed by soft drink industries to produce non-alcoholic carbonated beverages. The remainder is sold, principally, as seed powder for laboratories, drugstores and alternative products stores. Embrapa Western Amazon is the only research institution that have been maintaining a continuous plant breeding program and a germplasm collection since the 70's. As part of this program, a cloning technology via stem cuttings and the BRS clones were developed. The germplasm bank contain 246 accessions and the genetic diversity analysis using RAPD was performed for 100. Microsatellite *loci* that shall contribute to enlarge upon this data and to, e.g., evaluate self and outcross rates are being prospected in genomic libraries. This search, nevertheless, has not yet produced satisfactory results. Genomic libraries enriched in AG/TC-AC/TG repeats yielded 6.7% Sau3A1 fragments containing mostly imperfect and smaller than 11 di/trinucleotide blocks. From those, 75% were complementar to the probes and 25% were TA blocks. Specific primers were used to define and test five of these *loci*, which demonstrated complex patterns of polymorphism considered to be associated to the imperfections in the blocks. The aim of this work was to evaluate dinucleoride repears in 5,969 guarana fruits and seeds transcriptional units (ESTs singlets and contigs) recorded in the REALGENE databank and compare to the genomic survey. Sequences containing repeats were identified by using $(AC/TG)_{80}$; $(AG/TC)_{80;}$ $(AT)_{80}$ and $(CG)_{80}$ as queries to run BioEdit local Blast. Those containing perfect repeat blocks were selected. Recorded frequencies for different repeats were analyzed by Kruskal-Wallis ANVA (SigmaStat v. 2.0). Sequences containing repeat blocks were organized according to the number of repeats -7 to 21- per block and distribution of sequences in the most frequented classes was analyzed by the χ^2 test (Genes-Universidade Federal de Viçosa). This analysis revealed that 1.91% transcriptional units presented dinucleotide repeat blocks. The most frequent ($P < 0.001$) repeat was TA (0.97%) followed by AG/TC (0.77%), AC/TG (0.15%) and GC (0.02%). Sequences containing 7-repeats blocks (0.92%) were the most frequent, followed by those with 8-repeats blocks (0.28%), 9 (0.18%), 12 (0.17%), 10 and 11 (0.12%). These frequencies are different from the expected ($P = 0.05$), except for 8-repeats blocks. One $(TA)_{40}$ block was found. By screening 13,783 UniGene representative human protein-coding sequences, around 6.49 repeat blocks per sequence were found, in average, being principally trinucleotide repeats. From those, about 1.58% was considered to carry detectable polymorphisms in the copy-number. A higher frequency of polymorphism can be expected, since we are dealing with guarana UTR regions too, and can expect to define at least two polymorphic *loci*. ■

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