Aphicibles — SYMBIOSIS, DIGESTION AND REPRODUCTION AS APHID PHYSIOLOGICAL PROCESSES TO IDENTIFY NEW TARGETS FOR INSECTICIDES

Genoplante 2007, ANR-07-GPLA-002

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Aims

Transcriptomic identification of key genes in aphid biology (reproduction, symbiosis, sex cycle determinism)

Aphids are one of the most important groups of insect pests. Present chemical methods of control have important resistance and ecological toxicity drawbacks, thus inducing urgent research needs for alternative control strategies. This project has gathered three types of approaches, each studying the effects, on the physiology of the pea aphid *Acyrthosiphon pisum*, of:

i) **toxins** recently identified from diverse origins (bacterial origin; with the Cyt-like toxins of the typical phytopahogen *Dickeya dadantii* –syn *Erwinia chrysanthemi*- and plant origin with the A1b-like albumins from legume seeds).

ii) **nutritional deprivations**, typically implying essential amino acids synthesized by the obligate bacterial symbiont of aphids

iii) **hormones** or abiotic signals regulating sexual reproduction in the pea aphid species

These effectors and environmental stresses do target essential functions in aphids, such as the gut barrier, the *Buchnera aphidicola* symbiosis and the seasonal cyclical parthenogenesis. Our studies have therefore had the objective of characterizing the effects of such aphicidal toxins (A1b albumins and Cyt toxins from *D. dadantii*), as well as the identification of genes specifically implied in the cited biological functions, which are characteristic of aphids, of their phloem-restricted feeding habit and of their mode of reproduction alternating sex production and parthenogenesis.

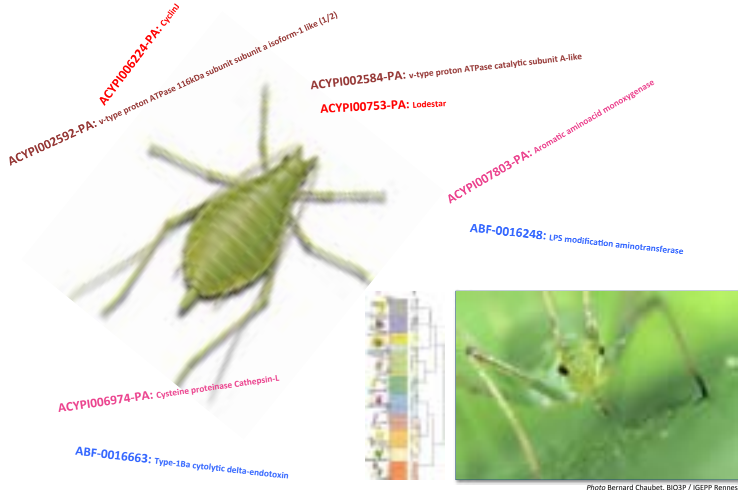

Photo Bernard Chaubet, BIO3P / IGEPP Rennes

Methodology & Results

The central methodologies used were based on transcript analyses of aphids (submitted to environmental or trophic stresses) and their interacting partner (plant, or bacterial pathogen). RNAi was also used to validate key gene functions and knock-out phenotypes in *Acyrthosiphon pisum*.

The pea albumin toxin PA1b was shown to be toxic for many clonal populations of the pea aphid *A.pisum*, including for members of the pea host-race. The Cyt-like toxins of *Dickeya dadantii*, homologous in sequence and structure to the cytolytic toxins of *Bacillus thuringiensis*, were shown to be one among many of the insect virulence factors harboured by this enterobacterial phytopathogen. These toxins are expressed mainly in the digestive tract of the invaded insect and are thought to help crossing this first barrier. Regulation networks of virulence within an insect host were globally found to be antagonistic to the ones governing plant virulence.

In the *Buchnera* symbiosis of aphids, plasmidic genes for leucine biosynthesis were shown for the first time to have a high and dynamically regulated transcriptional response, scaling biosynthesis to the external leucine excess or depletion, showing that the adaptive regulation of gene expression is conserved in a highly reduced genome. In the aphid host, the analysis, in single individuals, of tissue-distribution of gene knock-down after RNAi treatment (against a cathepsin-L target) revealed gene inactivation and phenotypes that were specific to the administration method (through ingestion with a main digestive target; through injection with a main moulting/cuticular target).

The first coupled transcriptomic/proteomic study of sex induction in aphids, relying on the recent annotated sequence for the pea aphid, have pinpointed an original response of cuticular metabolism, potentially associated with neuromediators acting on the autumn switch of reproductive mode (Illustration 2). Finally, the transcriptomic and *in situ* hybridization analyses of the embryonic development of sexual *vs* asexual phases have identified candidate genetic programs linked to cell division, and to the post-transcriptional and epigenetic regulations within the stem cells of ovocytes.

