

### Projects funded in 2008 (Summaries)

No	Title
0800-001 Completed	Comparison of three Arecaceae genomes, coevolution of sorgho and striga and genetic association studies in sorgho
0801-005	An international platform for the elucidation of gene function using rice as a model species : The RicE FUunctional GENomics (REFUGE) Platform
0801-006	Structuring scientific platform "Characterization of raw and biotransformed Plant Lipids and hydrophobic Polymers for a Green and sustainable chemistry" (PlantLipPol-Green)
0802-005 Completed	Response of natural Arabidopsis accessions to salt stress - A French-Chinese initiative
0802-006 Completed	Preparing an international networking project on rice blast diversity
0802-010	Collect of wild cocoa genetic resources related to aromatic chocolate qualities in Ecuadorian Amazonia.
0802-012 Completed	Analysis of <i>Xanthomonas albilineans</i> Gene Expression During Sugarcane Leaf Scald Pathogenesis
0802-019 Completed	AGRO2010 MONTPELLIER- The Scientific Week around Agronomy
0802-020 Completed	Integrative Proteomics Resources for Molecular Phenotyping of Plant Growth Responses to Environment and Climate Changes.
0802-021	Impact of changing environmental and anthropogenic constraints on the diversity of asexually propagated crops : towards an international research proposal on yam agrobiodiversity
0802-022 Completed	Adaptative responses of the N <sub>2</sub> -fixing symbiotic interaction between <i>Medicago truncatula</i> and rhizobia to whole plant N limitation
0802-023 Completed	Development of a system for high throughput functional analysis of <i>Magnaporthe grisea</i> virulence effector proteins
0802-027 Completed	Organisation of a workshop entitled "Reference sets of food crop germplasm for international collaboration"
0802-029	<i>Utilisation of new technologies for the international visibility of grape collections from INRA and ENTAV<sup>1</sup></i>
0802-030	Challenging theories and frameworks on socio-ecological systems with empirical research results
0803-007	Towards a federative research on modelling and simulation platforms
0803-008	Agropolis computational plant seminar
0803-012	Modelling and optimization of bioconversion of plant materials in inhomogeneous media
0803-013 Completed	Developing a statistical framework to study genotype x environment interactions in association studies

<sup>1</sup> Projects 0802-029 and 0803-019 have been merged into the PI@ntNet Flagship Project

0803-017	OpenAlea: An open software platform for plant modelling at different scales
0803-019	<i>Plant Computational Identification &amp; Collaborative Information System - PI@ntNet<sup>1</sup></i>
0803-021 Completed	Model level integration for understanding and managing human influenced ecosystems at the landscape scale
0803-022	Computational model for electrical signaling in plants in relation to polarized patterns of development and morphogenesis, and to adaptive responses to stresses
0803-024 Completed	Development of a toolbox for the modelling and simulation of retroactions between plant growth and plant biomechanics.
0803-027	3D virtual fruit - application to the control of fruit growth and quality

**Keywords:**

IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*)

STDI-2 (Socio-Technical Dynamics of Innovation 2: *Agri-food innovations, food and non-food use of plant crops*)

STDI-3 (Socio-Technical Dynamics of Innovation 3: *Innovation processes, social management of innovations*)

**Year of CP:** 2008

**Project No 0800-001 Completed**

**Project title:** Comparison of three Arecaceae genomes, coevolution of sorgho and striga and genetic association studies in sorgho

**Units submitting the project:** AGAP (Genetic improvement and Plant adaptation) (CIRAD, INRA, Montpellier SupAgro)

**Project leader:** Angélique D'Hont (angelique.dhont(a)cirad.fr)

**Countries involved in the project:** USA

**Research units from the Foundation's scientific network involved:** DIADE, LGDP

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*).

**Objectives, action carried-out and results obtained:**

**1 - Comparison of three *Arecaceae* genomes: oil palm (*Elaeis guineensis*), date palm (*Phoenix dactylifera*) and coconut tree (*Cocos nucifera*)**

The objective of the project is to compare five genomic regions in three *Arecaceae* species: oil palm (*Elaeis guineensis*), date palm (*Phoenix dactylifera*) and coconut tree (*Cocos nucifera*) and with rice. For the project, we used two BAC libraries own by Cirad: one for oil palm and one for coconut tree. The date-palm fosmid library was own by J. Bennetzen laboratory. The sequencing of the clones was obtained through a Genoscope project.

To select the BAC clones, we hybridized the BAC high-density filters with probes corresponding to a few genes:

- The ADH gene (alcohol dehydrogenase); this loci has already been largely studied in comparative studies. One probe has been derived from the sequence of the ADH gene in sugarcane and specific primers for ADH 1 and ADH 2 were defined from the alignment of several ADH genes.
- The genes EgDEF1 (MADS-box sub-family deficiens) et EgGLO2 (MADS-box sub-family Globosa) were selected since they are involved in the somaclonal variation "mantled", which induce a conversion of male floral component in female one with an important impact on production of palm oil. Specific probes were derived from the EgDEF1 and EgGLO2 cDNAs.
- The other regions were selected from the partial assembly of the date-palm sequence (<http://qatar-weill.cornell.edu/research/datepalmGenome/download.htm>). The scaffolds were annotated automatically with Fgenesh and Eugène. This annotation allowed identifying gene-rich scaffolds. Two gene rich scaffolds were chosen corresponding to the loci PEPC and FBP (phosphoenolpyruvate carboxylase and Fructose-bi-phosphate). Probes were developed from the genes on these scaffolds and PCR primers were designed for BAC validation.

ADH probes allowed identifying 18 oil palm BACs and 13 coconut tree BACs. BAC fingerprint and PCR analysis allowed distinguishing the BAC corresponding to ADH1 and ADH2 loci. Two oil palm BACs Eg033E10 (ADH1) and Eg060I13 (ADH2) and one coconut BAC comprising the ADH1 and ADH2 genes were selected for sequencing.

Probes corresponding to EgDEF1 and EgGLO2 allowed identifying 4 and 2 BAC respectively but did not allow identifying coconut tree BACs. Since these two genes correspond to multigenic family, we had to design very specific probes based on date palm. This may explained the absence of

hybridization on coconut trees. The oil palm BAC Eg133H20 (EgDEF1) and Eg033E06 (EgGLO2) were selected for sequencing.

Probe corresponding to the loci PEPC identified 4 oil palm and 5 coconuts BAC clones. PCR analysis and fingerprint allowed selecting BAC Eg132P05 (oil palm) and BAC Cn227M10 (coconut) for sequencing.

Probe corresponding to the loci FBP identified 5 oil palm and 3 coconuts BAC clones. PCR analysis and fingerprint allowed selecting BAC Eg172L05 (oil palm) and BAC Cn142B10 (coconut) for sequencing.

Selection of date-palm fosmid was performed in J. Bennetzen laboratory. Eight fosmids were selected Pd007D03\_D6 and Pd009A12 for the loci ADH; Pd005B81\_4D2 and Pd005B81\_5H2 for the loci EgGLO2; Pd009B12\_D2 and Pd009H8\_H8 for the loci FBP; Pd003C11\_1C9 and Pd006A61\_G9 for the loci PEPC.

We encountered an important problem of delay in the sequencing phase that took much more time than anticipated and lead to important delay in the progress of the project.

In addition, some of the fosmids sent by J. Bennetzen lab for sequencing to Genoscope appeared to be redundant: 4 fosmids (Pd007D03\_D6, Pd005B81\_4D2, Pd005B81\_5H2 and Pd003C11\_1C9) had the same sequence.

Finally, in total 6 oil-palm BAC, 3 coconut-tree BAC and 8 date-palm fosmids were sequenced

## **2. Co-evolution of sorghum and striga**

J. Bennetzen was interested to study sorghum/pearl millet/striga co evolution. Striga is a parasite plant very prejudicial in Africa. We provide him contact for his mission in Mali to collect samples.

## **3. Association analyses in sorghum and pearl millet**

K. Devos was interested to analyse gene involved in height variation through association study in sorghum and/or pearl millet.

Association analyses are done most effectively when a panel of varieties is shared between different research groups. In discussion with IRD, she adopted their association panel for pearl millet, and expanded it with varieties from the United States. her trait of interest is plant height. Three genes had been identified in her lab as underlying height variation in pearl millet. In first instance, we needed to isolate the pearl millet orthologs of those genes. Using grass comparative information, primers were designed against conserved regions in the candidate genes. Pearl millet amplification products were cloned and sequenced. Unfortunately, most of the cloned products appeared to be retro-elements and were thus the result from non-specific amplification.

### **4. Characterization of translocation break points in wheat**

K. Devos has been working on genomics in *Triticeae* for many years and more recently focused on the analysis of translocations. She wanted to use our expertise in molecular cytogenetics to be trained in new methods in molecular cytogenetic to continue this work in Montpellier.

The objective of the study was to determine the physical size of the region that spanned the 4L/5L translocation breakpoint in wheat. Using comparative information from rice, four markers (two for the group four and two for group five) had been identified that spanned the breakpoint. A partial contig for the breakpoint region had indicated that the region likely spanned a few hundred kilobases and consisted mainly of repetitive DNA. To determine the physical size of the region, in situ hybridization needed to be carried out with the markers flanking the breakpoint on both chromosomes 4 and 5. Visualization of in situ hybridization signals requires probe lengths of minimum 2 to 3 kb. The available probes were less than 500 bp long. To generate additional probes for the four loci, primers were constructed using the rice genes as template. Alignment of the rice genes with wheat ESTs allowed the design of primers that would amplify the orthologous sequences in wheat. The amplified fragments were cloned and sequenced to confirm their identity. This brought the total available probe length to 2 - 3.8 kb for each of the four loci. In order to increase the resolution with which two probes could be differentiated, in situ hybridization was carried out on flow sorted chromosomes, provided by J. Dolezel (IEB, Czech Republic) and we

tested the protocol of J. Dolezel to stretch the chromosome before hybridization. A series of labeling and hybridization procedures were tested to enhance the sensitivity with which hybridization signals could be detected. But the conclusion was that the probe length needs to be further increased to be able to obtain successful hybridization and detection at both two loci that flank a breakpoint.

**Prospects for the future:**

Comparison of three *Arecacea* genomes: we are currently annotating all the sequences then the comparison will be performed between the tree *Arecacea* species and with rice.

A publication on these results will be written jointly by the two research teams

**Total Agropolis Fondation funding:** € 93 706 (Bench fees, Sequencing, 2 CDD)

**Funding categorie(s):** Hosting of a senior scientist for a short period (less than 12 months)

**Project duration:** 01 may 2008 - 31 July 2010

**Keywords:** Association studies, *Arecaceae* genomes, coevolution, sorgho, striga

**Year of CfP:** 2008

**Project No** 0801-005

**Project title:** An international platform for the elucidation of gene function using rice as a model species: the Rice Functional Genomics (REFUGE) Platform

**Units submitting the project:** AGAP (Genetic improvement and Plant adaptation) (CIRAD, INRA, Montpellier SupAgro) and LGDP, Genome and plant development (CNRS, UPVD)

**Project leader:** Emmanuel Guiderdoni (guiderdoni(a) cirad.fr)

**Countries involved in the project:** Italy, Vietnam, Brazil, Spain, UK, Germany, Australia, Tunisia

**Research units from the Foundation's scientific network involved:** BPMP

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*).

**Objectives:**

Rice is both a crop of high agricultural, social and cultural importance and a model cereal species for studying genome organization and discovering gene function. Five years after the availability of its high quality genome sequence and following the development of numerous international biological, molecular and bioinformatic resources, and despite the ongoing delivery of sorghum, *Brachypodium* and maize genome sequences, rice remains a model system.

