

MOLECULAR MARKERS IN COFFEE (*Coffea arabica* L.)

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Coffee, being the beverage of tremendous economic importance, continuous efforts to develop better genotypes for sustainability is the need of the day. Genetic improvement of coffee is constrained predominantly due to narrow genetic base, limited molecular information, autogamous nature and long generation period. In the view of the above, our research is focussed particularly, to identify and exploit the largest possible number of polymorphic loci in cultivated species using molecular markers and to develop an inventory of the expressed genes through microarray based functional analysis of Expressed Sequence Tags (see poster Pallavicini et al.).

The development of microsatellites started with screening of two libraries; one enriched for TG repeats, derived from an *arabica* variety Caturra, the other enriched in dinucleotide and trinucleotide repeats, derived from an arabica variety Bourbon Tekisic. Until now, we have successfully developed a total of 159 *C. arabica* specific microsatellite markers and validated across different varieties. These markers were screened for parental polymorphism in 5 different crosses and a total of 17 polymorphic loci were identified, despite of limited genetic variation among *arabica* varieties. The selected polymorphic markers are being analysed in the segregating population for constructing map.

In previous studies, we developed a library of about 2000 ESTs from *C.arabica*. After a first and unsuccessful attempt at analyzing a selected panel of ESTs by PCR-RFLP, which did reveal significant polymorphism among species but not within *C. arabica*, we are sequencing the corresponding genomic loci to screen for Single Nucleotide Polymorphisms (SNPs). 8 different varieties have been screened in 17 loci and 3 SNPs have been detected. One SNP in particular is peculiar to the Mundo Novo cultivar and has been selected for further investigation.

We are starting to investigate the role of retrotransposons in the genomic rearrangements that occurred during and after the speciation of *C. arabica*. We used a protocol adapted from Pearce *et al.* (2001) to clone and sequence PCR products encompassing part of the LTR from the *Ty1-copia* retrotransposon family. 44 sequences were obtained, of which 36 from retrotransposons. 20 had a clearly identifiable polypurine tract, marking the start of the 3' LTR. We are currently using primers based on these sequences to perform S-SAP analysis on several varieties of *C. arabica* and closely related species. Preliminary experiments detected a low, although present, level of polymorphism among more distantly related varieties.

A query based user-friendly web-interface was developed, conceptually an effort for graphical display of large amount of information pertaining to coffee genomics generated from our lab. The database would also serve as a web portal for sequence analysis using in-house/web resources.