Publications:

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Conclusions - Perspectives

The Aphicibles project generated original and published data in most of its original thematic objectives: demonstration of original bacterial toxin activity (toxins recently imported by horizontal transfer) and their integration in a network of regulation of the recipient bacterium (Costechareyre *et al.*, 2010). In the same way, molecular analysis of signal transduction pathways of the determinism of sexual morph induction in the pea aphid pointed several groups of new actors in this process, such as the mobilization of cuticular proteins, a core CNS signalling pathway, an insulin-like endocrine signal transduction pathway (Le Trionnaire *et al.*, 2009) and genetic programs of early oogenesis. Finally, aphid symbiosis was first proved to respond early and dynamically to leucine stress by a fast mobilization of its symbiotic bacterium *Buchnera* plasmid gene expression (Viñuelas *et al.*, 2011). It is the first evidence of a strong and specific regulation to the stress imposed on the host in a bacterium with reduced genome, implying selective pressures on this regulatory trait (counteracting the genetic drift driving such genomes' evolution). Moreover, the conditions of use of the gene silencing by RNAi were analysed systematically across various individuals and tissues in the pea aphid; this helped to define correlations in efficacy between method and tissue expression. Interesting phenotypes on larval molts and cuticular targets was highlighted for the control gene used (the gene encoding the lysosomal enzyme Cathepsin L).

Overall, this work opens new avenues in terms of both targets and mechanisms of functional targeting in aphids and similar sap-feeding insects.

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BrassiNAM

Development of a **N**ested **A**ssociation **M**apping population for complex traits dissection in ***Brassica napus***

ANR-10-GENM-001

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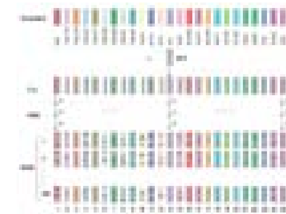
Context

Oilseed rape is the main oil crop cultivated through Europe grown on nearly 1.5 million hectares in France in 2007 (Agreste Infos, April 2008), producing around 4.6 million tonnes of seed, with a worldwide production in 2007 of around 47.6 million tonnes. Recently the demand for arable products and vegetable oil in particular, has increased dramatically. This is mainly due to the growing world population and the related demand for food as well as to the increasing demand for bioenergy. At the same time inputs need to be reduced for both economic and ecological reasons, creating yet another challenge for the farming community and the crops they are growing. To meet these challenges, a large improvement of the genetic potential of the oil crop species is required, which is impossible without strong support of the breeding process.

Winter OSR crop yields in main EU growing countries over the last 40 years. (Sources: Eurostat / Agreste / UFOP / DEFRA)

Objective

The objective of theses project is to develop a NAM population in order to dissect complex traits, as yield, in winter oilseed rape. The concept of NAM population is to join the genome wide QTL detection power of linkage analysis, and more particularly issued from multiple lines cross, to the high resolution provided by association studies. The NAM population consists in multifamily Recombinant Inbred Lines (RIL). A set of founder lines is defined as potentially source of different alleles. Each founder line is then crossed to the same pivotal line and a recombinant inbred line population is built for each cross. The subsequent generations of progeny of the crosses can then be used as association populations with a better mapping resolution than in bi-parental population. The originality of the method in NAM, is that the nucleotide polymorphisms within tagging SNPs can be tested directly because high-density SNPs on founders is available and this information can be projected onto the progeny through the flanking Common-Parent-Specific (CPS) SNPs. The NAM construction scheme also avoids biais linked to population structure that could lead to false positive in association mapping.



NAM Principle from Yu et al., 2008

Description

The project contain 5 work packages :

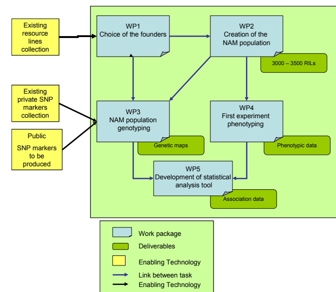
WP1 : selection of founder lines and the pivotal genotype in order to maximize the genetic diversity within the NAM population.

WP2 : development of a large collection of 3000 to3500 RILs connected, well adapted to agronomical experiment to obtain accurate result of phenotyping.

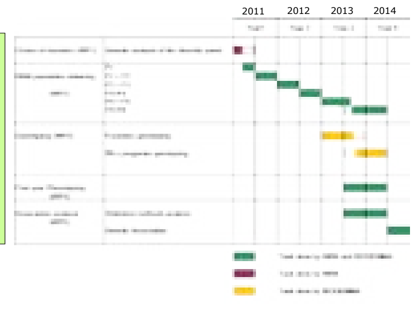
WP3 : genotyping of the whole population (Founders and RILS) using SNP markers

WP4 : first round of phenotyping experiment to validate if the population is well adapted to detect numerous QTLs.

WP5 will evaluate statistical procedures, already existing or under development, to exploit the NAM population created.

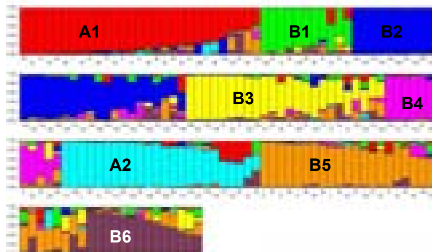


Workflow



Schedule

Results



Structure of *Brassica napus* genetic diversity obtained using a set of 50 SSR well spread over the genome (COREBRAS project, funded by PROMOSOL) A collection of 280 accessions was considered. A1 group gathered spring oilseed rape; A2 groups gathered exotic accessions, originated mainly from Japan and China. B groups are representative of winter type. B1 to B6 groups mainly differed by the accession origin (France and Germany for B2, eastern Europe for B6), or by erucic acid and glucosinolate profiles ("++" for B1, "00" for B2, B3,…).