Numerous laboratories from the South have invested in molecular biology techniques and are performing excellent research, sometimes taking advantage of unique genetic resources but do need an access to facilities, equipment and consumables that will allow them to complete their experiments to prepare high quality publications, further facilitating their access to national and international research grants.

The aim of this project is the establishment of a Rice Functional Genomics platform (REFUGE) widely open to the scientific community. This platform will offer training and support service for the generation and characterization of transgenic plants, as well as the characterization of existing biological resources (e.g. insertion lines) to facilitate and enhance the functional analysis of genes identified either in rice, other cereals or *Arabidopsis*.

More precisely, the specific objectives of the REFUGE project are:

- to make available to visitors of the local, national and international plant science communities various tools to carry on in rice the functional analysis of genes of agronomic importance - identified either in rice, cereals or *Arabidopsis*- in taking advantage of the biological resources, bioinformatics and molecular tools existing in the model cereal species and available in our unit. To share performing infrastructures, know-how and expertise unique in the public sector in Europe for the production and characterization of transgenic rice and insertion lines to support investigation on gene function.
- To improve local capacity to organize an international training in functional genomics targeting African scientists.

**Total Agropolis Fondation funding:** € 328,640 (salary of an engineer for 36 months, acquisition of small equipment, running costs)

**Funding categorie(s):** Agropolis Fondation grants for scientific platform

**Project duration:** 1 January 2009 – 31 March 2012

**Keywords:** rice – genomics – molecular biology – platform

**Year of CfP:** 2008

**Project No** 0801-006

**Project title:** Structuring scientific platform “Characterization of raw and biotransformed **Plant Lipids** and hydrophobic **Polymers** for a **Green** and sustainable chemistry” (PlantLipPol-Green)

**Unit submitting the project:** IATE, Agropolymer Engineering and Emerging Technologies (CIRAD, INRA, Montpellier SupAgro, UMII)

**Project leader:** Eric Dubreucq (Eric.Dubreucq(a)supagro.inra.fr)

**Countries involved in the project:** Brazil, India, Portugal, Thailand, Uruguay, USA

**Research units from the Foundation’s scientific network involved:** SPO, BPMP, DIADE

**Sub-thematic axes:** IBP1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), STD12 (Socio-Technical Dynamics of Innovation 2: *Agrifood innovations, food and non-food use of plant crops*)

**Objectives:**

Green and sustainable production of chemicals and materials relies on the development of eco-efficient technologies for the transformation of agro-resources into biofuels, biomaterials and other biomolecules.

In order to meet technological, industrial and economic requirements of sustainable biorefinery-based transformation, a reliable scientific knowledge of the composition and the properties of the plant materials and of the products obtained is necessary. This knowledge is the main basis for the definition of criteria for the (i) selection and the improvement of plants used as bioresources (ii) selection of suitable microorganisms and enzymes for biotransformations (iii) conception and optimization of suitable transformation processes.

The project concerns more specifically lipids and hydrophobic biopolymers (polyisoprene, polyesters, hydrophobic proteins, complex lipids, waxes...), for which the need of new high-end analytical tools has been identified within a large scientific community. The funds are used for the acquisition, the setting-up and the operation of the analytic chains as the basis of a scientific platform for collaborative research projects.

The platform has several objectives:

- to provide a unique set of powerful and innovative tools for (i) the analysis of structure and architecture of lipids and hydrophobic plant biopolymers and derivatives (polyisoprene, polyesters, complex lipids, hydrophobic proteins...) in complement to the existing facilities within partner units; (ii) the identification, study, isolation and modification of new molecules issued from the biodiversity of Mediterranean and tropical plants.
- to contribute to the development of the interface between plant sciences and the biotransformation of plant lipids and polymers in the context of green and sustainable chemistry.

**Funding by Agropolis Fondation:** € 436,800 (acquisition of high-end equipment, functioning costs, maintenance contracts, salary of an engineer for 24 months)

**Funding categorie(s):** Structuring scientific platform

**Project duration:** 01 February 2009 – 30 April 2013

**Keywords:** lipids – platform – sustainable chemistry – green chemistry – Mediterranean crops

**Year of CfP: 2008**

**Project No 0802-005 Completed**

<b>Project title:</b> Response of natural <i>Arabidopsis</i> accessions to salt stress – A French/Chinese initiative
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**Unit managing the project:** BPMP, Plant Molecular Physiology and Biochemistry (Montpellier SupAgro, INRA, CNRS, UMI1)

**Project leader:** Christophe Maurel (maurel(a)supagro.inra.fr)

**Country involved in the project:** China

**Sub-thematic axis:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

**Objectives:**

Environmental constraints (and water stress in particular) represent major limitations for crop production worldwide. In particular, these constraints manifest their effects by harming plant water status. Understanding molecular mechanisms that are involved in water stress perception and signaling and eventually trigger adjustments of plant water relations is a scientific challenge of prime importance.

The aim of this project is to explore the use of a recently identified natural accession of *Arabidopsis thaliana*, as a unique material to identify master regulators of plant responses to water stress. This approach is based on the atypical regulation of root hydraulic conductivity exhibited by this accession in response to salt stress. The genetics of this trait and transcriptomic responses of the accession of interest will be investigated.

This project will be conducted in collaboration between the BPMP unit in Montpellier and a Chinese group working at the Shanghai Institute for Biological Sciences. These two groups share a common interest for the study of stress responses in *Arabidopsis*. Two Chinese scientists will visit the Montpellier unit: a senior scientist for one week, and a post-doctoral student for nine (9) months.

**Action carried-out and results obtained:**

SCIENTIFIC PROGRAMME: Previous studies on 13 natural accessions of *Arabidopsis* have revealed a marked variability of the water transport properties (i.e. hydraulic architecture) of the roots of these plants. In this project, we have combined molecular and biophysical approaches to characterize the response to salt stress of the roots of 5 of these accessions. Measurements of the root hydraulic conductivity (L<sub>pr</sub>) by means of a pressure chamber technique revealed that this parameter shows a typical inhibition in response to salt in all accessions, except in one (Y) which shows a unique L<sub>pr</sub> up regulation. To refine these analyses, we have measured the hydraulic conductivity of root cortical cells (L<sub>pcell</sub>) using a cell pressure probe. These measurements turned out to be very difficult in salt stress conditions, because the cell turgor is then very low. Nevertheless, we were able to show that salt stress down regulates cell water transport in 3 of the 5 investigated accessions. The accession Y, and another one (Z), did not show any L<sub>pcell</sub> reduction. This cell assay points to a novel aspect of the natural variability of salt stress responses in *Arabidopsis*. Our analyses indicate however that the whole root and cell levels do not reveal similar variability profiles. This discrepancy may be explained by differences in root suberisation that were also observed among accessions. To search for molecular markers of root hydraulics in normal and stress conditions, the expression in roots of 17 aquaporin genes was also characterized by quantitative RT-PCR. The regulation of these genes in response to salt stress was not different between the 5 accessions of interest. By contrast, a study in 13 accessions grown in standard conditions revealed that 2 aquaporin genes can be taken as relevant markers of the , L<sub>pr</sub>. In

conclusion, our set of physiological and molecular data reveals a high diversity of hydraulic strategies in the roots of *Arabidopsis* plants grown in standard or salt stress conditions. The aquaporins but also certain anatomical and morphological characteristics of the root contribute to this diversity of functional adaptations.

**COOPERATION INITIATIVES:** The junior scientist (Dr Guowei LI) started his stay in France on December 1st, 2008. After 9 months devoted to the present project, he now continues his stay in our laboratory within the frame of an ANR project. The senior scientist (Dr Weining Sun) stayed in Montpellier from May 24 to May 31, 2009. She delivered a seminar and visited 5 local laboratories. The French group leader (C. Maurel) traveled to China to attend the international InterDrought III congress (<http://www.interdrought.org/index.jsp>) and visited the partner laboratory in Shanghai at this occasion.

**Publications:**

Moira Sutka, Gwowe Li, Julie Boudet, Yann Boursiac, Patrick Doumas, Christophe Maurel (Mar 2011) Natural variation of root hydraulics in *Arabidopsis* grown in normal and salt-stressed conditions. *Plant Physiol.* 155(3):1264-1276.

**Prospects for the future:**

This work has led to a very precise description of root hydraulics in *Arabidopsis*. In particular, it revealed a marked natural variability of this function. We could not confirm the idea that the previously identified Y accession could be a unique material for studying the response of plants to salt stress, as only Lpr but neither Lpcell nor aquaporin gene regulation showed an atypical regulation. By contrast, the identification of gene markers for Lpr, in combination with an increased throughput of Lpr measurements, pave the way to exploring water transport in the *Arabidopsis* root by quantitative genetics. The present project also reinforced the cooperation with our partner laboratory in Shanghai and allowed to recruit valuable collaborators for other projects in our laboratory.

**Total Agropolis Fondation funding:** € 37,358 (salary of a Chinese Junior scientist for 9 months, travel expenses for 2 scientists between France and China)

**Funding categorie(s):** Agropolis Fondation visiting fellowship, Agropolis Fondation small grants (support for exploratory, risky and innovative projects)

**Project duration:** 23 September 2008 – 22 September 2009

**Keywords:** *Arabidopsis* – salt stress – water stress – transcriptomics

**Year of CfP: 2008**

**Project No 0802-006 Completed**

<b>Project title:</b> Preparing an international networking project on rice blast diversity
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**Unit managing the project:** BGPI, Biology and Genetics of plant/pathogen interactions (CIRAD, INRA, Montpellier SupAgro)

**Project leader:** Didier Tharreau (tharreau(a)cirad.fr)

**Countries involved in the project:** Vietnam, Nepal, India, Laos, Philippines, China

**Sub-thematic axis:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

**Objectives:**

The rice blast disease, caused by the fungus *Magnaporthe oryzae*, is the main fungal disease of rice all over the world. This plant pathogen is known to reproduce asexually in all rice growing areas studied. However, sexual reproduction can be achieved in controlled conditions, and different genetic and biological results suggest that sexual reproduction could take place in limited areas of the Himalayan foothills. This area is a centre of diversity of the pathogen and is suspected to be its centre of origin. Rice was domesticated in the same area. It is demonstrated that intercontinental migrations have shaped the structure of the rice blast populations and allowed dissemination of specific virulent genotypes around the world. If sexual reproduction takes place, even limited in frequency and distribution, because of long distance migrations, it is likely to have an impact on the durability of rice resistance to the blast disease. Thus, determining if sexual reproduction takes place is an important research objective.

Tools in population genetics allow to detect traces of sexual reproduction. These techniques will be applied to selected populations of the blast pathogen in the presumed centre of origin of the pathogen. A first step is to better delimit this centre by studying populations from different countries on the South border of Himalaya. The second step is to sample more populations in the best candidate areas previously identified. Sampling requires partners in the different countries targeted.

The objective of this project is to set up contacts and initiate collaboration with partners in South Asia to identify valuable partners to prepare a future collaborative project on rice blast diversity. The scientists involved in this project will identify and train partners on various genotyping techniques being used routinely to characterize the rice blast fungus. The final goal is to develop a network of partners and an international platform to study the diversity of the most damaging fungal pathogen of rice, *Magnaporthe oryzae*.

