

Nucleotide diversity and molecular evolution of 5 genes involved in the sucrose biosynthesis pathway of *Coffea canephora*

Bouchet Sophie¹, Marraccini Pierre², Jourdan Isabelle¹, Leroy Thierry¹, Vieira Luiz G.E.², Ferreira Lucia.P.², Musoli Pascal³, Pot David¹.

¹CIRAD-CP, UMR T52 PIA, TA 80/03, Avenue Agropolis, 34398 Montpellier Cedex 5, France.

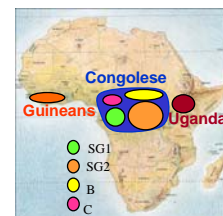
²Laboratório de Biotecnologia, Instituto Agrônomo do Paraná, Rod. Celso Garcia Cid, km 375, CP 481, 86001-970 Londrina - PR - Brasil. ³NARO-CORI, P.O. Box 185, Mukono, Uganda.



Coffee is one of the world's heavily traded commodities. However, little is known about the genomic control of cup quality and abiotic stresses tolerance in *Coffea sp.* Sucrose is the most important soluble sugar in plant. It is considered as the major precursor of coffee flavor and aroma and it is also involved in abiotic stress tolerance. Identification of the genes controlling its variation would be interesting for *Coffea* breeding. On an evolutionary point of view, according to its central role in fruit development/maturation and abiotic stress tolerance, the genes involved in its genetic control are likely to present signatures of natural selection at the molecular level.

❖ Aims of the study

- Evaluate the level of polymorphism of 5 genes involved in sucrose metabolism in *C. canephora*.
- Estimate the potential consequences of these polymorphisms on the proteins.
- Analyse the nucleotide diversity structure of these genes
- Test if signature of departure from selective neutrality is detectable at the molecular level within these genes.



❖ Material and methods

- 6 *Coffea canephora* populations representing the different genetic groups previously identified (Montagnon 2000)
- 5 genes (partial sequences) involved in sucrose metabolism
 - 2 Sucrose synthase (Susy1 and Susy2)
 - 1 Sucrose Phosphate Syntase (SPS)
 - 1 Cell Wall invertase (CWI)
 - 1 Vacuolar Invertase (INV)
- Sequence alignments were manually edited with Sequencher (<http://www.genecodes.com>), Dnasp (Rozas and Rozas, 1999) was used for all the analyses, i.e. nucleotide diversity estimates, neutrality tests and population structures.

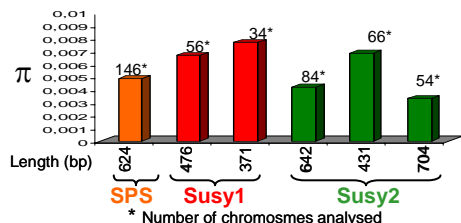
❖ Nucleotide diversity

▪ Summary

	# polymorphisms	Frequency
S	21	1 / 265 bp
NS	9	1 / 601 bp
non coding	76	1 / 65 bp
total	106	1/ 90 bp

- 9590 bp explored (8 to 162 chromosomes analysed)
- Low level of non synonymous polymorphism
- No protein modification

▪ Fine analysis of SPS, Susy1 and Susy2



- Different levels of variation within and between genes

❖ Population structure

- Good correspondence with the species' geographical distribution (Cubry 2005 $F_{st} = 0.4$)
- Susy1 promoter exhibits an extremely high level of genetic differentiation ($F_{st} = 0.83$) indicating a potential diversifying selection acting on this fragment

❖ Tests of selection

Gene	Tajima's D (1989)	# population in which the test is significant (P<0,05)
SPS	<0	1
Susy1	>0	0
Susy2	>0	0

- Signature of a selective sweep for SPS in one population

Sucrose metabolism genes are polymorphic in *Coffea canephora*. Although their nucleotide diversity structure globally fits a neutral model of evolution, signatures of natural selection were detected in Susy1 and SPS. Susy1 promoter seems to undergo diversifying selection whereas the coding region of SPS presents a signature of positive selection. These results indicate that these two fragments are good candidates for sucrose accumulation in *Coffea canephora*. These genes will be mapped in order to test their co-localisation with QTLs. Sequencing efforts will continue in order to estimate nucleotide diversity all along these genes in *Coffea* species. The relevant polymorphic sites will be used in association genetic approaches for cup quality and abiotic stress tolerance.

References

Cubry P. (2005). Analyse de la diversité et évaluation du déséquilibre de liaison chez quelques populations naturelles et cultivées de caféiers *Coffea canephora*. Master Recherche 2, Université Montpellier II.
 Montagnon C. (2000). Optimisation des gains génétiques dans le schéma de sélection réciproque de *Coffea canephora* Pierre. Thèse, ENSA Montpellier.
 Montagnon C, Guyot B, Cilas C, Leroy T. (1998). Genetic parameters of several biochemical compounds from *Coffea canephora* green coffee. Plant breeding. 117, 576-578.
 Rozas J. and Rozas R. (1999). DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15: 174-175.
 Tajima F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.



French
Agricultural
Research
Centre for
International
Development