Group	Type	Line	Cross	Sowing F1	F2>F3	F3>F4	F4>F5	F5>F6
A1	Spring	Tower	INRA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
A1	Spring	Crésor	INRA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
Australien	Spring	Groose	INRA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
B1	WOSR	Sarepta	INRA	Oct-2009	Dec-2010	Juil-2011	Jan-2013	Sept-2013
B2	WOSR	Bristol	INRA	Oct-2009	Dec-2010	Juil-2011	Jan-2013	Sept-2013
B2	WOSR	Tosca	INRA	Oct-2009	Dec-2010	Juil-2011	Jan-2013	Sept-2013
B3	WOSR	Express	INRA	Oct-2009	Dec-2010	Juil-2011	Jan-2013	Sept-2013
B5	WOSR	Quinta	INRA	Oct-2009	Dec-2010	Juil-2011	Jan-2013	Sept-2013
A2	WOSR	Norin9	BGA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
B1	WOSR	Jet-Neuf	BGA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
B2	WOSR	ES-Astrid	BGA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
B2	WOSR	Mohican	BGA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
B4	WOSR	Jupiter	BGA	Juil-2011	Mars-2012	Oct-2012	Mai-2013	Dec-2013
B5	WOSR	Lembkes	BGA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013
B6	WOSR	Bolko	BGA	Dec-2010	Juil-2011	Mars-2012	Jan-2013	Sept-2013

Design : All the 15 lines have been crossed with the pivotal line Aviso a « 00 » wosr from the B3 group with a good agronomic value. Crosses are done in greenhouse

Conclusions and perspectives

The BRASSINAM project is ongoing according to the schedule and the all NAM population will be used in the recently fund RAPSODYN project (Biotechnologies et bioressources-2011) in order to dissect the genetic determinism of rapeseed yield under nitrogen constraints.

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CEREALDEFENSE

Transgenesis and genomics for a better understanding and use of pathogen resistance in rice and wheat

Génomique et Biotechnologies Végétales 2010

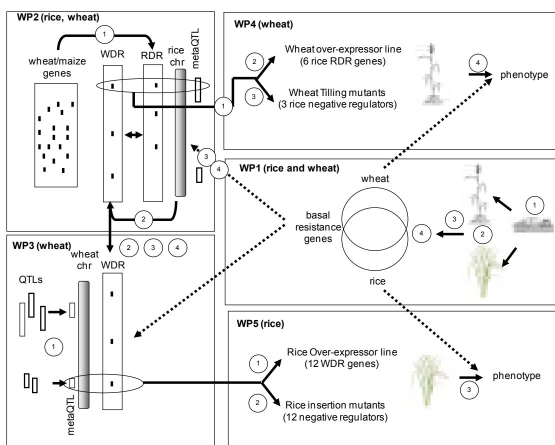
ANR

Coordinateur: Jean-Benoit MOREL, INRA-Montpellier

Partenaires: Biogemma et INRA

Project's objectives

Because it is scarcely understood, resistance to biotic stress is often misconsidered in the breeding programs. Biotic stress plant breeding of non-model crop species like wheat is still limited by our insufficient knowledge in these systems. In contrast, large investments were done in the past decade in the model crop specie rice in order to identify key components of disease resistance in cereals. We are now at the stage where several important genes required for disease resistance have been identified in rice and could be used in different cereals. Conversely, functional validation of wheat candidate genes remains tedious in these species and rice seems to be a good alternative for this validation steps. Finally, exploring basal immunity against fungal pathogens in cereals has probably been limited by our limited knowledge on the pathogen side.



The 5 main objectives of this project are:

Workpackage 1- Identifying basal resistance candidate genes by wheat and rice transcriptome analysis

We will compare rice and wheat transcriptome in order to identify components of basal resistance in both species. For this purpose, we will use pathogen mutants affected in the early steps of infection and/or in secretion of effectors. This should provide information on basal defense systems that are usually masked by other defense systems.

Workpackage 2- Transferring knowledge on disease resistance between rice and wheat. We will convert all information available in genes required for disease resistance in rice into operational markers for wheat by identifying the wheat putative orthologs. This should provide for the first time the comparative repertoire of disease resistance regulators cereals

Workpackage 3- Validating wheat orthologs of rice disease regulator (RDR) by association mapping. We will assess the involvement of wheat putative orthologs in pathogen response through development of molecular markers and association mapping with known pathogen resistance QTLs.

Workpackage 4- Improving wheat by using RDR genes. We will transform wheat with up to 6 positive regulators of disease resistance found in rice. This will be the first large scale demonstration of the use of model crop for improvement of disease resistance in wheat.

Workpackage 5- Improving rice by using wheat disease regulator (WDR) genes. We will validate in rice up to 24 candidates for disease regulators found in wheat in previous work and WP1. This will provide functional information on wheat candidate genes for disease resistance.

Methods and Results

WP1- We have analyzed the transcriptomic response of rice to two *Magnaporthe oryzae* mutants M1 and M2 unable to infect rice. For *M1*, there are preliminary data suggesting that this mutant may over-induce basal defense while for *the M2* our preliminary data suggest that the expression of several small secreted proteins is impaired in the early infection process. For *M1*, the data indicate that this mutant triggers over-induction of chitin-induced genes. Consistently, we further showed that the *M1* mutant can infect the rice chitin-receptor mutant *cebip*. Using *M2*, we could identify 1703 rice genes. Among them, we identified an ABC transporter that is induced more by wild-type *Magnaporthe* strain than by the *M2* mutant. KO plants for this gene were produced and show enhanced levels of resistance. This suggests that this ABC transporter gene could be a susceptibility factor. Overall, the expression of 25 rice genes could be validated by QRT-PCR.