**Action carried-out and results obtained:**

The objectives of this project were to set up contacts and initiate collaboration with partners in Continental South Asia to identify valuable partners to prepare a future collaborative project on rice blast diversity. The final goal is to develop a network of partners and an international platform to study the diversity of the most damaging fungal pathogen of rice, *Magnaporthe oryzae*. We contacted more than 20 partners to explore the possibility of setting up collaborations. We realized prospective missions to Yunnan province of China, Laos, and Nepal to visit different preselected research groups working on rice and/or rice blast. These missions were followed by a collaborative work, that consisted in obtaining rice diseased samples, isolating rice blast strains, and characterizing their fertility and genetic diversity with molecular markers. This resulted in the isolation of 335 strains and characterization of 235. Among these samples, we identified a particular population that shows all characteristics of a sexually reproducing population. This is the

first time that such a population is unambiguously identified for the rice blast fungus. We could welcome a Nepalese colleague in Montpellier and train him to our biological and molecular methods of characterization. We also realized a mission to the Philippines to try to involve IRRI in the network construction.

The expected output to submit to funding agencies an ambitious international collaborative project could not be reached because there were no appropriate calls for proposals. But, the prospective missions allowed us to identify valuable partners and to initiate collaboration. The preliminary data obtained orientated the choice of geographic areas to further investigate. The results were presented in different national and international conferences and part of them are presented in a paper submitted for publication in international peer-reviewed journal.

**Prospects for the future:**

The project has generated effective collaboration and promising scientific results. We aim at strengthening collaboration and at comforting the scientific results. We will maintain our efforts to set up new collaborations Continental South Asia. We will go on looking for calls for proposals that would support either bilateral cooperation or networks on rice blast diversity in this area.

**Total Agropolis Fondation funding:** € 20,800 (travel expenses)

**Funding categorie(s):** Agropolis Foundation small grants (support to prepare applications for national or international calls for proposals, e.g. ANR and EU FPs)

**Project duration:** 18 September 2008 – 31 March 2010

**Keywords:** rice blast – fungus – network – phenotyping – genotyping – population genetics – diversity

**Year of CfP: 2008**

**Project No 0802-010**

**Project title:** Collect of wild cocoa genetic resources related to aromatic chocolate quantities in Ecuadorian Amazonia.

**Unit managing the project:** AGAP (Genetic improvement and Plant adaptation) (CIRAD, INRA, Montpellier SupAgro)

**Project leader:** Claire Lanaud (claire.lanaud(a)cirad.fr)

**Country involved in the project:** Ecuador

**Research units from the Foundation's scientific network involved:** Qualisud

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), STDI-2 (Socio-Technical Dynamics of Innovation 2: *Agrifood innovations, food and non-food use of plant crops*)

**Objectives:**

Food quality improvement, nutritional as organoleptic, is now a strong demand of the society. *Theobroma cacao* is mainly cultivated in West Africa, Latin America and Asia and according to the International Cocoa Organization (ICCO), 14 million farmers are involved on cocoa culture.

Flavor is among the main criterion of quality for chocolate manufacturers. Flavor components depend strongly on conditions of post-harvest processing (environmental effects, storage, fermentation, drying, roasting). However, the genetic origin is also a strong determinant of flavor, independently of the conditions of post-harvest processing. Cocoa could be classified in two main categories : the standard and bulk cocoa representing the main part of the market, and the fine flavor cocoa, used to produce fine flavor chocolate, and produced by 2 main varieties: Criollo and Nacional (from Ecuador). Nacional cocoa has been classified as "fine flavor cocoa" and benefits for this reason of a higher price in the market. However, due to the introgression of foreign varieties, part of Ecuador cocoa was declassified and considered as "bulk cocoa" at lower price.

The objectives of this project are to collect a large and extensive pool of wild *Theobroma cacao* genetic resources, closely related to the aromatic Nacional variety and potentially rich in aromatic cocoa flavors, in 2 regions of Amazonia from Ecuador recently identified by our team as the potential region of origin of the Nacional variety. After morphological characterization of the samples, they will be propagated by bud grafting. Biochemical and molecular analyses will then be performed to study their diversity. This enrichment of genetic resources will facilitate the improvement of new resistant and productive Nacional varieties displaying a strong floral aroma similar to the original Nacional variety.

**Total Agropolis Foundation funding:** € 45,672 (travel expenses of three French scientists to Ecuador for one month, small equipment, running costs, one month fellowship for a junior scientist from Ecuador)

**Funding categorie(s):** Agropolis Fondation small grants (support for small exploratory, risky and innovative projects), Agropolis Fondation visiting fellowship

**Project duration:** 1 January 2009 – 31 December 2011

**Keywords:** *Theobroma cacao* - cocoa - genetic resources- diversity - aroma

**Year of CfP: 2008**

**Project No 0802-012 completed**

<b>Project title:</b> Analysis of <i>Xanthomonas albilineans</i> Gene Expression during Sugarcane Leaf Scald Pathogenesis
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**Unit managing the project:** BGPI (Biology and Genetics of plant/pathogen interactions) (CIRAD, INRA Montpellier SupAgro)

**Project leader:** Monique Royer (monique.royer(a)cirad.fr)

**Country involved in the project:** USA

**Research units from the Foundation's scientific network involved:** LGDP

**Sub-thematic axis:** IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

**Objectives:**

The xylem-invading mechanisms of plant pathogenic bacteria are still not well known. *Xanthomonas albilineans* is the causal agent of leaf scald disease of sugarcane, one of the major diseases of sugarcane, and it is one of the most ancient species of the *Xanthomonas* genus. This pathogen multiplies in the xylem and systemically colonizes the entire host plant. *X. albilineans* has several unusual characteristics that distinguish its pathogenicity from other *xanthomonas*.

The recent sequencing and annotation of the genome of *X. albilineans* revealed that the size of this genome (3.7 Mb) was reduced in comparison to the sizes of the genomes of other *xanthomonas* sequenced to date (approximately 5 Mb). Additionally, *X. albilineans* possesses 518 genes that are not conserved in other *xanthomonas*, but is missing the Hrp type III secretion system (T3SS) which is found or thought to be present in most other pathogenic *xanthomonas*. The Hrp system is used for injection of protein pathogenicity effectors into plant cells.

It is indispensable to identify new candidate genes potentially involved in pathogenesis. The objective of this project is therefore to investigate, using microarray technology, the full breadth of the response of *X. albilineans* to sugarcane host environment during the colonization of the xylem and also during the epiphytic life of the pathogen.

Bacterial gene expression will be quantified using the microarray technology that was recently successfully used by Professor Caitilyn Allen to investigate the full breadth of the response of *Ralstonia solanacearum* to tomato environment.

**Action carried-out and results obtained:**

Action 1: Carrying out experiments to study gene expression during sugarcane leaf scald pathogenesis

*Xanthomonas albilineans* is the causal agent of leaf scald disease of sugarcane, and a xylem-invading plant pathogenic bacterium. The objective of this project is to identify candidate genes by investigating the full breadth of the response of *X. albilineans* to sugarcane host environment during the colonization of the xylem.

Bacterial gene expression will be quantified using the microarray technology that was recently successfully used by Professor Caitilyn Allen to investigate the full breadth of the response of *Ralstonia solanacearum* to tomato environment.

Custom microarrays specific to the transcriptome of the *X. albilineans* strain GPE PC73 were designed and produced by NimbleGen (Madison, WI, USA). These arrays are specific to following sequences:

- the 3209 protein-coding sequences annotated in the genome of the *X. albilineans* strain GPE PC73,
- all intergenic regions identified in the genome of the *X. albilineans* strain GPE PC73,
- 505 ESTs of *Saccharum officinarum* corresponding to either orthologs of rice genes involved in defense mechanisms or either EST present in many copies (30 à 300) in the databases which therefore may be constitutively expressed.

We prepared sugarcane plants infected by the *X. albilineans* strain GPE PC73. We prepared total RNA from several samples collected on the stem of these sugarcane infected plants. To prepare these total RNA, we used the protocol developed by Professor C. Allen to prepare RNA from samples collected on the stem of tomato plants infected by *R. solanacearum*, a pathogenic bacterium that spreads into the xylem. We also prepared total RNA from liquid cultures of the *X. albilineans* strain GPE PC73. These RNA will be soon analysed by Nimblegen using the arrays produced as part of this project. The Professor Caitilyn Allen's team will participate to the statistical analyses of the microarray experiments. Differential analyses of microarrays experiments of RNA prepared from samples collected on the stem of infected sugarcane plants or from liquid cultures will lead to the identification of gene expressed during sugarcane leaf scald pathogenesis.

Action 2: Teaching Activities at Montpellier SupAgro

- Diagnostique et gestions des maladies bactériennes des plantes tropicales (Diagnosis and management of bacterial diseases of tropical crops). Three-hour intensive module for 22 Tropical IPM Masters students from francophone Africa at Montpellier SupAgro (January 26, 2009).
- Biologie évolutive et diversité du vivant (Evolutionary Biology and Diversity of Life) – Two-hour lecture on the evolution and phylogeny of plant pathogenic bacteria. Fifteen Montpellier SupAgro Master's students (April 20, 2009).
- Member of the Advisory Committee of Ph.D. student Melanie Marguerettaz (ED SIBAGHE, Université Montpellier 2, France; Monique Royer, Thesis Advisor)

#### **Prospects for the future:**

Action 1: The candidate genes identified by the microarray analyses will be further studied. The expression of these genes will be studied by quantitative RT-PCR in the samples collected from the stem of sugarcane plants inoculated with the *X. albilineans* strain GPE PC73. Functional analysis of these genes will be performed with knockout mutants in which these genes will be inactivated. Professor Caitilyn Allen will participate to this future project.

Action 2: Professor Caitilyn Allen will participate to the future student exchange programs between University of Wisconsin-Madison and Montpellier SupAgro. She also will participate to the Advisory Committees of the future Ph.D. students of BGPI who will study pathogenicity of *X. albilineans*.

**Total Agropolis Fondation funding:** € 21,360 (travel, housing and direct costs link to the conduct of experiments)

**Funding categorie(s):** Agropolis Fondation visiting fellowship (senior scientist, less than 12 months)

**Project duration:** 15 October 2008 - 30 June 2010

**Keywords:** *Xanthomonas albilineans*, pathogenicity, gene expression, microarrays

**Year of CfP: 2008**

**Project No 0802-019 Completed**

<b>Project title:</b> AGRO2010 MONTPELLIER – The Scientific Week around Agronomy
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**Unit managing the project:** UMR SYSTEM (Functioning and management of tree-based Planted Ecosystems) (CIRAD, INRA, Montpellier SupAgro)

**Project leader:** Jacques Wery (wery(a)supagro.inra.fr)

**Countries involved in the project:** International

**Research units from the Foundation's scientific network involved:** AMAP, BGPI, CBGP, AGAP, ECO&SOLS, EMMAH, GREEN, INNOVATION, LAMETA, LEPSE, LISAH, LSTM, PSH

**Sub-thematic axis:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*), STDI-3 (Socio-Technical Dynamics of Innovation 3: *Innovation processes, social management of innovations*)

#### **Objectives:**

The objective assigned to Agronomy by the society was to improve production on the basis of external input (fertilizers, pesticides, energy). During the past 20 years Agronomy came back progressively to its original concept with the integration of environmental impacts in the assessment or in the design of cropping systems. With the launching of the *Agence Nationale de Recherche* (ANR) Call "Systerra", the challenge is now to design agro-ecosystems with high production performance based on limited input and environmental impacts. This has to be conducted into a sustainable development context thereby adding socio-economic criteria to this multi-functional vision of agronomy. Although it remains deeply anchored in the biophysical functioning of the cultivated field, the science of agronomy is, today, a multi-scale and multi-criteria systemic approach.

