

## Development and Characterization of Coffee Specific Microsatellite Markers for Use as Potential Genetic Markers

R. K. AGGARWAL<sup>1</sup>, A. BARUAH<sup>1</sup>, V. NAIK<sup>1</sup>, P. S. HENDRE<sup>1</sup>, A. ASHRAF<sup>1</sup>,  
P. RAJENDRAKUMAR<sup>1</sup>, R. RAJKUMAR<sup>1</sup>, V. ANNAPURNA<sup>1</sup>,  
R. PHANINDRANATH<sup>1</sup>, N. S. PRAKASH<sup>2</sup>, C. S. SRINIVASAN<sup>2</sup>, L. SINGH<sup>1</sup>

<sup>1</sup>Centre for Cellular and Molecular Biology, Uppal Road, Tarnaka, Hyderabad-500007, India

<sup>2</sup>Central Coffee Research Institute, Chikamagalur-577117, India

### SUMMARY

Availability of informative genetic markers is prerequisite for effective genetic-linkage studies. Recent advances in molecular biology have led to the development of a plethora of DNA variation based efficient marker systems that promise-impetus, dependability and directionality to the genetic improvement efforts. Among various types of DNA markers the short-sequence repeat (SSR) based microsatellite markers have proven to be the most desirable for genetic studies due to their codominant nature, stability, abundance, sensitivity, ease and speed of analysis, minimal sample requirements and suitability for automation. Despite their potential and desirability, very few such markers have been developed and described in the literature for coffee till date. Under the first Indian initiative on Coffee Genomics, we have been able to develop 150+ new coffee microsats that provide potential genetic markers for germplasm characterization and molecular linkage studies in coffee.

For development of microsatellite markers, small-insert, partial, genomic libraries (comprising ~ 75,000 plasmid clones) were constructed from total DNA of *Coffea arabica* var.HdeT (Hibrido-de-Timor) and *C. canephora*. These libraries spotted on high-density nylon filters were screened for SSR positive clones through Southern hybridization with different synthetic oligonucleotide repeat probes. Based on hybridization signals, a large number of putative repeat-positive clones were selected and sequenced. The sequence data revealed relatively low abundance of SSR motifs in coffee genome, with AG di-repeat being most frequent. The clone sequences having 18+ bp repeat-motifs were used for designing PCR primers, which were subsequently validated using panels of elite coffee genotypes for their suitability as genetic markers. In total > 170 informative markers could be developed that also showed broad cross-species transferability. It is hoped that this new set of markers would serve as an important resource for coffee genomics.