WP2- From wheat to rice: we have expertized a set of 365 wheat probes that show expression levels before infection correlated or anti-correlated with resistance levels to *Fusarium* (a phenomenon called preformed defense). Among these genes, we found an homolog of the rice 33 kDa gene that was known by P1 to have a similar pattern. Rice plants over-expressing the rice Os33kDa gene showed enhanced resistance to *Magnaporthe*. Another gene, involed in amino acid metabolism, was shown to display an expression pattern anti-correlated with *Fusarium* resistance. About 20 SNPs were developed using this gene list ("preformed defense" wheat genes).

From rice to wheat: a set of more than 60 known regulators of disease resistance in rice were converted into putative wheat orthologs. For about 35 of these, SNPs were produced and 25 were mapped in wheat.

WP3- The 55 SNPs developed in WP2 were used for association genetics' studies in wheat using the Biotech panel. Ten of these SNPs showed significant association with *Fusarium* resistance, among which the chitin receptor CEBIP and a putative K-transporter.

WP4- Three genes that were previously shown in rice to be involved in disease resistance were over-expressed in wheat. Plants silenced for the amino acid metabolism related gene are being produced.

WP5- Besides the KO line for the rice ABC transporter, we started the analysis of three rice mutants for the orthologous copy of the amino acid metabolism related gene. Four other genes (a nodulin, a PDR transporter and two transcription factors) identified in WP1 were mutated with insertion lines.

Conclusions and perspectives

WP1- For wheat, a similar approach than in rice will be undertaken. For that purpose, we are producing the *M1* and *M2* mutants in *M. oryzae* strains that are able to infect wheat. We will also explore the preformed defense phenomenon in wheat leaves using an efficient strategy developed in rice. For rice, a subset of the 25 genes validated will be used for over-expression and/or KO analysis in rice and wheat.

WP2- Genes differentially expressed in wheat upon *Mycosphaerella graminicola* (from TWIST project) were brought to the project and will be analyzed. A set of more than 12200 wheat probes (from P1's in-house analysis) will also be added to the project.

WP3- This WP is almost finished.

WP4- Wheat over-expressor lines will be phenotyped for fungal resistance.

WP5- Insertion lines will be evaluated for *Magnaporthe* resistance. Over-expression rice lines will be produced with genes identified in WP1.

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Chloro-types
Chloroplast adaptation to abiotic stresses: use of proteomics to reveal molecular phenotypes

Programme: Génomique Végétale. Edition 2010

Coordinateur: **Laboratoire de Physiologie Cellulaire & Végétale, CNRS UMR 5168 / INRA UMR1200 / CEA / Université Joseph Fourier, Grenoble**
James Connorton, Gilles Curien, Elisa Dell'Aglio Giovanni Finazzi, Cécile Giustini, Marcel Kuntz, Michel Matringe, Stéphane Ravanel, Daniel Salvi, Daphné Seigneurin-Berny, Maritino Tomizioli and Norbert Rolland

Partenaire : **Laboratoire Biologie à Grande Echelle (équipe EDyP), CEA / INSERM U1038 / Université Joseph Fourier, Grenoble**
Claire Adam, Christophe Bruley, Sabine Brugière, Florence Combes, Véronique Dupierris, Alexandra Kraut, Christophe Masselon, Yves Vandenbrouck, Jérôme Garin and Myriam Ferro

Aims of the project

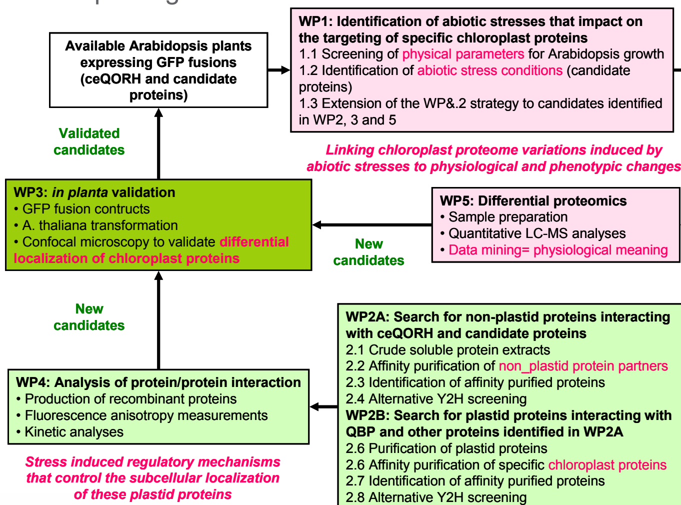
Most chloroplast proteins contain a N-terminal transit peptide that is lost upon import into the organelle. Among the components of the chloroplast envelope, we identified the ceQORH protein which contains a central transit sequence which is not cleaved during the import of the protein into the chloroplast [1,2]. Study of ceQORH revealed a previously unknown mechanism controlling protein trafficking between the cytosol and the chloroplast. In that context, the Chloro-types project aims to:

- Identify abiotic stress affecting the targeting of some chloroplast proteins,
- Determine regulatory mechanisms induced by abiotic stress and controlling sub-cellular localisation of some chloroplast proteins,
- Understand how these regulatory mechanisms, induced by stress, affect chloroplast physiology,
- Use large-scale proteomics approaches to analyze the chloroplast proteome in identified stress conditions.

Chloro-types (ANR N° 2010-GENOM-BTV-002-02) is a 4-year project (01/2011 – 12/2014).