This project will use the opportunity of the 11<sup>th</sup> Congress of the European Society for Agronomy (ESA) in 2010 in Montpellier to create a major international scientific event around Agronomy (Agro2010). This event includes a set of symposia and workshops covering (i) the scientific challenges for agronomic research in the context of sustainable development, (ii) the presentation and discussion of recent advances in the interfaces of agronomy and neighboring disciplines, and (iii) the major advances and research agenda for agronomy in Europe and in the developing world.

Specifics workshops will be organized for young scientists and students from developing countries, in order to help them to manage their research project and establish networks with the Agropolis Fondation scientists.

#### **Action carried-out and results obtained:**

The International Scientific Week Around Agronomy, Agro 2010 (Montpellier August 29 - September 3, 2010), has been co-organized by Agropolis International and the European Society for Agronomy (ESA) for its XIth congress, with the help of SupAgro, Inra and Cirad under the chair of Professor Jacques Wery (Executive Secretariat of ESA). The aim of this congress was to demonstrate the scientific grounds, the applied results and the ambitions of Agronomy to address the burning questions of the XXIst century for agriculture and its contribution to sustainable development. It was more specifically dealing with adaptation to climate change, ecological intensification of cropping systems, yield gap analysis and the design of productive and resilient cropping systems with low use of pesticides, fertilizers and energy. The design of this scientific event allows to follow a large range of trails with specific combinations of the following elements:

- an International symposium "Agronomy for Sustainable Development" with high level contributions opening the dialogue between scientists and stakeholders on the major challenges for Agronomic Research in Europe and in the developing countries
- Three Parallel symposia on scientific advances and challenges of interdisciplinary research between Agronomy and other disciplines:
  - o Plant Biology and Genetics: Co-designing plants and cropping systems for new targets
  - o Ecology: Ecological intensification of cropping systems
  - o Economy and Social Sciences: Co-assessment and co-design of technological innovations and agro-environmental policies
- Three parallel symposia organized by the scientific division of ESA on the three pillars of modern agronomy:
  - o Plant System Biology: the cultivated plant in a field (ESA - Division 1),
  - o Field Scale Agroecology: the cultivated field as an ecosystem (ESA - Division 2),
  - o Cropping Systems at Farm, Regional and Global Scales: the Cropping System in its embedding systems (ESA - Division 2),
- A symposium for the dialog between researchers and extension services on "Decision-Aid in Agriculture: which type of research and how to better use it in extension" with specific sessions for Europe and Africa,
- Three field trips combining meetings with farmers, tasting of regional products and visits of monuments
- Two international master classes on crop modelling and integrated assessment of agricultural systems, given for young scientists with priority given to developing countries,
- Two seminars aimed to develop international collaborations on Adaptation of Agriculture to Global Changes in OECD countries and on Ecological Intensification of cropping systems in the tropics.
- This congress was opened by an evening show to open the discussion between scientists and the Montpellier inhabitants on agriculture and the role of research.

Agro2010 has gathered 590 participants, which is a record in ESA congresses history. 56 countries were represented, 75% from Europe and 25% from all over the world, Africa being represented by more than 30 participants

Across the first four days Agro2010 organized 29 scientific sessions of two hours each. 470 papers have been published in the proceedings from which 160 oral presentations have been selected for an oral presentation and 370 have been presented as posters. All these communications and a large set of films are available on the congress web site [www.agropolis.fr/agro2010](http://www.agropolis.fr/agro2010)

#### **Prospects for the future:**

A specific brochure of 68 pages dedicated to the expertise in Agronomy of 13 Montpellier's research teams, and their partners, was especially edited in French and English by Agropolis International and distributed to all participants. It is available at <http://www.agropolis.org/publications/thematic-files-agropolis.php>

During Agro2010, and thanks to the involvement of Agropolis International, the European Society for Agronomy (<http://www.european-agronomy.org>) has established its headquarters in Montpellier and will continue on the international momentum of this congress.

**Total Agropolis Fondation funding:** € 61,136 (edition, invited speakers fees, scientific committee members fees, grants for 20 young scientists from developing countries)

**Funding categorie(s):** Agropolis Fondation visiting fellowships, Agropolis Fondation small grants (support for the organisation of high-level scientific events, support for publication and dissemination of results)

**Project duration:** 1 September 2008 – 01 April 2011

**Keywords:** Agronomy – sustainable development – AGRO2010

**Year of CfP: 2008**

**Project No 0802-020 Completed**

**Project title:** Integrative Proteomics Resources for Molecular Phenotyping of Plant Growth Responses to Environment and Climate Changes

**Unit managing the project:** Proteomique (INRA)

**Project leader:** Michel Rossignol (rossignol(a)supagro.inra.fr)

**Countries involved in the project:** Belgium, Germany

**Research units from the Foundation's scientific network involved:** BPMP, LEPSE

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

**Objectives:**

Our goal is to build proteomics resources suitable to characterize simultaneously the various transporters present in the *Arabidopsis* plasma membrane in order to study their responses to environmental stresses and correlate them to macroscopic phenotypic features of aerial parts of plants.

A targeted proteomics strategy relying on mass spectrometry in the Multiple Reaction Monitoring (MRM) mode will be selected for its high specificity and sensitivity. This method requires three consecutive steps: the bioinformatics identification of peptides of interest together with the prediction of their behavior in LC-MS/MS, their synthesis in light or heavy form (by incorporation of heavy isotopes) and tuning of the mass spectrometer for every peptide.

This should generate proteomics integrative information about the networking of transport systems involved in the responses to changes in nutrient availability and introduce into molecular phenotyping strategies a proteomics dimension at a scale compatible with current macroscopic plant phenotyping approach.

**Action carried-out and results obtained:**

- ▶ Identification of peptides of interest.

Three main types of transporters were selected for this proof of concept study: the H<sup>+</sup>-ATPases proton pumps as primary transporters (AHA family), the influx or efflux nitrogen (as well nitrate as ammonium) transporters (AMT, NRT and POT families) as example of secondary transporters, and aquaporins as channels (PIP family). For these different families, bioinformatics analysis first allowed to identify ca 800 tryptic peptides as unique in the *Arabidopsis* genome, and being thus usable to specifically identify isoforms in a given family. Their propensity to be analyzable by LC-MS/MS was then calculated using the various predictors. The resulting data were then inspected by partner teams expert for each transporter family in order to select the most pertinent protein and peptides on a knowledge basis (available expression data, known post-translational modifications, availability of mutants). Finally, a total of 30 transporters were selected for MRM analysis.

- ▶ Peptide synthesis.

Thirty-two peptides covering the 30 accessions (5 AHAs, 13 PIPs, 5 AMTs, 5 NRTs and 2 POTs) were synthesized in light and heavy forms.

- ▶ MS optimization.

For every peptide, optimal sensitivity requires tuning both ionization and fragmentation instrumental parameters. Under optimized conditions, response curves of peptides must then be checked for robust statistical significance of detection and quantification thresholds (LOD, LOQ). In addition, different quantification methods (using either light or heavy peptides) were compared. The method finally selected as the more robust relies on (i) spiking samples with known amount of the mixture of heavy peptides and (ii) using a digest of biological samples as a matrix for establishing dilution curves of heavy peptides.

#### ► Results.

In terms of resource (identification of usable sequences, availability of heavy peptides, peptide-dependent optimized conditions), this work resulted in a tool allowing the simultaneous characterization of the 28 accessions, excepted for the ammonium transporter AMT1.1. For this latter, most of the 7 peptides unique in the Arabidopsis genome display unfavorable predicted features, whereas others may co-exist under various states due to post-translational or chemical modifications. The less unfavorable peptide was initially selected but turned out to be convenient only in quite simple mixtures (such as that of the synthetic peptides used here) and no longer robustly detectable in a complex matrix (like a digest of membrane proteins). This peptide was discarded. Such situation is illustrative of a potential limitation of the strategy for highly homologous multigene families where the identification of favorable sequences may be problematic.

In terms of molecular phenotyping, due to the late availability of some synthetic peptides, the resource was little used with plants submitted to simultaneous macroscopic phenotyping under salt stress. Molecular phenotyping was performed, at the root level, on plants grown in hydroponics and exposed to a short salt stress (150 mM NaCl, 4 hours). Three AHAs, all the 13 PIPs, 2 AMTs and 2 NRTs were simultaneously identified and quantified in roots, with accumulation differences covering 3 orders of magnitude. The abundance of 11 among them appeared to be significantly lowered under salt stress. These results are novel in several concerns:

- For a number of transporters, they correspond to the first experimental observation as proteins (available antibodies not allowing until now to resolve isoforms);
- They constitute the first simultaneous analysis of several transporter families covering the main types (primary transporters, secondary active transporters, channels);
- They enable functional analysis (expression regulation) accessible to date only at the transcript level. In this view, excepted for a limited number of accessions, results (i) show a weak disconnection with available data for the accumulation of PIPs transcripts in roots, and (ii) demonstrate for all families a fair overall correlation between transcriptome and proteome responses to salt stress.

#### ► Conclusions.

This project allowed developing a unique resource for the simultaneous molecular phenotyping of various transporter families. This resource is innovative, including outside the plant area, and reusable. It has been already taken to profit for investigating the effect of nycthemeral status on the expression of PIPs in the leaf. In addition, the approach is generic and, at the possible reserve of specific cases within some multigene families, is portable at larger scale as well to address other transporter families as more generally for targeted proteomics strategies.

#### Publications

Monneuse JM, Sugano M, Bécue T, Santoni V, Hem S, Rossignol M. Towards the profiling of the *Arabidopsis thaliana* plasma membrane transportome by targeted proteomics. Proteomics, in press.

**Total Agropolis Fondation funding:** € 39,297 (running costs)

**Funding categorie(s):** Agropolis Fondation small grants (support for small exploratory, risky, and innovative projects)

**Project duration:** 1 January 2009 – 15 December 2010

**Keywords:** Proteomics – phenotyping – transporters

**Year of CfP: 2008**

**Project No 0802-021**

**Project title:** Impact of changing environmental and anthropogenic constraints on the diversity of asexually propagated crops: towards an international research proposal on yam agrobiodiversity

**Unit managing the project:** DIAPC (Diversity and Adaptation of Cultivated Crops) (CIRAD, IRD, INRA, Montpellier SupAgro, UMII)

**Project leader:** Jean-Louis Pham (pham(a)ird.fr)

**Countries involved in the project:** The Netherlands, UK, Guinea, Côte d'Ivoire, Ghana, Benin Togo, Nigeria, Cameroon, International

**Research units from the Foundation's scientific network involved:** AGAP, CBGP

**Subthematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*),

**Objectives:**

While yam is a very important crop for food security in Africa (which accounts for 90% of the total world production), the research dedicated to its improvement and its diversity conservation is poorly organized. The germplasm collections in Africa are generally understudied. No international network has actually been set up to address African crops and research has been conducted at limited geographical scales. The agronomic importance of the crop justifies the development of international initiatives which foster partnership among stakeholders at a large geographical scale.

The aim of this project is to prepare a research proposal on the impact of anthropogenic and natural environmental changes on the agrobiodiversity of vegetatively propagated crops. This project will then be submitted to an international call (European Union (EU), Biodiversa, Agence National de la Recherche ANR).

