MINUTES OF ICGN MEETING HELD AT THE PLANT AND ANIMAL GENOME MEETING (PAG), SAN DIEGO, CALIFORNIA JANUARY 15, 2008

Participants

Alan Andrade	EMBRAPA, Brazil
Marco Cristancho	CENICAFE, Colombia
Alexandre de Kochko	IRD, France
Perla Hamon	IRD, France
Ray Ming	University of Illinois, USA
David Pot	CIRAD, France
Nicolas Roux	Bioversity International, France
Alberto Pallavicini	University of Trieste, Italy
Lukas Mueller	Cornell University, USA
Philippe Lashermes	IRD, France

Marcela Yepes Cornell University, USA

Agenda ICGN meeting San Diego

1-Request feedback from participants to the first coffee genomics workshop organized during the PAG meeting.

2-Update the ICGN on progress of working group III of the ICGN towards an international initiative for the construction of a coffee physical map in collaboration with Rod Wing at the Arizona Genomics Institute, and explore the use of novel genome sequencing technologies to sequence the coffee genome.

3-Propose a meeting for the whole ICGN at the upcoming ASIC meeting in Brazil, September 14-19, 2008.

Summary meeting

First Coffee Genomics Workshop at PAG

At least 50 scientists, including 12 ICGN members, participated in the first coffee genomics workshop held at the Plant and Animal Genome Meeting in San Diego January 13, 2008. The co-organizers of the workshop Rod Wing, Philippe Lashermes, and Marcela Yepes appreciated the participation of the invited speakers and their contributions, as well as, the participation of representatives of several funding agencies in the US including the National Science Foundation (NSF), the US Department of Agriculture (USDA) and the InterAmerican Development Bank (IDB). Program and abstracts of presentations and posters are inserted in annex of this document. We received very positive feedback from workshop participants to continue the organization of this event during future PAG meetings (http://www.intl-pag.org/). The coffee genomics workshop is a good opportunity to present the ICGN and advances in coffee genomics research to the International Plant and Animal Genomics Community and can help us attract funding for an international project on coffee genomics.

Construction of a coffee physical map and genome sequencing

Rod Wing, Director of the Arizona Genomics Institute (AGI) at the University of Arizona, has agreed to work with working group III (http://www.coffeegenome.org/research/physical_mapping_wg3.htm) of the ICGN towards an international initiative for the construction of a coffee physical map and the

development of a cost-efficient strategy for sequencing the coffee genome.

Construction of two *Coffea canephora* BAC libraries (*Eco* RI and *Hind* III) has been contracted with Rod Wing by Alexandre de Kochko and Philippe Lashermes. The cost of the construction of the *C. canephora* BAC libraries will be jointly covered by the research teams of Alex (IRD) and Philippe (IRD-CIRAD). The BAC libraries will be constructed from a doubled haploid genotype available at IRD. The BAC libraries will be kept at AGI for three years and will be obtainable (clones and filters) to anyone from the ICGN community at a cost recovery base and through signing a simple MTA. It was agreed that a robust physical map of this genotype should be completed as a preliminary step towards sequencing the coffee genome using a hybrid approach: an optimized BAC pool sequencing strategy coupled with next generation sequencing technologies. On behalf of the ICGN working group III, Philippe Lashermes and Marcela Yepes will take the lead with Rod Wing to search for funding for this international initiative. Also they will draft the white paper that will support this strategy. The white paper will be circulated among ICGN members for input and to receive endorsement from the ICGN steering committee and members interested to help us obtain the funding for this international effort.

In addition, a pilot project based on shotgun sequencing using the FLX454 technology was also discussed and several ICGN members expressed their interest. We are looking at costs and strategy, and will share the information once available among ICGN members in order to develop a coordinated strategy.

ICGN meeting at the upcoming ASIC meeting in Brazil Sep 14-19, 2008

The group agreed to propose the organization of a general assembly of ICGN during the up coming ASIC meeting in Brazil (14-19 September 2008). Alan Andrade and Philippe Lashermes will contact the ICGN executive secretary and the ASIC organizers for this matter. In particular, during this meeting, the funding and communication capacities of the ICGN will have to be discussed to find ways to improve them. Nicolas Roux proposed to use the Bioversity office in Montpellier to arrange more regular telephone conferences between the Steering Committee members as well as with any other members of the ICGN if needed.