Methodologies and Results

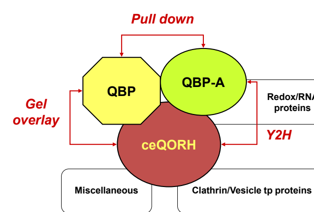
The Chloro-types project has been designed according to 5 work packages.



Current status and main results

WP1: Set up of a system which allows both the observation of the GFP-ceQOR fusion and the measurement of the redox status of the chloroplast (likely to be involved in the differential subcellular localization of some plastidial proteins).

Gas tight chamber mounted on a microscope slit to control gas composition. The set up is compatible with spectrophotometric (assessment of the redox status), and confocal microscope (visualization of sub-cellular protein localization) measurements



WP2: 1- Identification, by proteomics, of ~200 proteins interacting with QBP, a protein which controls the subcellular localisation of ceQOR. 2- Identification, by Y2H, of ceQOR partners including the QBP-A protein which interacts with both ceQOR and QBP.

Conclusions and perspectives

Proteomics and Y2H experiments have allowed the identification of proteins likely to be involved in protein trafficking between the cytosol and the chloroplast. Ongoing and future experiments are now aimed at deciphering the role of some of these candidates.

Future work

WP1: Screening of alternative stress conditions on Arabidopsis growth and subcellular localization of the proteins of interest.
WP2: Identification of new proteins interacting with ceQOR and other proteins that interact with QBP (proteomics, Y2H). Publication of present results of WP2.
WP3: *in planta* validation of the interaction of some selected proteins (identified in WP2)
WP4: Production of the QBP-A protein to raise antibodies and to perform its functional characterization.
WP5: Differential proteomics using chloroplast sub-fractions of Arabidopsis grown in various stress conditions (use of the AT_Chloro database [3]).

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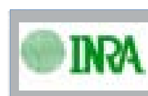

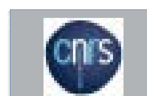

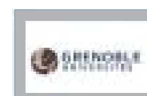
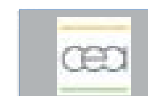

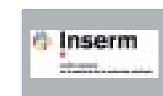

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DURAVITIS

The developmental, molecular & genetic bases of grapevine adaptation to thermal stress

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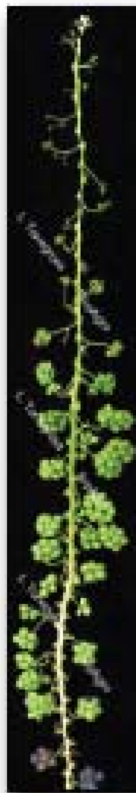
ANR
Genom-BTV 2010

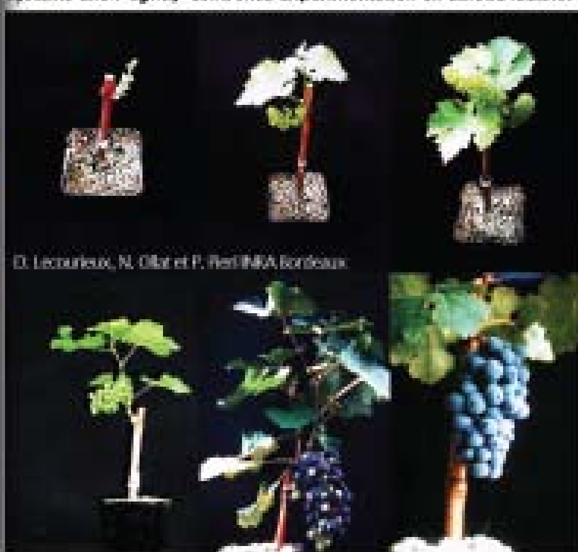
Project position

In the long term, breeding is the more relevant strategy to mitigate the effect of global warming on vine industry. To identify traits of tolerance to heat, the project aims :

1) Model T° effects on plant development
2) Parameterize the effects of C flow variations induced by heat on berry respiration, growth & metabolite accumulation
3) Diversity study to map QTL of adaptation



The project uses 2 original models: i) the microvine (Chaib et al., 2010) which exhibits a dwarf stature & continual flowering (DRCF) and ii) fruiting cuttings (Mullins and Rajasekaran, 1981), both systems allow tightly-controlled experimentation on abiotic factors.



Methods & results

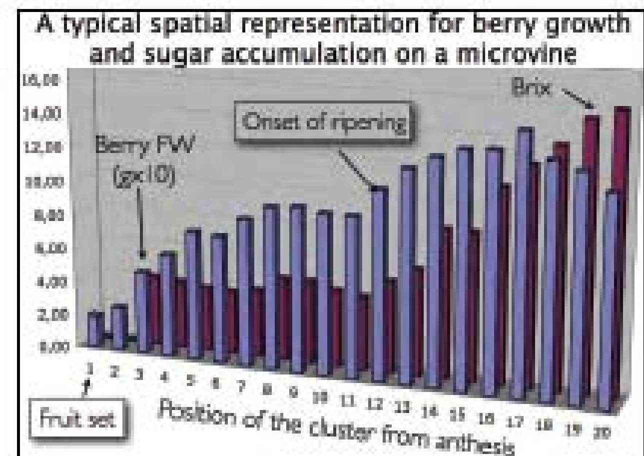
1.1. Microenvironmental stresses: Elevated T° (+8°C) was applied to clusters of Cabernet-Sauvignon at 3 stages (middle-green, veraison, ripening).