Crops need to adapt through natural or human-driven evolutionary processes to new environmental conditions, e.g. global warming, emerging pests or new agronomic objectives. The diversity of crops and their wild relatives is threatened by changes in the management of agroecosystems or the fragmentation of natural habitats which affects the interplay within the wild-cultivated complexes. *In situ* adaptation will have to happen with less genetic diversity, i.e. less material for adaptative changes. How can such adaptation happen for vegetatively-propagated crops, which do not benefit from the "innovation power" of genetic recombination? In simple terms, the diversity of vegetatively-propagated crops will inevitably be eroded because of genetic drift and farmers' decisions to discard varieties and only mutations will enhance genetic diversity. The reality is more complex, however. For a few root and tuber crops, studies demonstrated that sexual reproduction still plays a role in the dynamics of their diversity.

This study will look at how these complex processes interact, in order to develop scenarios on the evolution of agrobiodiversity of yam --a tuber crop of major importance for food security in tropical countries--in changing agroecosystems in Africa, and to help manage its conservation and use at a large geographical scale.

The project will bring together identified partners and resource persons to discuss the following:

- Main drivers of change in yam agrobiodiversity

- State of the art in spatial/landscape genetics, including the availability of asexually-propagated crops
- Phylogeny of yam relevant for genetic conservation objectives
- Evaluating the adaptive potential of yam
- Tools to integrate genetic, socioeconomic and environmental data
- Connecting these tools (databases, GIS) to modeling approaches

**Total Agropolis Fondation funding:** € 21,029 (travel expenses)

**Funding categorie(s):** Agropolis Fondation small grants (support to prepare applications for national and international calls for proposals)

**Project duration:** 15 November 2008 – 15 July 2010

**Keywords:** Yam – agrobiodiversity – crops

**Year of CfP: 2008**

**Project No 0802-022 Completed**

<b>Project title:</b> Adaptative responses of the N <sub>2</sub> -fixing symbiotic interaction between <i>Medicago truncatula</i> and rhizobia to whole plant N limitation
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**Unit managing the project:** LSTM (Laboratory of Tropical and Mediterranean Symbiosis) (CIRAD, INRA, IRD, Montpellier SupAgro, UMII)

**Project leaders:** Gisèle Laguerre (gisele.laguerre(a)supagro.inra.fr) and Marc Lepetit (BPMP, Plant Molecular Physiology and Biochemistry)

**Research units from the Foundation's scientific network involved:** BPMP

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*)

**Objectives:**

In soil, the roots are constantly subjected to biotic and abiotic stresses that locally suppress the acquisition of N. Moreover, in legumes, roots formed nodules in the presence of heterogeneous and fluctuating bacterial populations consisting of several strains of rhizobia whose efficiency for symbiotic nitrogen fixation is variable. The continuation of plant growth in such situations depends on its ability to offset the deficit by increasing the acquisition capacity of the roots remained unstressed. Recent work has characterized these adaptive responses in *Medicago truncatula* (Ruffel et al., 2008, Plant Physiol 146: 2020-2035; Jeudy et al., 2010, New Phytol 185: 817-828). In the case of plants fed NO<sub>3</sub><sup>-</sup>, the plant compensates very quickly and very efficiently the N deficit, first by increasing the absorption capacity of roots remained supplied then preferentially stimulating their proliferation. There is also a compensatory response in N<sub>2</sub>-fixing plants (in symbiosis with the model strain *Sinorhizobium meliloti* RCR2011 / local deficit caused by treatment Ar/O<sub>2</sub>), but it is much less efficient and slower. It is not accompanied by any increase in the specific activity of existing fixing nodules (which seems to be always at its maximum) and only depends on the stimulation of nodule development in non-stressed roots remained supplied. Our objective was to determine in *M. truncatula* the impact of variability in bacterial efficiency to fix N<sub>2</sub> on the functional (nodular activity, acquisition of N in whole plant) and developmental responses (nodules and roots) exploiting the natural genetic variability exists among compatible rhizobia.

Studies will be conducted on the model species *M. truncatula* (*Mt*) and a unique collection of compatible rhizobial partners that has been constructed by the LSTM. The effect of genetic variability of rhizobia on the responses of the structures (nodule size, nodule number, root/nodule architecture) and the functions of the symbiotic organs (nodule activity, N intake) will be explored. This approach is the first step of a more ambitious project aiming to unravel bacterial mechanisms that contribute to the adaptation of plant to fluctuations of environmental factors.

**Action carried-out and results obtained:**

Screening of a collection of rhizobia (*S. meliloti* and *S. medicae*) was used to select strains with different levels of efficiency to fix N<sub>2</sub> in symbiosis with *M. truncatula* (efficiency lower or higher than that of RCR2011). The compensatory response of the plant has been characterized in plants nodulated with each of these strains in split-root devices. The effect of local suppression of N<sub>2</sub> fixation applied to half of the root system has been studied on the roots of the same plant remained fixing. For short-term responses (4 d), the suppressive treatment was to replace the air by a mixture of argon and oxygen. For long-term responses (30 d), nitrogen limitation has been

obtained by nodulation of half of the root system with a strain of *S. meliloti* unable to fix N<sub>2</sub> (mutant fixJ of RCR2011).

The results show that the general pattern described above for the *M. truncatula* -RCR2011 symbiosis is conserved whatever the strain and its effectiveness for N<sub>2</sub> fixing. In our study, the responses observed at 30 days showed that the plant has fully compensated the local limitation of nitrogen uptake since biomass and nitrogen contents of plants subjected to treatment are equivalent to those of control plants regardless of the strain considered. For all strains the compensatory response to the localized nitrogen limitation depends only on the stimulation of nodule development and not on any increase of nodule specific activity on untreated (nitrogen fixing) roots. The observed responses are consistent with a systemic negative feedback exerted by the N assimilates of the plant on nodule development.

The magnitude of the adaptive response to long-term depends on the activity of N<sub>2</sub>-fixing strain: indeed, more the strain is efficient, more the compensatory response is important. However, the response is not solely determined by the N status and N demand of the plant, but is also strongly dependent on the efficiency of the strain in interaction with the plant. Indeed, for poorly effective strains, although the overall level of fixation of N is insufficient to meet the N demand of the plant, nodule development reached a threshold. This suggests either a response limited by nutritional factors (limitation in C, for example) or a limitation by mechanisms linked to autoregulation of nodule number (AON) that seem to be partly determined by the nature of the symbiotic interaction. Studies with plants grown in an atmosphere enriched in CO<sub>2</sub> and / or using mutants affected in the AON (hypernodulant mutant) could help evaluate these two hypotheses.

#### **Prospects for the future:**

Various studies have shown that the host plant preferentially associated with certain bacterial genotypes from the genetic diversity available in the in the free-living soil populations. However, recent studies indicate that the population structure changes in the nodules during the vegetative cycle, varies according to changes in nodule development, and may vary with nutritional constraints experienced by the plant (Depret and Laguerre, 2008, *New Phytol* 179:224-235; Kiers et al., 2007, *Proc. R. Soc. B* 274:3119-3126). These results suggest that the various components of the rhizobial populations can be differentially mobilized by the symbiotic interaction in response to environmental conditions or plant development and thus contribute to the plasticity of plant adaptation to a changing environment for N supply. Therefore, our current work now aims to determine whether the plant, according to its nitrogen demand, is able to select the most effective bacteria for the formation of nodules and / or to favor the growth of existing nodules formed by the most effective strains. In the longer term, according to the results obtained, it is planned to develop a project to decipher the molecular basis of these adaptive responses.

**Total Agropolis Fondation funding:** € 8,840 (running costs)

**Funding categorie(s):** Agropolis Fondation small grants (support for small exploratory, risky and innovative projects)

**Project duration:** 1 October 2008 – 31 December 2009

**Keywords:** symbiosis – nitrogen – rhizobia – *Medicago truncatula* -

**Year of CfP: 2008**

**Project No 0802-023**

**Project title:** Development of a system for high throughput functional analysis of *Magnaporthe grisea* virulence effector proteins

**Unit managing the project:** BGPI (Biology and genetics of plant/pathogen interactions) (CIRAD, INRA, Montpellier SupAgro)

**Project leader:** Thomas Kroj (kroj(a)supagro.inra.fr)

**Country involved in the project:** China

**Research units from the Foundation's scientific network involved:** LGDP

**Sub-thematic axis:** IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*),

**Objectives:**

The aim of this project is to understand the molecular bases of virulence of phytopathogenic fungi. Plant pathogens like bacteria, oomycetes and fungi secrete numerous proteins during infection which serve to manipulate host defense and to exploit plant resources. These so-called effector proteins are therefore central elements in microbial virulence. In bacteria, a certain class called type III effectors are directly injected into host cells. In fungi, this system does not exist. While, some secreted proteins seem to accumulate and act inside host cells. With the objective of understanding to which extent and by which mechanisms they act as effectors of virulence and contribute to fungal pathogenicity, investigations will analyze proteins that the rice pathogenic fungus *Magnaporthe grisea* secretes into plant tissue during infection.

Functional analysis of fungal effector proteins is severely hampered by their huge number and their apparent functional redundancy. Fungal strains mutated for individual effectors are generally not altered in their virulence and it is necessary to develop functional screening systems to elucidate their role in fungal virulence and to decipher their mode of action.

In the present project, a high throughput screening system for the functional analysis of fungal candidate effector proteins based on the use of the bacterial rice pathogens *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) will be developed. It will allow the identification of *Magnaporthe* effector proteins that have a strong impact on virulence or act as avirulence proteins.

Multiple indications now suggest that virulence effectors from one organism can function in other species even if they are phylogenetically unrelated. Fungal candidate effector proteins will thus be analysed for their role in virulence by expressing them as a fusion with a signal for bacterial type III secretion system in *Xoo* and *Xoc*. *Magnaporthe* candidate effectors are then added to the arsenal of *Xanthomonas* virulence factors. Pathogenicity assays will enable the analysis whether this affects (negatively or positively) the capacity of *Xanthomonas* to colonize host tissue and to cause disease symptoms.

Fungal effector proteins showing interesting phenotypes in those functional assays will be analyzed in more detail, e. g. by localizing them during infection, by measuring their contribution to fungal virulence by reverse genetics or by identifying the plant processes they target and the plant target molecules they interact with.

**Action carried-out and results obtained:**

The aim of the project was the development of a heterologous system for the functional analysis of fungal candidate effector proteins. The initial project was to develop a system based on the use of the bacterial rice pathogens *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv. *oryzicola*

(Xoc) for the identification of *Magnaporthe oryzae* effector proteins with a strong contribution to virulence or acting as avirulence proteins. For the development of the system, translational fusions between the N-terminal parts of the *Xanthomonas* avirulence (Avr) proteins AvrBs2 or AvrXa10 comprising signals for secretion by the bacterial type III secretion system (T3SS), and intracellularly acting *M. oryzae* Avr proteins Avr-Pita, Avr-Pia, Avr-Pii, Avr-Pik, Avr-Pizt and AvrCO39 were expressed in Xoo and Xoc. It was expected that these strains became avirulent on rice varieties carrying the cognate resistance genes due to specific and intracellular recognition of Avr proteins injected by Xoo or Xoc. However, even if fusion proteins were produced properly by Xoo, as demonstrated by western blot experiments, they did not confer avirulence indicating that they were not functional. Most probably this may be due to endogenous Xoo and Xoc effectors acting as potent suppressors of rice defence and suppressing resistance induced by *M. oryzae* Avr proteins. Due to these negative results, development of the Xoo- and Xoc-based system will not be pursued.

