

Characterization of the first *Coffea canephora* coffee-tree BAC library

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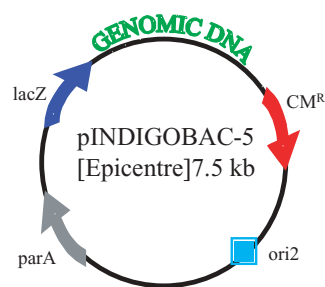
Coffee is an extremely important agricultural crop with more than 7 million tons of green beans produced every year, generating incomes to lots of farmers in tropical countries. *Coffea canephora*, growing in lowlands, is more resistant to pests and diseases than *Coffea arabica*. However, Robusta coffee, produced by the diploid species *Coffea canephora*, could be improved for its organoleptic and technological characteristics.

The construction of a BAC library for this species has been initiated to study associations between markers and characters. This BAC library will also allow to study synteny and allelic variations between Robusta and Arabica, and to achieve gene discovery and gene analysis in *Coffea canephora*, related to the improvement of Robusta coffee cup quality.

Material and methods

Plant material

- Species: *Coffea canephora*
- Genotype: 126
- Geographical origin: West Africa
- Ploidy: diploid
- Genome size: 8.10⁸ bp
- Chromosome number: n=11
- Scientific interest: biodiversity studies, agronomic traits, coffee bean quality.



Methods

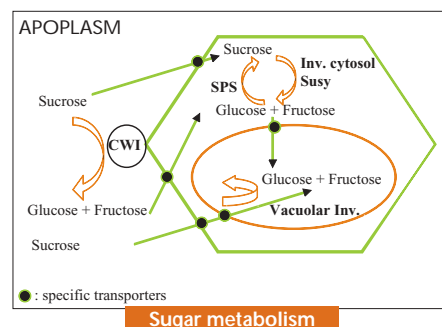
A BAC library of the heterozygous diploid genotype of *C. canephora* 126 was constructed starting from DNA extracted from young leaves (Zhang *et al.*, 1995).

The validation and characterization of the BAC library has been achieved with three types of probes:

- organelle (chloroplastic and mitochondrial) DNA, to estimate the rate of contamination;

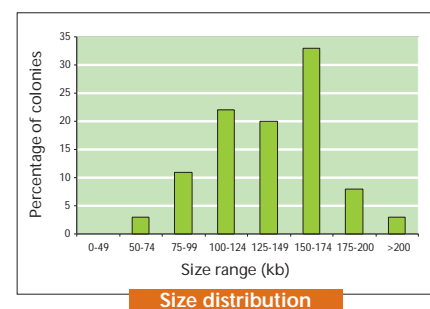
- RFLP probes (13 single-copies and 4 duplicated) located on different chromosomes (Lashermes *et al.*, 2001) to experimentally validate the genome coverage of the BAC library;

- cDNA probes of sugar metabolism enzymes expressed during bean maturation (Marraccini *et al.*, 2003).



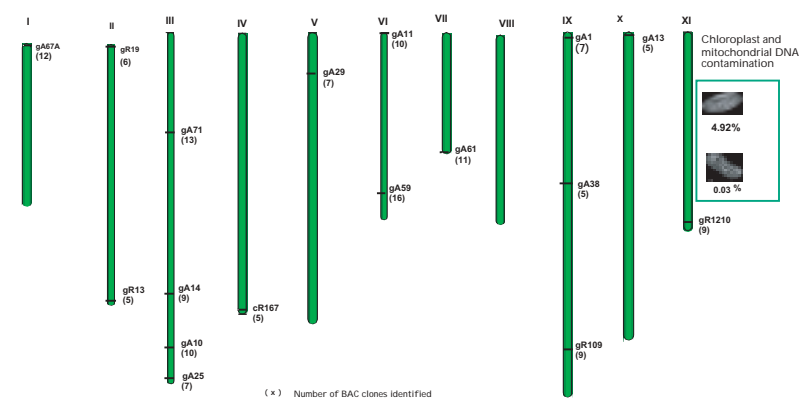
BAC library construction

- Number of clones: 55296
- Average size: 135 Kb
- Clones picked only from TOP band
- Genome equivalents: 9 x
- Construction date: 06/2002



Coffea canephora BAC library characterization

- Mitochondrial (0.03%) and chloroplastic (5%) contaminations were low.
- Confirmation of copy number of RFLP probes.
- Number of clones per single-copy probe: 8.5.
- Invertase probes were single-copy as confirmed by Southern blots (CWI and Vacuolar Invertase).
- Sucrose-synthase (SUSY) copy number (2 copies, 17 hits) to be confirmed by Southern analyzes.



Discussion

- This BAC library represent a potential tool for linkage disequilibrium studies in wild populations of *Coffea canephora*. Other major outcomes will concern positioning of QTLs for agronomic traits as well as characterization of genes of interest controlling sucrose accumulation during fruit maturation.
- This BAC library is a publicly available genomic resource.

References

Lashermes P., Combes M.C., Prakash N.S., Trouslot P., Lorieux M., Charrier A. (2001). Genetic linkage map of *Coffea canephora*: effect of segregation distortion and analysis of recombination rate in male and female meiosis. *Genome* 44 : 589-596.
 Marraccini P., Pereira L.P.P., Ferreira L.P., Vieira L.G.E., Cavalari A.A., Geromel C., Mazzafera P. (2003). Biochemical and molecular characterization of enzyme controlling sugar metabolism during coffee bean development. ISPMB conference, Barcelona (Spain), 23-28 June 2003, poster S19-14.
 Zhang H.-B., Zhao X., Paterson A.H., Wing R.A. (1995). Preparation of megabase-size DNA from plant nuclei. *Plant J.* 7 : 175-184.



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