Expression patterns of genes of the flavonoid pathway revealed strong differences, depending on the developmental stage of the berries. However, these expression profiles did not fully explain the dramatic reduction in anthocyanin and flavonol concentrations, suggesting additional regulation processes such as post-translational modifications.

A transcription factor associated to thermal stress in the berry was identified and its functional characterization has been started.

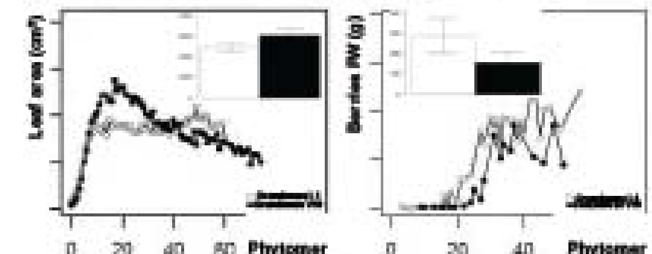
1.2. Whole plant stresses: The possibility to substitute a spatial gradients of vegetative and reproductive development of DRCF genotypes for temporal development at one phytomer was evaluated for 450°C.d^{-1} under 2 T° charts (12°/22°C vs 20°/30°C n/d).

A typical spatial representation for berry growth and sugar accumulation on a microvine



First developmental phenotypes associated with elevated T° were: decrease of plant fresh weight, inflorescence and berry numbers while leaf area was increased. At metabolic level: tartrate was not impacted while kinetic of malate accumulation was deeply modified.

1.2. Genetics of adaptation traits: Several crosses were started with genotypes whose sequencing is underway. Phenotyping of the DRCF progenies revealed a segregation for fruit load, berry volume, organic acids and II^{ary} metabolites (anthocyanins & tannins).



Perspectives

The specific response of berries to localized thermal stresses will be performed through a combination of transcriptomic (30K), proteomic (ITRAQ) and metabolomic analyses (UPLC-MS-MS).

At plant level, developmental and metabolomics traits will be further investigated and patterns of tannin accumulation will be established. Transcriptomic (30K) of reproductive organs (anthesis-ipening) will be analysed under thermal stresses.

The phenotyping of DRCF progenies will be completed to identify QTLs for physiological traits that are potentially impacted by T°. Genotyping will be done with the new grape Illumina chips (20K SNP).

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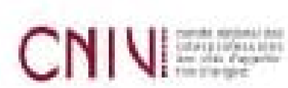
Partnership: UMR AGAP, UMR SPO, UMR LEPSE & UMR EGFV
Co-funding: INRA GAP-EA, SupAgro, Fondation Poupelain, CNIV



DELICAS Association mapping and model phenotyping for the characterization of molecular markers associated with sugarcane yield formation and limitation

Génomique végétale 2009

ANR AGENCE NATIONALE DE LA RECHERCHE

Coordinateur : CIRAD, UMR PVBMT
Partenaires : eRcane, CIRAD UMR AGAP, CIRAD UR SCA

Objectives of the project

The DELICAS project aims at identifying molecular markers associated with genes involved in the elaboration of sugarcane yield or in resistance to some pests or diseases. Two main innovative strategies will be used to achieve this objective. First, the elaboration of yield will be decomposed into elemental processes using two ecophysiological models, Mosicas and EcoMeristem. Second, phenotype–genotype association will be studied within an 180 international cultivar core collection rather than within biparental progenies.

Methodology and Results

Methods and tools for model assisted phenotyping
Elaboration of method and tools for model assisted phenotyping is based on morphogenesis data collected in one field trial comparing two contrasted cultivars and two field trials comparing 20 cultivars in two locations.
The adaptation to sugarcane of the model EcoMeristem and the coupling with an optimization module devoted to parameter estimation has been completed. The elaboration of statistical methods for estimation of model parameters using generalized least squares is under progress.

Phenotyping of the core collection
Two 1.5 ha field trials were planted in two contrasted environments to record growth and development data on the core collection. The resistance to the viral Yellow Leaf Disease and to its aphid vector *Melanaphis sacchari*, has been quantified in a field trial.

Genotyping of the core collection and identification of marker-trait associations
3,307 AFLP and DART markers have been scored in the core collection
First analyses of marker-trait association were performed with Yellow leaf and yield components data. Although PCA revealed no stratified structure within the panel, the cryptic structure described by the Principal Components explained a significant part of the phenotypic variation. Detection of marker-trait associations is under progress.
The fine mapping of two resistance genes, *Bru2* (resistance to brown rust) and *Ryl1* (resistance to SCYLV), has been carried out through exploitation of sorghum – sugarcane syntheny. Densification of markers in the target regions was attempted using SSAP and TRAP. SSAP was unsuccessful, but the test of 308 TRAP markers allowed the identification of 17 and 5 markers segregating respectively with *Bru2* and *Ryl1*.

Conclusions and prospects

During the last year of the project, the planned activities are:
- completion of the methodological studies on model assisted phenotyping (test of EcoMeristem optimization module, statistical methods)
- estimation of model parameters for the core collection,
- analysis of marker-trait association,
- continuation of the fine mapping of *Bru2* and *Ryl1*,
- elaboration of a module for the integration of the results of the project in the web database TropGene.