An in vitro system allowing to determine the capability of pathogen effectors to cross plant plasma membranes has been developed by the group of Prof. Tyler. Recombinant fusion proteins between effectors suspected to be translocated into host cells and GFP are produced in *E. coli* and added to roots of in vitro grown plants. Translocated effectors accumulate inside root cells resulting in fluorescent staining of the plant cytoplasm. A collaboration with the group of Prof Tyler was established in order to identify potentially translocated *M. oryzae* effectors. Three out of six tested *M. oryzae* effectors accumulated inside root cells, suggesting their translocation into host cells during rice infection. Host cell translocation of one of these effectors named PWL2, has been recently demonstrated by life cell imaging supporting significance of the results of the in vitro translocation assay.

**Prospects for the future:**

At present, additional *M. oryzae* effectors are tested for translocation in the in vitro system and motifs necessary and sufficient for translocation of *M. oryzae* effectors are searched. In addition, complementary experiments aiming to confirm translocation of candidate effectors by independent approaches, in particular life cell imaging, are under way.

**Total Agropolis Fondation funding:** € 36,452 (equipment, travel expenses, financial support for 2 master students)

**Funding categorie(s):** Agropolis Fondation small grants (support for small exploratory, risky and innovative projects)

**Project duration:** 15 October 2008 – 31 December 2010

**Keywords:** rice – fungus – virulence – secretion – *Xanthomonas* – effector

**Year of CfP: 2008**

**Project No 0802-027 Completed**

<b>Project title:</b> Organisation of a workshop entitled "Reference sets of food crop germplasm for international collaboration"
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**Unit managing the project:** AGAP (Genetic improvement and Plant adaptation) (CIRAD, INRA, Montpellier SupAgro)

**Project leader:** Jean-Christophe Glaszmann (jean-christophe.glaszmann(a)cirad.fr)

**Country involved in the project:** Mexico

**Research units from the Foundation's scientific network involved:** DIADE

**Sub-thematic axis:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*),

**Objectives:**

*Organisation of an international workshop on reference sets of food crop germplasm*

Although core collections is a widely used concept, as far as representation or even preservation of genetic diversity is concerned, the choice and use of reference collection is less well understood once it deals with association studies.

Within the context of the Generation Challenge Program (GCP), a large effort has been made to characterize the genetic diversity of a "composite set" of 21 crop species. It is expected that from this set, collections that could be used for trait of interest and thus association studies, can be defined.

This led to a major GCP product: germplasm reference samples with validated data of reference markers, accessible as a global public good in a robust form. It is hoped that these samples will be widely shared and used, so that new data can be integrated in order to derive biological understanding useful for germplasm diversity management and use.

This project consists in organizing and holding a workshop where all these steps and aspects are described and discussed, as well as the perspectives and the mode of organization that is necessary for taking full advantage of the initiative. This will be an opportunity for coordination among various players engaged in germplasm management in international programs. Venue: Montpellier, France, November 13-17, 2008.

**Action carried-out and results obtained:**

71 participants from 28 countries, including crop specialists, breeders and germplasm managers have exchanged their background in term of definition and use of crop reference germplasm. This was done either through oral presentations and/or posters, and was structured as communications and brainstorming sessions. The main conclusions are :

- The Generation Challenge Program approach was affirmed in exploring, evaluating and using germplasm diversity, in defining reference sets on the principles that all GCP products must be delivered to users.
- The expectation extends also to related initiatives in the System-Wide Genetic Resources Program (SGRP) of the CGIAR, the Global Crop Diversity Trust and the Global Partnership Initiative for Plant Breeding Capacity Building (GIPB). GCP-derived reference sets can play

a prominent role provided that there is clarity on political availability of the resource and the attached data by placing them under the Multi-Lateral System and that research continues on their use as an entry to the broader collections. Their description and access have a natural place in the web system being put in place by the SGRP and Bioversity, which can therefore be used for publicising and ordering.

- Information has been shared about how many reference sets are available, how they were compiled and what their status is. Together with this, we have been made aware of the broad diversity of situations among crops and some limitations that need to be overcome, on representation and comprehensiveness of original composite collections, choice and justification of methodology to select reference sets, data quality of both collections, composite and reference, traceability of germplasm, availability of the minimum required information to be distributed with the material and quality control (label, certification, ...) of the material to be distributed

### **Prospects for the future:**

Issues to be addressed and actions to be taken

- Governance: Who will decide which sets are ready to get the GCP seal of quality and approval? How will this decision be made?
- Multiplication: Who will do it? Who will finance it? (CG Centers vs NARS)
- Development of the sets and ensuring their quality and their readiness should go together with the development of the information systems to support them (to be developed in conjunction with Bioversity, Compliance with Multilateral System (MLS))
- Availability of methodologies is a must
- Survey on the situation crop by crop and past and current demand for minicore/reference sets

This WS has initiated the establishment of a Genetic Resource Support Service (GRSS) in the frame of the Generation Challenge Program.

**Total Agropolis Fondation funding:** € 36,031 (travel expenses)

**Funding categorie(s):** Agropolis Fondation small grants (support for the organisation of high-level scientific events e.g. conferences, seminars)

**Project duration:** 1 October 2008 – 30 September 2009

**Keywords:** germplasm – workshop – crop – genetic diversity

**Year of CfP: 2008**

**Project No 0802-030**

**Project title:** Challenging theories and frameworks on socio-ecological systems with empirical research results

**Unit managing the project:** GREEN (Renewable resources and environment management) (CIRAD)

**Project leaders:** Martine Antona ([martine.antona@cirad.fr](mailto:martine.antona@cirad.fr)) and François Bousquet ([francois.bousquet@cirad.fr](mailto:francois.bousquet@cirad.fr))

**Countries involved in the project:** USA, Australia, Norway

**Research units from the Foundation's scientific network involved:** CBGP, B-AMR, LAMETA

**Subthematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*), STDI-3 (Socio-Technical Dynamics of Innovation 3: *Innovation processes, social management of innovations*)

**Objectives:**

For the last twenty years, the research units participating in this project have been developing research activities on socio-ecological systems aiming at understanding relationship between agriculture and biodiversity, policies and landscapes dynamics, watershed management, ecosystem management and health risk, etc. At the same time diverse schools of thought have developed theories and frameworks to analyze the dynamics and the management of socio-ecological systems.

The objective of this proposal is to assess the relevance and the complementarities of these theoretical frameworks by applying and testing them on the empirical research done in Montpellier for the last 20 years. This will enhance the value of the empirical research and will provide a clear view on the potential of the theoretical frameworks. The lessons from this project will also provide the basis for new conceptualization of socio-ecological systems dynamics and management.

Senior researchers will be hosted for short periods (about two months) with the aim of interacting with various research units. The researchers from Montpellier will select several case studies (on-going or concluded) which will be analyzed from the invited researcher's point of view. The same case studies will be presented to all invited researchers. Thus the relevance, strengths, weaknesses and complementarities of the theoretical frameworks proposed by the visiting fellows will be assessed. Each case study will be analyzed from different angle leading to progress in the understanding of the dynamics and management of the socio-ecological system under study.

**Total Agropolis Fondation funding:** € 280,800 (travel expenses for senior scientists, publication costs)

**Funding categorie(s):** Agropolis Fondation visiting fellowship, Agropolis Fondation small grants (support for the organisation of high-level scientific events e.g. conferences, seminars)

**Project duration:** 01 February 2009 - 30 September 2011

**Keywords:** theoretical frameworks – socio-ecological systems

**Year of CfP: 2008**

**Project No 0803-007**

<b>Project title:</b> Towards a federative research on modeling and simulation platforms
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**Units managing the project:** GREEN, Renewable resources and environment management (CIRAD), and LISAH, laboratory for soils, agrisystems, hydrosystems interaction studies (INRA, IRD, Montpellier, Montpellier SupAgro)

**Project leaders:** Jean-Pierre Müller (GREEN, jean-pierre.muller(a)cirad.fr) and Jean-Christophe Fabre (LISAH)

**Research units from the Foundation's scientific network involved:** AMAP, AGAP, GREEN, LISAH, SYSTEM, LAMETA

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*)

**Objectives:**

In the domain of plant, environment, and landscape simulation, several software platforms have been developed independently by different teams, with different focuses. Sharing of methodologies and/or tools has been limited, thus leading to

- Different programming languages, hence the need for ways of packaging models in using independent languages, or at least the definition of interoperable structures.
- Lack of agreement despite each platform providing a middleware for coordinating modules on (1) what the middleware is in charge of (e.g. information flows, spatio-temporal structures, coherent schedules, etc.) and (2) what should be specified in the components (i.e., inputs/outputs, events, meta-information)

The objective of this proposal is to make an inventory of these platforms and to start a first analysis of existing solutions to develop a synergy between these projects. This includes analyzing their complementary features, studying their interoperability, comparing their weak and strong points and finding out how common elements could be shared in order to improve software development.

**Total Agropolis Fondation funding:** € 25,637 (salary for an engineer, travel expenses)

**Funding categorie(s):** Agropolis Fondation grants for scientific platform, Agropolis Fondation small grants (support for the organisation of high-level scientific events e.g. conferences, seminars)

**Project duration:** 1 November 2008 – 31 December 2010

**Keywords:** modelling – simulation – platform – landscape – software

**Year of CfP: 2008**

**Project No 0803-008**

<b>Project title:</b> Agropolis computational plant seminar
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**Units managing the project:** AGAP (Genetic improvement and Plant adaptation) (CIRAD, INRA, Montpellier SupAgro), LEPSE (Ecophysiology of Plants under Environmental Stresses) (INRA, Montpellier SupAgro), DIADE (Diversity, Adaptation and development of Plants) (IRD, UMII)

**Project leaders:** Yann Guédon (AGAP, guedon(a)cirad.fr), Christine Granier (LEPSE), Laurent Laplaze (DIADE)

**Sub-thematic axis:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*), STDI-3 (Socio-Technical Dynamics of Innovation 3: *Innovation processes, social management of innovations*)

**Objectives:**

Many researchers from different disciplines (biology, computer science, applied mathematics) are involved in the development of mathematical models and methods applied to plant structures and functions. These researchers are scattered in different research units in Montpellier and are not always well connected with the computer science and applied mathematics scientific communities. While internal seminars already exist in research units and seminars attached to well-defined scientific disciplines exist in Montpellier (different domains of plant biology, applied statistics and computer science), there is a lack of a multidisciplinary seminar devoted to the modelling of plant structures and functions.

This proposal aims to organize monthly seminars and a yearly one-day workshop in Agropolis. This workshop will be devoted to a specific topic selected each year and keynote speakers will be invited. One objective is to make this workshop a national scientific event. All the scales from cell and tissue to the entire plant will be of interest. Equilibrium between presentations devoted to sub-organ scales and presentations devoted to macroscopic scales will be ensured.

All plant types will be of interest (tropical, Mediterranean and temperate plants, model plants, annual plants, fruit and forest trees). The scope of the seminar will not only concern models of plant structures and functions of different nature but also mathematical and computer tools for analyzing digitized plant structures and the integration of heterogeneous tools and models in software platforms.

A web site will be created for the seminar. This web site, preferentially hosted by Agropolis, will contain the program, the summaries of the past and planned presentations with links to the speaker's personal pages and the announcement for the yearly workshop.