Program First Coffee Genomics Workshop, San Diego, California

(http://www.intl-pag.org/16/16-coffee.html)

Sunday Afternoon, 13 January, 2008 3:35 pm to 5:45 pm

Coffee Workshop - Pacific Salon 2

Co-Organizers:
Philippe Lashermes, L'Institut de recherche pour le développement (IRD), France
(philippe.lashermes@mpl.ird.fr)
and
Marcela Yepes, Cornell University
(my11@nysaes.cornell.edu)
and
Rod Wing, University of Arizona
(rwing@Ag.arizona.edu)

Speakers:

1- Alan ANDRADE, EMBRAPA (alan@cenargen.embrapa.br)
"Integrated Molecular Analysis of Drought Stress Responses in Coffee Plants"

2- David POT, CIRAD (dpot@cirad.fr)
"Genetic structure of *Coffea canephora* Pierre species assessed by microsatellite markers"

3- Philippe LASHERMES, IRD (Philippe.Lashermes@mpl.ird.fr)
"Exploitation of synteny for positional gene cloning in Coffee"

4- Isabelle PRIVAT, NestlÃ© (Isabelle.Privat@rdto.nestle.com)
"The Coffee Microarray Project : A new tool to discover candidate genes correlated to quality traits"

5- Gabriel CADENA et al., CENICAFE (Gabriel.Cadena@cafedecolombia.com)
" Study of the genomes of Coffee (*Coffea arabica*), its major Insect pest the Coffee Berry Borer (*Hypothenemus hampei*), and its biological control agent (*Beauveria bassiana*) "

6- Rod WING, Arizona University (rwing@Ag.arizona.edu)

"Towards an international initiative for the construction of a physical map of coffee"

ABSTRACTS COFFEE GENOMICS WORKSHOP PAG MEETING SAN DIEGO, AND ABSTRACTS PRESENTED AS POSTERS

Integrated Analysis Of Drought Stress Responses In Coffee Plants

Pierre Marraccini[1] , Humberto Ramos[2] , Luiz G. E. Vieira[2] , Maria Amélia G. Ferrão[3] , Felipe R. da Silva[4] , Carlos Bloch Jr.[4] , Alan C. Andrade[4]

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Coffee is an important commodity worldwide with about 125 million people depending on this crop for their livelihoods. Drought stress significantly affects coffee yield with losses estimates in Brazil, varying from 10 to 15 % in recent years. Therefore, the development of molecular tools for rapid generation of drought-tolerant coffee varieties is amongst the priorities of the Brazilian Research Program on Coffee Genomics. The principal aim of this study was to investigate the molecular mechanisms underlying the response to drought stress in coffee plants by an integrated approach. As part of the Brazilian Coffee EST project, two EST libraries from leaves of drought-stressed plants of *Coffea arabica* cv. Rubi (drought sensitive) and *C. canephora* clone 14 (drought tolerant), were generated. The plant material of *C. arabica* and *C. canephora* were obtained from field and pot trials, respectively. More than 15,000 clones were sequenced and, after trimming and clustering, resulted in 10,924 reads grouped on 6,141 unigenes (1,779 contigs and 4,362 singlets). The approaches used to identify candidate genes (ESTs) underlying stress responses in coffee consisted of an in silico analysis of ESTs generated from drought-stressed and control libraries, physiological characterization, transcription profiling of drought-stressed and control tissues and protein profiling by 2-DE coupled with tryptic peptide identification by MALDI-TOF-MS/MS. These integrated analysis resulted in the identification of several candidate drought-responsive genes. In addition, a mapping population from crosses of *C. canephora* clones contrasting for this trait is also under way for future association studies and QTL mapping.

Genetic Structure Of Coffea canephora Pierre Species Assessed By Microsatellites Markers

Philippe Cubry[1] , David Pot[1,2] , Fabien De Bellis[1] , Hyacinte Legnathe[3] , Pascal Musoli[4] , Thierry Leroy[1]

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4 Coffee Research Institute, P.O. Box 185, Mukono, Uganda

Coffee is one of the most important cash crops for numbers of countries in the intertropical zones all over

the world. *Coffea canephora* is responsible for about 35% of the total world production of coffee. Natural area of this species corresponds to the actual zone of tropical rainforest in Africa.

In order to better assess genetic resources and natural history of this species, we analysed a sample of 285 individuals from different sampling points on the repartition area, including some cultivated genotypes. A set of 39 nuclear microsatellites markers was genotyped in order to investigate species genetic structure and population history. An integrate approach combining both distances (factorial analysis) and bayesian model (Structure) methods was used to study the species structure. We shown that 2 major groups can be clearly discriminated, those two groups correspond to previous work led by Berthaud. However a finest structure has been shown, dividing the previous groups in a total of 6 groups, whereas previous studies have shown 5). We tried to investigate those groups history by computing the dm^2 statistic of Goldstein in our sample. Results show the possible effect of glaciation refuge areas on the elaboration of the *Coffea canephora* genetic structure, separating a guinean region composed by 2 groups and a Congolese one composed by four groups. Consequences on species actual diversity and breeding are discussed.