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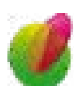


EFG-MIG

Evolutionary and Functional Genomics of Modified Indole Glucosinolate Biosynthesis

ANR-2010-GENOM-BTV

AGENCE NATIONALE DE LA RECHERCHE ANR

Juergen KROYMANN
Bertrand GAKIÈRE, Marina PFALZ
With contributions from Marine PAUPIÈRE and Maisara MUKHAIMAR

Objectifs

The glucosinolate-myrosinase system is an activated defense system in the model plant *Arabidopsis thaliana* and related species from the order Brassicales. This system protects plants effectively from most herbivorous insects and other enemies. It relies on the generation of toxic effector molecules from biologically inactive precursors upon enemy attack. While the basic functional principle of this activated defense is simple, the system itself is nonetheless extremely complex and displays an enormous amount of structural and regulatory variation within and among species. This project pursues the following aims:

1) Identify the genes that control structural and quantitative variation in indole glucosinolate biosynthesis with quantitative genetic approaches

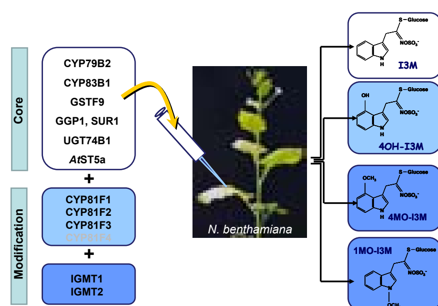
2) Understand the mechanistic role of these genes and their gene products with tools from molecular biology and biochemistry

3) Investigate the potential ecological impact of these genes in plant-enemy interactions

4) Decode the evolutionary trajectory of these genes by analyzing patterns of genetic variation within *Arabidopsis thaliana* and among close and distant relatives, utilizing comparative genomics and statistical methods from molecular population and evolutionary genetics

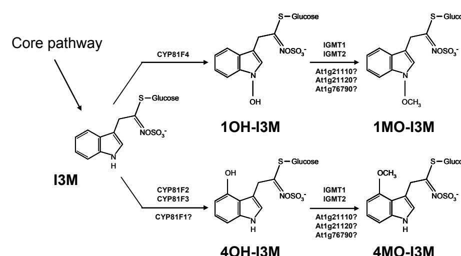
Methods and Results

We have disentangled the function of all four members of the *CYP81F* subfamily in *Arabidopsis thaliana* using Arabidopsis mutant lines and a transient *Nicotiana benthamiana* expression system in which we engineered the entire core pathway for Arabidopsis indole glucosinolate biosynthesis.



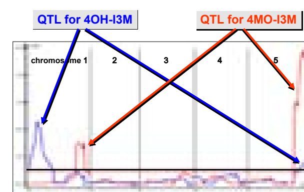
All *CYP81F* gene products catalyze the hydroxylation of indole-3-yl-methyl glucosinolate (I3M) towards hydroxy-indole-3-yl-methyl glucosinolates.

CYP81F1, F2, and F3 carry out the hydroxylation at position 4 of the indole ring, leading to 4-hydroxy-indole-3-yl-methyl glucosinolate (4OH-I3M), while CYP81F4 hydroxylates at position 1, leading to 1-hydroxy-indole-3-yl-methyl glucosinolate (1OH-I3M). Moreover, we have identified an additional gene family which is involved in the generation of modified indole glucosinolates. This gene family encodes *O*-methyltransferases (termed indole glucosinolate methyltransferases, IGMT) and consists of five members in *Arabidopsis thaliana*. These IGMTs utilize hydroxyl-indole-3-yl-methyl glucosinolates as substrates, and generate 4-methoxy-indole-3-yl-methyl (4MO-I3M) and 1-methoxy-indole-3-yl-methyl glucosinolates (1MO-I3M), from 4OH-I3M and 1OH-I3M, respectively.



Conclusions and Perspectives

We have fine-mapped a second QTL for modified indole glucosinolates. Arabidopsis lines with mutations in the candidate gene display an altered glucosinolate phenotype. We are in the progress of conducting a QTL complementation assay to verify that the candidate gene causes the QTL.



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FungIsochores

Isochores and effectors : genome reshaping and the birth of highly pathogenic species in fungal phytopathogens

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Background and Objectives

FungIsochores develops a comparative and evolutionary genomics approach to assess the role of fungal genome reshaping following massive transposable element (TE) invasion on generation of novel species better adapted to new hosts or with increased fitness on a given host plant.

The original fungal model for this study is a pathogen of oilseed rape, *Leptosphaeria maculans* 'brassicae' (Figure 1), whose genome sequence analysis strongly suggested the following events in the course of evolution :

i. massive invasion of the genome by TEs linked with a probable incidence on acquisition of novel effector-encoding genes

ii. TE degeneracy by repeat-induced point mutations (RIP) generating a compartmentalised genome into isochores (Figure 3)

iii. diversification of effector-encoding genes following mild RIP mutation

A second plant pathogenic fungus, *Venturia inaequalis*, the agent of apple scab (Figure 2) is also concerned since preliminary sequence data indicated that its genome is structured into isochores that were postulated to specifically host effector-encoding genes.

This project aims at sequencing and analysing the genomes of three members of the *L. maculans* – *L. biglobosa* species complex, choosen because they show a divergent adaptation towards oilseed rape and for which preliminary data indicated a low level of invasion by TEs, and at contributing to the sequencing of *V. inaequalis* to validate that its genome is structured into isochores.

Figure 1. Oilseed rape stem canker caused by *Leptosphaeria maculans* 'brassicae'.

Figure 2. Apple scab caused by *Venturia inaequalis*.