**Total Agropolis Fondation funding:** € 31,200 (workshops and speaker invitations)

**Funding categorie(s):** Agropolis Fondation small grants (support for the organisation of high-level scientific events e.g. conferences, seminars)

**Project duration:** 1 January 2009 – 31 December 2011

**Keywords:** plant – computer science – seminar

**Year of CfP: 2008**

**Project No 0803-012**

<b>Project title:</b> Modelling and optimization of bioconversion of plant materials in inhomogeneous media
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**Units managing the project:** IATE (Agropolymer Engineering and Emerging Technologies) (CIRAD, INRA, Montpellier SupAgro, UMII), UMR ASB (*Analyse des Systèmes et Biométrie*) (INRA, Montpellier SupAgro), EPI MERE (INRA, INRIA)

**Project leaders:** Eric Dubreucq (IATE) (Eric.Dubreucq(a)supagro.inra.fr), Jérôme Harmand (jerome.harmand(a)supagro.inra.fr) and Alain Rapaport (ASB et EPI MERE INRIA) (rapaport(a)supagro.inra.fr)

**Countries involved in the project:** Canada, Tunisia

**Sub-thematic axis:** STDI-2 (Socio-Technical Dynamics of Innovation 2: *Agrifood innovations, food and non-food use of plant crops*)

**Objectives:**

The biotransformation of plant materials consists of using microorganisms or enzymes to carry out a reaction that is more costly or not feasible non-biologically (A typical instance of such a process is the enzymatic hydrolysis, where switch-grass is mixed with well-selected enzymes that convert cellulosic material into sugars that will be fermented into ethanol). In an industrial context of "green chemistry", these reactions are elaborated and piloted in "bioreactors". From an economic point of view, using very large tanks filled with high substrate concentrations appears to be the most efficient. However taking into account spatial effects, managing the overall process is a challenging issue.

The aim of this project is to study the effect of spatial structuration on the dynamics of biocatalyzed reactions in non homogeneous reactors through a low complexity modelling approach. This problematic of generic concern (including industrial and natural reaction systems,) will be in a first approach based on the study of a model system. This system will be in an enzyme-catalyzed reaction performed in multi-fed stirred-tank bioreactors connected in series to mimic non homogeneous phenomena.

**Total Agropolis Fondation funding:** € 121,380 (salary for a post-doctoral fellow, travel, conference fees, laboratory expenses)

**Funding categorie(s):** Agropolis Fondation visiting fellowship, Agropolis Fondation post-doctoral fellowship

**Project duration:** 1 December 2008 – 31 January 2011

**Keywords:** bioconversion – biotransformation – bioreactors – modelling – green chemistry

**Year of CfP: 2008**

**Project No 0803-013 Completed**

**Project title:** Developing a statistical framework to study genotype and environment interactions in association studies

**Units managing the project:** DIAPC (Diversity and Adaptation of Cultivated Plants) (CIRAD, IRD, INRA, Montpellier SupAgro, UMII), Institute of Genomic Diversity, Cornell University, USA

**Project leaders:** Yves Vigouroux (yves.vigouroux(a)mpl.ird.fr)(DIAPC), Anne-Céline Thuillet (DIAPC) and Edward Buckler (Cornell University)

**Country involved in the project:** USA

**Sub-thematic axis:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

**Objectives:**

During the last five years, new promising methodologies have been developed to identify genes that play a significant role in plant adaptation: 1) methods allowing the identification of the signature of selection events in a high number of markers or genes 2) association detection between phenotypic variation and genetic polymorphism in structured populations, so called association studies.

Association studies offer new opportunities to assess the role of a particular gene on a phenotype. Compared to QTL analyses, which deal with the descent from two known parents, association studies have the advantage to take into consideration a much broader diversity, and to rely on a much more recombined material. The DIAPC laboratory is involved in the development of an association framework in pearl millet (*Pennisetum glaucum* L.) for the study of genetic basis of flowering time differences between varieties. The strategy relies on the observation of flowering time variability on the one hand, and of existing polymorphism on candidate genes on the other hand. Testing for an association between phenotypic and genetic variability requires appropriate statistical models. The current state of the art of the corresponding statistical framework allows taking into account the population structure and the existence of kinship in the population. To date, interaction terms of the model are not considered. However, genotype x environment and/or structure/kinship x environment interactions may exist, and may interfere with our ability to detect genotype-phenotype association. In addition, ignoring these interactions limits our understanding of complex traits expression.

The proposed research will lead to assess the possibility And /or limits to detect genotype x environment interaction in genetically structured populations.

**Action carried-out and results obtained:**

We developed a statistical model to perform association study taking into account genetic and environment interactions. A mixed linear model which uses population structure and kinship matrix to perform an association between phenotype and genotype was extended to take into account interactions with the environment. To assess the power of this new model, we simulated a thousand datasets showing a given genetic/environment interaction effect. The statistical model was then used to detect genetic/environment effect on these simulated datasets. This analysis helps understand when such interactions can be readily detected (Figure 1). Finally, we used the model on two real datasets (maize and pearl millet) and identified some flowering time gene showing significant interactions with the environment.

**Prospects for the future:**

The relationship between genotype and phenotype is a central question in biology. Taking into account gene/gene and gene/environment interactions is important to understand this relationship. One of the main challenges in the next years will be to adapt the model to process genome wide genetic data.

**Total Agropolis Fondation funding:** € 6,240 (travel expenses for a PhD student)

**Funding categorie(s):** Agropolis Fondation small grants (overseas travel grants for Doctoral and Post-doctoral scientists)

**Project duration:** 15 January 2009 – 31 December 2009

**Keywords:** statistics – genotype – environment – association studies

**Year of CfP: 2008**

**Project No 0803-017**

<b>Project title:</b> OpenAlea: Open software for plant modelling at different scales
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**Units managing the project:** AGAP (Genetic improvement and Plant adaptation) (CIRAD, INRA, Montpellier SupAgro) and LEPSE (Ecophysiology of Plants of Environmental Stresses) (INRA, Montpellier SupAgro)

**Project leaders:** Christophe Gaudin, DAP, EPI VIRTUAL PLANT INRIA (christophe.gaudin(a)inria.fr), Christophe Pradal, DAP, (christophe.pradal(a)inria.fr), Christian Fournier, LEPSE (christian.fournier(a)supagro.inra.fr)

**Countries involved in the project:** Canada, Germany, The Netherlands, USA

**Research units from the Foundation's scientific network involved:** PSH

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

**Objectives:**

Functional-structural plant models aim at simulating understanding the biological processes involved in the functioning and development of plants. This requires efficiently using and combining models or computational methods from different scientific fields in order to analyze, simulate and understand complex plant processes at different scales. Due to the different constraints and background of the teams, these models are developed using different programming languages, with different degree of modularity and inter-operability. In order to increase the interaction between these models, their reusability, the possibility to compare them on identical datasets, efficient and flexible computational frameworks are required.

The objective of the open source project *OpenAlea* is to provide an easy-to-use environment for plant modellers through a visual programming interface to efficiently using and combining models or computational methods from different scientific fields in order to represent, analyze and simulate complex plant systems at different scales, from meristems to plant canopy; and to understand processes occurring within such systems or between plants and their environment. Data and tools for the analysis, modelling and simulation of plants will thus be made available to plant scientists through an integrated software platform. Such a software environment is targeted not only for developers and computer scientists but also for biologists, which may be able to assemble models while minimizing the programming effort.

**Total Agropolis Fondation funding:** € 198,002 (salary for an engineer, computers, workshops)

**Funding categorie(s):** Agropolis Fondation grants for scientific platform

**Project duration:** 1 January 2009 – 31 July 2012

**Keywords:** platform – plant modelling – simulation – plant development

**Year of CfP: 2008**

**Project No 0803-021 Completed**

**Project title:** Model level integration for understanding and managing human influenced ecosystems at the landscape scale

**Units managing the project:** LISAH, Laboratory for soils, Agrosystems, Hydrosystems Interaction studies (INRA, IRD, Montpellier SupAgro) and AMAP, Botany and computational plant architecture (CIRAD, CNRS, INRA, IRD, UMII)

**Project leaders:** Xavier Louchart, LISAH (louchart(a)supagro.inra.fr) and Marc Jaeger, AMAP and EPI Digiplante INRIA (marc.jaeger(a)cirad.fr)

**Research units from the Foundation's scientific network involved:** DIADE, Innovation

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*), STDI-3 (Socio-Technical Dynamics of Innovation 3: *Innovation processes, social management of innovations*)

**Objectives:**

There is great need for scientific-based, user-friendly, game-like platforms for integrative landscape modelling, which would enable either scientists or multiple stakeholders to test their landscape design ideas in the light of economic, ecological and environmental targets. Such platform development needs to integrate knowledge from different disciplines in order to provide effective solutions for real-world problems. A few interdisciplinary studies have already been conducted related to integrative landscape modeling. They generally consisted in integrating social, economic, and ecological aspects for a sustainable development. It appears however that no study has been carried out on complete integrative landscape approaches including bio-physical, ecological and socio-economic processes.

One of the strongest barrier for discussing the concepts in interdisciplinarity or transdisciplinarity project is the lack of understanding. The notion of ontology in computer science is more and more advocated for representing the domain knowledge and supporting multi-disciplinary integration but few works have been carried out in this area.

We propose in this project to tackle the issue of integrative ecosystems & landscape modelling by first studying states-of-art in the various concerned disciplines, and by a prior analysis of the main conceptual gaps. In most projects concerned with the development of integrative landscape modelling, the approach was either to extend a prior model in a given scientific domain by coupling it with models from other domains, or to couple in a weak manner existing models in order to obtain an integrative model. In both cases, the developed modelling structures often lack genericness and/or capacity to represent accurately the main multidisciplinary interactions between processes. By starting prior to platform and model development, an in-depth evaluation of the interdisciplinary conceptual gaps and needs for designing an integrative landscape modelling platform, it is expected to overcome the above mentioned problems and to design the main desirable characteristics of a generic and shared platform.

This work will allow defining the conceptual basis of an integrative and interdisciplinary (socio-economy, agronomy, biology, ecology, hydrology...) approach, in order to further develop a modelling platform of "ecosystems & landscape functioning under human influence".

**Action carried-out and results obtained:**

There is definitely a great need for scientific-based, user-friendly, game-like platforms for integrative landscape modelling, enabling either scientists or multiple stakeholders to test their

landscape design and management ideas in the light of economical, ecological and environmental constraints. The development of such platforms needs to integrate knowledge from different disciplines in order to provide effective solutions for real-world problems. For this purpose, two projects were set up within the advanced thematic research network "Montpellier Agricultural Sciences and Sustainable Development", funded by Agropolis Foundation. The projects are entitled "Integrative landscape modelling" and "Towards a federative research on modelling and simulation platforms".

Their objective was to put together various teams, each of them having developed, with a specific scientific focus, its own model or platform for simulating landscape structure and functioning. The challenge is to manage to link these models and platforms in a general conceptual and modelling framework, enabling to consider the main biological, physical, geographical and socio-economical interactions impacting ecosystems and landscape functioning and management. These projects constituted a first step for all partners to share their background experience in their own discipline (ecology, agronomy, hydrology, socio-economy) and further try to define a roadmap for building an integrative modelling platform.

These two projects have been carried-out in very close cooperation, the first one focusing on thematic issues and the second one being its methodological counterpart. In consequence, the results of the two projects are presented together.

The projects started in February 2009 and ended in December 2010. During the time-course of the projects, three thematic workshops have been organized, implying a total of around 50 members of the participating research units. An international conference on integrative landscape modeling was also set up, with a worldwide-recognized scientific committee. The event attracted more than one hundred participants from all over the world. Fifty-two researchers presented their ongoing work and three round-table allowed discussions about hot topics of landscape modeling and simulation. In total, sixteen scientists were invited to present their work and feed the discussions.

As a result, the proceedings of the international conference were published, gathering thirty-seven scientific communications ([www.symposcience.org](http://www.symposcience.org)). A website was built-up to capitalize information and discussions generated by the projects (<http://www.umr-lisah.fr/rtra-projects/>). A state of the art of integrative landscape modeling was written, from bibliographic data and exchanges within the scientific community.