Exploitation Of Synteny For Positional Gene Cloning In Coffee

Philippe Lashermes , Marie-Christine Combes , Laetitia Mahé , Alessandra Ribas , Eveline Dechamp , Hervé Etienne

UMR RPB (CIRAD, IRD, UM2), Centre IRD de Montpellier, BP 64501, F-34394, Montpellier, France

Coffee leaf rust caused by the obligate parasitic fungus *Hemileia vastatrix* is an economically important disease and a major limiting factor for arabica coffee (*Coffea arabica*) production. While the rust resistance genes identified in C. arabica, a recent allotetraploid species, have not provided durable resistance, resistance genes from diploid related coffee species such as *C. liberica* (i.e. SH3 gene) and *C. canephora* have provided long-lived protection under field conditions. Positional cloning of the SH3 gene has been therefore undertaken in order to enhance opportunities for genomics-enabled breeding and to gain molecular insight into rust durable resistance. Hence, we explored the possibility to utilize the exponentially increasing sequence information from model plants such as Arabidopsis and Tomato. By combining a search of Arabidopsis sequences homologous to coffee BAC-end sequences belonging to the related SH3 BAC contig and use of orthologous sequence markers, we demonstrated microsynteny between coffee and Arabidopsis duplicated counterparts. The complex duplication history of Arabidopsis did not prevent the use of Arabidopsis as a genetic and genomic model for coffee species. Furthermore, an extended colinearity between the coffee and tomato genomes was revealed for the chromosome arm carrying the SH3 locus using comparative genetic molecular mapping. In particular, plant disease resistance (R gene) loci appeared to be positionally well conserved and several candidate genes have been identified. These finding highlight the possibility to develop detailed comparative genome study and to share genomic and genetic information among these two related crop plants.

The Coffee Microarray Project: A New Tool To Discover Candidate Genes Correlated To Quality Traits

Isabelle Privat1 , Benoit Bertrand2 , Philippe Lashermes3
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Coffee is a product of mass consumption, with worldwide consumption estimated at 2.2 billion cups per day. The annual turnover generated is approximately 25 billion Euros and so coffee is the third biggest source of international trade, after oil and cereals. Coffee trees have not, however, resulted in any significant seedling industry involvement, which is in sharp contrast with their economic importance. However, biotechnology tools used for improving other species with economic impact are gradually adapted to coffee trees and used for guiding and improving coffee trees performances. Over the past few years, Coffee research programs also include Functional Genomics studies for the discovery of the genes and biosynthesis pathways involved in characteristics of agricultural, industrial or qualitative interest. The Coffee Microarray Project is based on scientific collaboration between NESLTE and CIRAD/IRD granted by ANR (National Research Agency) via GENOPLANTE.
PUCE CAFE project has two main objectives 1) Create the first Coffee 16 K oligo microarray and 2) validate and use this new tool to analyze gene expression patterns during coffee grain maturation in *Coffea arabica* and *Coffea canephora* (robusta).

Study Of The Genomes Of Coffee (Coffea arabica), Its Major Insect Pest The Coffee Berry Borer (Hypothenemus hampei), And Its Biological Control Agent (Beauveria bassiana)

Gabriel Cadena1 , Pablo Benavides1 , Marco Cristancho1 , Pilar Moncada1 , Carmenza Gongora1 , Ricardo Acuna1 , Alvaro Gaitán1 , Huver Posada 1 , Diana Villareal 1 , Diana Molina1 , Jerson Dominguez1 , Juan-Carlos Herrera1 , Herb Aldwinckle2 , Marcela Yepes2

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The Colombian National Coffee Research Center (CENICAFE) was funded by the Colombian Coffee Growers Federation in 1938 with the mission to generate scientific knowledge and technologies for sustainable coffee production in Colombia while preserving the environment. The Coffee Genome Project was launched by CENICAFE in 2003 co-funded by the Colombian Ministry of Agriculture. It has as fundamental goals to identify the location of genes of interest (cup quality, resistance to diseases and pests, yield, etc.) on the chromosomes, their sequences (structural genomics) and function (functional genomics). This initiative has been developed in collaboration with Cornell University, University of Maryland, the Institut de Recherche pour le Développement (IRD), The Institute for Genomic Research (TIGR), and the University of Arizona. A major focus of this project has been the development of genomic tools for coffee including ESTs from cDNA libraries to describe the coffee transcriptome, a large-insert genomic library (BAC), and expression profiling to identify genes of interest. Molecular markers are being developed for the construction of high-density genetic maps, and to enhance the use of germplasm for the development of

improved varieties. Our project also targets the genome of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae), the most devastating insect pest of coffee worldwide. Genomic and population studies are being conducted to help elucidate the biological mechanisms involved on the insect-coffee interaction. Genomic studies on *Beauveria bassiana*, the main biological control agent against this pest, are being conducted to identify which genes are involved on pathogenicity.

