ASIC 2008

# 22nd International Conference on Coffee Science

## Campinas, SP - Brazil

September 14 - 19, 2008

# PROGRAMME
# ABSTRACTS

ASIC

Association for Science and Information on Coffee

Association pour la science et l'information sur le café

## PB633 USING A *COFFEA ARABICA* BACTERIAL ARTIFICIAL CHROMOSOME LIBRARY FOR GENE CLONING AND INTEGRATIVE MAPPING APROACHES

CAÇÃO, Sandra M.B.*, SILVA, Nathalia V.*, ALVES, Gabriel S.C.**, VINECKY, Felipe**, DINIZ, Leandro E.C.***, ANDRADE, Alan C.**, PEREIRA, Luiz Filipe P.****, VIEIRA, Luiz G.E.*

*Instituto Agronômico do Paraná, Londrina, PR, Brazil, **EMBRAPA Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil ***EMBRAPA Tabuleiros Costeiros, Aracaju, SE, Brazil. ****EMBRAPA Café, Brasília, DF, Brazil,

Genomic libraries with large insert-DNA are useful tools for studies of the plant genome, including the production of physical maps, integration studies of physical and genetic maps, genome structure analysis and gene isolation. As an initial step towards the integration of strategies for physical and genetic mapping, as well as to perform map based cloning and identification of genes of interest, a BAC library of *Coffea arabica* Timor Hybrid 832/2 was constructed. High molecular DNA fragments digested with *Hind*III were cloned into the pCC1BAC vector and transformed into *E.coli* DH10B. The clones were manually picked and transferred to 96 well plates. Automatic compression of the library to 384 well plates was performed with a GENETIX Q-Bot resulting in 55.778 clones distributed in 148 plates. Random sampling of 80 clones showed an average size of 118 KB in a range of 80 to 250 KB. The coverage is estimated to be 5 to 6x of the *C. arabica* haploid genome. BAC clones were gridded in high-density filters (18.432 clones /filter). Pooling of BAC clones was performed for each 384 well plate. After DNA extraction, the pools were grouped to form 15 super-pools of BAC-DNA, each one representing more than 3500 clones. Screening of BAC pools by PCR or hybridization of the membranes allowed the identification of BAC clones with genes involved in sugar metabolism, abiotic stress response, bean and fruit development. Integration of genetic and physical mapping has been initiated by selecting markers from linkage maps which will be used for identified and anchor BAC clones selected by both PCR and hybridization strategies.

**Financial Support:** Brazilian Consortium on Coffee Research and Development (CBP&Café); Embrapa - MP2.

## PB634 TRANSCRIPTION ANALYSIS OF ONE PECTIN METHYLESTERASE ISOFORM IN *COFFEA ARABICA* L.

LEITE, Tiago F.*, BUDZINSKI, Ilara G.F.*, CAÇÃO Sandra M.B.*, PEREIRA, Luiz F.P.**; VIEIRA, Luiz G.E.*

*Plant Biotechnology Laboratory, Agronomic Institute of Paraná (IAPAR), Londrina, PR, BR, **Embrapa – Café, Brasília, DF, BR.

The coffee quality has been directly related to the ripening stages of fruits during harvesting. Non uniform maturation of the coffee fruits, combined with inadequate harvest and post harvest practices, may negatively affect the final quality of the product. Pectin methylesterase (PME - EC 3.2.1.11) has an important role in fruit softening. This enzyme catalyzes the methyl esterification of esters from polygalacturonic acid, increasing the susceptibility of pectins for the activity of polygalacturonases during rippening. In order to study the changes occurring during the coffee fruit maturation in silico and in vivo studies on PME genes were initiated. Trought in silico analysis on Brazilian Coffee Genome Project database we identified 31 PME contigs, however only eight of them had ESTs from fruit libraries. One of those PME isoform, CaPME12 was selected for further characterization . CaPME12 (1946 nucleotides) has a multicopper oxidase domain and a pro-region that is cleaved when the protein is mature. The pro-region shares some homology with PME inhibitors and probably acts as an intracellular inhibitor of PME activity while the protein is not mature. To analyze CaPME12 transcription during coffee ripening, fruits were monthly collected, after flowering, from *Coffea arabica* cv IAPAR-59. Northern Blot analysis was performed from total RNA of pulp, perisperm and endosperm tissues and from different tissues of coffee plant. Specific spatial transcription of PME12 was found in pulp at 210 days after flowering (DAF). We also observed low levels of PME12 transcripts in endosperm at 210 DAF and high transcription in bud flowers. These results suggest that this isoform acts specifically during the later stages of fruit ripening and probably contributes to the coffee fruit softening.

**Acknowledgements:** Brazilian Consortium on Coffee Research and Development