The projects allowed reinforcing the links both within the Montpellier-based research community and with the national and international scientific community (METISSE Network, Réseau National des Systèmes Complexes, Global Land Project, etc.). In addition, the outputs of the projects have been highlighted through various publications and communications towards the local, national and international communities around landscape modeling. Finally, the projects gave rise to several concrete research collaborations at various levels.

## **Publications**

Farolfi S., Müller J.P., Bonte B.. 2010. An iterative construction of multi-agent models to represent water supply and demand dynamics at the catchment level. *Environmental modelling and software*, 25 (10) : 1130-1148.

Gumiere, S., Raclot, D., Cheviron, B., Davy, G., Louchart, X., Fabre, J. C. & LeBissonais, Y., 2010. MHYDAS-Erosion a distributed single-storm water erosion model for agricultural catchment. *Hydrological Processes*, <http://dx.doi.org/10.1002/hyp.7931>.

Lagacherie, P., Rabotin, M., Colin, F., Moussa, R. & Voltz, M. Geo-MHYDAS: A landscape discretization tool for distributed hydrological modeling of cultivated areas. *Computers & Geosciences*, August. 2010, 36, 1021-1032.

**Total Agropolis Fondation funding:** € 60,125 (salary for an engineer, travel expenses, one international and 3 internal workshops organisation)

**Funding categorie(s):** Agropolis Fondation small grants (support for the organisation of high-level scientific events e.g. conferences, seminars)

**Project duration:** 1 November 2008 – 07 April 2011

**Keywords:** ecosystems – landscape – modelling – platform

**Year of CfP: 2008**

**Project No 0803-022**

<b>Project title:</b> Computational model for electrical signaling in plants in relation to polarized patterns of development and morphogenesis, and to adaptive responses to stresses (ELEXIGNAL)
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**Units managing the project:** BPMP (Plant Molecular Physiology and Biochemistry) (CNRS, INRA, Montpellier SupAgro, UMII) and University of Ottawa

**Project leaders:** Jean-Baptiste Thibaud, BPMP (thibaud(a)supagro.inra.fr), Anne-Gaëlle Rolland-Lagan Department of computer sciences, University of Ottawa (arolland(a)uottawa.ca)

**Countries involved in the project:** Canada, Germany

**Research units from the Foundation's scientific network involved:** GREEN, SYSTEM, LAMETA

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

**Objectives:**

Plant ion transport systems are well studied with respect to their role in plant mineral homeostasis and nutrition. But ion transport through cell membranes are intrinsically dependent on membrane potential and, in turn, do contribute to both the steady-state and the transients of membrane potential. Electrical signaling, which is mostly studied in animals (and especially in excitable cells) is not foreign to plants. Electrical signals do occur at both the plant cell and plant tissue levels and can even propagate at the whole plant level from one organ to another and participate in stress signaling. Electrical fields within plant tissues, in relation with the activity of ion transport systems and with propagation of electrical signals, are also believed to be involved in the control of polarized patterns of growth, development and morphogenesis. The molecular basis of these peculiar aspects of plant physiology is as yet poorly understood.

An increasing number of plant ion transport systems have been identified at the molecular level and their individual biophysical characteristics have been described in detail. Particularly, a number of voltage-dependent ion channels and other ion transport systems have been identified in plant cells which are analogous to animal counterparts responsible for electrical signaling in excitable cells. It is now important to collect quantitative data on ion transport and electrical signaling, and to use these data as a basis for developing simulation models of the control of cell membrane potential.

The aim of this project is:

- to implement and validate a mathematical model for the integration, at both the cell and tissue scales within the plant, of the individual contributions of ion transport systems to the steady-state and transients of the cell membrane potential
- to explore, describe and demonstrate the role of electrical signals in the polarization of development and morphogenesis and in stress signaling within plants (especially water and biotic stresses)

**Total Agropolis Fondation funding:** € 266,760 (salary for a Junior Fellow for three years, travel expenses, running costs)

**Funding categorie(s):** Agropolis Fondation Junior Fellowship

**Project duration:** 1 January 2009 – 30 June 2012

**Keywords:** electrical signaling – computational modelling – development – morphogenesis – stresses – ion channels

**Year of CfP: 2008**

**Project No 0803-024 completed**

**Project title:** Development of a tool box for the modelling and simulation of retroactions between plant growth and plant biomechanics

**Units managing the project:** AMAP, Botany and computational plant architecture (CIRAD, CNRS, INRA, IRD, Montpellier Supagro, UMII), LMGC CNRS, Laboratoire de Mécanique et Génie Civil

**Project leaders:** Thierry Fourcaud, AMAP (Thierry.fourcaud(a)cirad.fr), Tancrede Almeras LMGC

**Countries involved in the project:** China, UK

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

**Objectives:**

The response of plants to mechanical stress is of major interest in number of research fields. Despite many experimental investigations already carried out on this phenomenon, the underlying physiological mechanisms are still poorly understood. This project aims at developing a library of biomechanical tools devoted to the simulation of retroaction processes between plant growth and its mechanical state at the individual scale.

This library will be developed in Java programming language (compatible with current growth models and platforms that are developed in other projects) based on previous studies carried out by the involved teams. It will be designed in order to allow the interactions with plant growth simulators to be easily performed via

- The stepwise extraction of structural information
- The stepwise modification of growth parameters (e.g. maturation strains, branching probabilities, sinks of assimilates, allometric rules) with regards to the current mechanical state of the plant.

The biomechanical model will be based on the beam theory and the solver derivate from the Finite Element Method. The toolbox can be split into several independent modules (mesh, beam section, material properties, biomechanical pre-stresses for woody plants, assembly of the stiffness matrix, assembly of the force matrix, solver of linear and non-linear systems for static and dynamic problems, field and history outputs, manager of cumulated field output, connections with plant growth parameters), which could be developed separately by different people.

**Action carried-out and results obtained:**

The project was divided into 4 main steps:

1 - Definition of the structure of the library: it was mainly to distinguish the different core modules (reference materials, beam cross sections, mesh, loads, scheduler, solver, post-GPU ) and associated data structures (representation of the topology, list of applied constraints, list of events, functional links between the different modules).

2 - Implementing the library under the platform Scilab, evaluation tests and coupling with the plant growth simulator GreenScilab (<http://liama.ia.ac.cn/wiki/projects:greenscilab:home>). The Scilab toolbox is operational and can calculate the bending of growing stems as well as the field of mechanical stress (see internship report of Miss Yang Ming, Ecole Centrale Pékin), through the implementation of the transfer matrix method. It was used to evaluate the risk of tree breakage within a forest stand submitted to strong winds, according to the allometry of each individual. This

work was conducted in partnership with the Department of Wood Science and Forestry, Faculty of Forestry, Geography and Geomatics, Laval University, Quebec, Canada;

3 - Development of the library in Java and of a "stand alone" simulator with a proper graphical user interface (see report of William and Benjamin Surroca Train, and site development <http://amap-dev.cirad.fr/projects/plantbiom/wiki>).

4 - Interfacing with the software XPLO (<http://amap-dev.cirad.fr/wiki/xplo>), which is devoted to the representation of plant architecture, via the data structure Architree? This work is still underway as part of a TER M1 internship at the University of Montpellier 2. Meanwhile, the library PlantBioM was translated in C language and implemented within the simulation platform for plant growth Qing Yuan, developed by partners LIAMA in Beijing (see visit of Dr. Mengzhen Kang and internship of Mr Haoyu Wang).

A modeling work has been carried out ahead of this development work, as part of Thomas Guillon's PhD thesis. It was proposed a new theoretical framework to explicitly calculate the mechanical response of a growing beam by defining a virtual reference configuration. An article describing this framework and giving examples of calculation has been submitted to the journal "Biomechanics and Modeling in Mechanobiology".

#### **Prospects for the future:**

The project PlantBioM continues in the form of a MASTER internship in Computer Sciences, which would achieved the integration of the library in the software XPLO.

The prospects in the short and medium term are:

- Implementing Thomas Guillon's results in a new PlantBioM solver. An algorithm has already been developed in Java and tested for solving equations of biomechanics;
- Provide a tool for training and expertise for the management of urban trees, based on the software XPLO-PlantBioM. This tool will allow testing the impact of different types of intervention (pruning, bracing, etc ...) on the tree mechanical factor of safety.
- The above point should also be developed with the METLA (Finland) and the University of Gottingen (Germany). A thesis proposal is being drafted and should be submitted in 2011 as part of an ITN European project supported by INRA on Virtual Plants.
- The PlantBioM toolbox would be used as part of research partnerships with LIAMA, China, if the cPlant project (EU INCO-LAB) submitted in early 2011 is accepted. This research includes modeling the architectural plasticity of plants and in particular dynamic feedbacks between bending of stems and development of branching.

**Total Agropolis Fondation funding:** € 21,330 (travel expenses)

**Funding categorie(s):** Agropolis Fondation small grants (support for exploratory, risky and innovative projects)

**Project duration:** 1 November 2008 – 31 December 2009

**Keywords:** modelling – simulation – plant – biomechanics – growth

**Year of CfP: 2008**

**Project No 0803-027**

<b>Project title:</b> 3D virtual fruit – application to the control of fruit growth and quality
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**Units managing the project:** PSH (Horticultural Plants and culture Systems)(INRA), INRIA

**Project leaders:** Nadia Bertin, PSH (nadia.bertin@avignon.inra.fr) and Christophe Godin, EPI Virtual Plant INRIA (christophe.godin(a)inria.fr)

**Country involved in the project:** Poland

**Sub-thematic axes:** IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

**Objectives:**

Appearance (size, shape and color), taste (determined principally by sugar and acid contents), and texture are main components of the perception of fresh fruit quality. Fruit size and composition depend on cell division, cell expansion (driven by the cell turgor pressure and cell wall properties) and water and carbon accumulation and partitioning, some processes already described in ecophysiological models. Fruit texture, for which no model exists, depends on the rheological properties of tissues which rely on their structural units, including cells, cell walls and conducting vessels. Despite the identification and modelling of many individual processes, the control of quality on its whole is still poor because it is orchestrated by a variety of physiological interacting processes temporally and spatially organized during fruit development. This organization is not or only partially described in current fruit models and cross-talks among the different scales described in the fruit system are insufficient.

This project will gather the competence of plant modellers, physicists and ecophysicologists to build-up a spatial and temporal model describing physical and biological laws involved in tissue differentiation and cell growth, in relation to fruit growth and compartmentation, and a number of related traits of quality (e.g. size, composition and texture). Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI) techniques will be employed so as to provide an *in vivo* validation of the model by non invasive measurement.

This 3D model will account for the fruit compartmentation into differentiated tissues and for the cellular organization of main tissues. Such a model will be a powerful tool to analyze and understand complex interactions at the cell, tissue and fruit levels, by coupling different levels of control and in particular by describing the mechanical constraints associated with the development of different tissues and with cell division and expansion in a given tissue. In the future, the integration of other processes or signals will provide an accurate description of the fruit functioning. This new multidisciplinary approach in fruit modelling is expected to provide with new hypotheses to better understand and manage fruit quality and with new methods to assess it *in vivo*.

**Total Agropolis Fondation funding:** € 218,141 (postdoctoral fellowship, travel expenses, equipment, running costs)

**Funding categorie(s):** Agropolis Fondation Post-doctoral fellowship

**Project duration:** 1 January 2009 – 30 April 2012

**Keywords:** virtual fruit – fruit quality – fruit growth – modelling