POSTER ABSTRACTS

Differential Expression Of A Prolyl Oligopeptidase Gene In 'Tall Mokka' And 'Kona-Typica' Coffee

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In Hawaii, coffee (*Coffea arabica L.*) cultivars Tall Mokka (MA2) and Kona-Typica (KO34) produce high quality beans with distinct flavors. MA2 differs from KO34 in having smaller organs, including leaves, fruits, and seeds, resulting in lower yield. Comparison of leaf epidermal cell size and number between the two cultivars indicated that cell size is probably the major organ size determinant. Our goal is to identify genes related to organ size control in these two cultivars. Forty-five genes with ≥ two-fold difference in expression were identified using potato cDNA microarrays from TIGR as a cross-species platform. Homologous coffee sequences were identified for 28 of these genes in the publicly available databases. Differential expression was confirmed for only one of the 28 genes using qRT-PCR with an approximately four-fold higher expression in MA2. This gene is homologous to prolyl oligopeptidase (POP) of *Arabidopsis*. POP expression is also higher in ´Laurina´ and ´Mokka´, two other *arabica* coffee varieties with small organs, as compared with KO34. In both MA2 and KO34, the expression of POP in leaf tissue increases as the leaves mature but decreases during the first 8 weeks of fruit development. The difference in POP expression between MA2 and KO34 is greater at the very early stages of development for both leaves and fruit. We are in the process of cloning and sequencing POP gene and cDNA from coffee. This study will increase our understanding of the role of POP in plants. The gene(s) controlling organ size may be used in coffee breeding to improve bean size and yield.

Genomic Strategies To Detect Genes Involved In Resistance To The Coffee Berry Borer Hypothenemus hampei

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The coffee berry borer, Hypothenemus hampei Ferrari (Coleoptera: Curculionidae: Scolytinae), is the most devastating insect pest of coffee worldwide. To identify sources of resistance, we have conducted field evaluation of genotypes exhibiting antibiosis, QTL analysis to detect genes involved, analysis of genetic expression of coffee to insect attack, construction of cDNA libraries to identify candidate genes for resistance, and studied heterologous proteinaceous compounds against the pest (proteases, amylases, xylanases, chitinases).
Oviposition reduction (27-35%) of H. hampei was observed on Coffea arabica accessions (CCC363, 359, 534) and C. liberica compared to the susceptible C. arabica Caturra variety. These genotypes have been used to develop F1 and F2 populations for QTL analysis to identify the type of inheritance, the number of genes and the regions of the genome involved on that reduction. The analysis of the F1 plants showed that the lower oviposition is conserved in H. hampei progeny.
In addition, cDNA libraries of coffee beans (28 weeks old) of CCC 534 and C. liberica have been constructed and sequenced. EST analysis indicated the presence of chitinases, proteinases, gammathionins, phytocystatin and cisteins known to be associated to plant defense response against insects. Differentially expressed libraries were developed for Caturra and C. liberica, and CCC363, 359 and 534 after 72 hours of H. hampei infestation. The sequences from C. liberica were grouped on 55 unigenes: 31 were present on C. arabica libraries and 24 were unique to C. liberica. Further analysis with real time PCR and microarrays will allow verification of their function.

An Integrated Web-Based Bioinformatics System For Genome Sequences, Gene Expression Data, And Molecular Genetic Markers For Coffee

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We have implemented a web-based Bioinformatics system that functions as a genomics information resource for coffee and other organisms studied at the Colombian National Coffee Research Center (CENICAFE), in collaboration with TIGR and Cornell University.
The new Bioinformatics platform includes a Laboratory Integrated Management System (LIMS), the implementation of wEMBOSS, home-developed perl tools for data analysis, and InterProScan for annotation of sequence domains. The main backbone of the system has been developed for analysis of ESTs, molecular markers, and BAC end-sequences. The system is based on the postgresQL relational database using perl scripts for the manipulation of data, the Apache Web server with the mod_perl integrated perl interpreter, and the servers run the Debian distribution of

the GNU/Linux operating system.

The CENICAFE coffee database includes 81,378 ESTs representing the coffee transcriptome with 30,646 unigenes. The majority of the coffee ESTs were derived from cDNA tissue specific and normalized libraries of Coffea arabica, and some from the diploid species C. liberica and C. kapakata. In addition, CENICAFE has generated 4,186 Beauveria bassiana ESTs representing 2,401 unigenes, and 4,870 Hypothenemus hampei (coffee berry borer) ESTs representing 1,766 unigenes. The coffee database also includes over 80,000 BAC–end sequences of C. arabica.

The sequences are annotated based on Solanaceae, Arabidopsis, Swissprot and Genbank sequence comparisons using BLAST homology searches, aminoacids are predicted using ESTScan and the domains are annotated using InterProScan. We have incorporated a GBrowse graphics visualization tool for the display of large sequences such as whole BAC sequences and chloroplast DNA