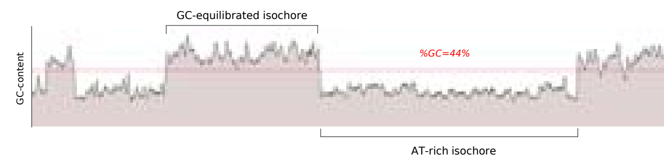

Figure 3. The isochores-structured genome of *Leptopshaeria maculans* 'brassicae'.

Results

- Three members of the *Leptosphaeria* species complex, *L. maculans* 'lepidii', *L. biglobosa* 'thlaspii' and *L. biglobosa* 'brassicae', have been sequenced, assembled and annotated. All isolates have a compact genome sized 31-32 Mb and show a low TE content, only 2-3.5%, compared to 30% in the *L. maculans* 'brassicae' genome (Table 1).

  Comparative genomics between the *Leptosphaeria* genomes indicates a high conservation of chromosomal synteny. This is mainly the case between *L. maculans* 'lepidii' and *L. maculans* 'brassicae' (Figure 4) for which gene order and content are extremely conserved whereas sequence divergence between orthologues is important.

  Very few families of TEs are common between the different species and most of TE invasion took place after the separation between *L. maculans* and *L. biglobosa*, and was not accompanied by massive chromosomal rearrangements. This invasion may have favoured reproductive isolation and recent speciation between the weakly pathogenic *L. maculans* 'lepidii' and the highly pathogenic *L. maculans* 'brassicae'.

  Comparative genomics between the *Leptosphaeria* genomes indicate a highly divergent content in effector-encoding genes.

  While a comparable number of predicted effector-encoding genes is present in the different isolates (ranging from 650 to 740), only 20% are common to all isolates and up to 40% of these genes are isolate- or species-specific. This number is even higher when analysing effector genes hosted in TE-rich genomic landscapes, and TE invasion is shown to be accompanied by « generation » of novel effector genes in a few cases.

	L. maculans 'brassicae'	*L. maculans* 'lepidii'	*L. biglobosa* 'thlaspii'	*L. biglobosa* 'brassicae'
Genome size (Mb)	**45.12**	**31.53**	**32.1**	**31.79**
No of scaffold	76	123	237	606
SC N50 (Mb)	1769.6	1356.3	715.1	779.1
GC content (%)	44.1	47.3	46.9	47.6
TEs (%)	**30.4**	**1.9**	**3**	**3.5**
No of gene models	12543	11272	11691	11390
No of putative effectors	**651**	**737**	**676**	**665**

Table 1. Genome statistics of the sequenced members of the *Leptosphaeria* species complex.

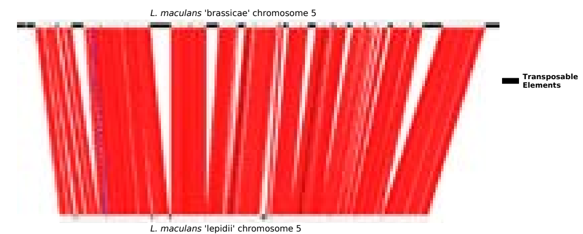

Figure 4. Chromosomal synteny between *L. maculans* 'brassicae' and *L. maculans* 'lepidii'.

- The genome sequence of *V. inaequalis* substantiate our initial postulate that it is structured into contrasted isochores (Figure 5) as the genome of *L. maculans* 'brassicae' is. The genome assembly covers 73.2 Mb, which is much higher than most currently known fungal genomes (45 Mb for *L. maculans* 'brassicae'). But it is still very fragmented and large TE-rich regions are poorly assembled or completely missing.

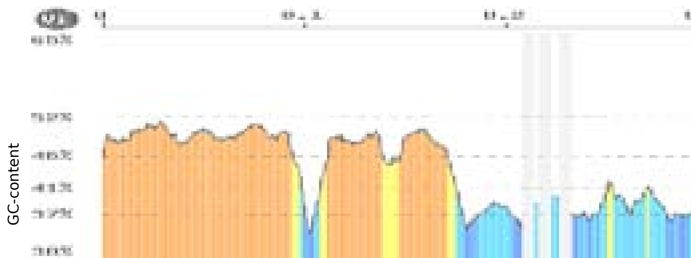

Figure 5. The genome of *V. inaequalis* is structured into isochores (exemple of scaffold00008).

- Automated annotation and setting up of the genome browser has been done for *V. inaequalis*, and a comparative genomics browser (Figure 6) is currently being setup for all *Leptosphaeria* isolates.

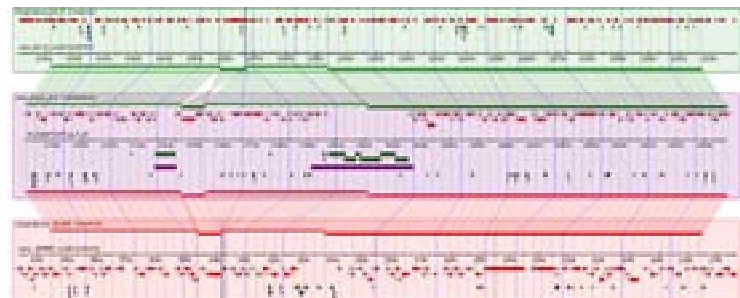

Figure 6. The synteny browser dedicated to sequenced isolates of the *Leptosphaeria* species complex.

Perspectives

Improvment of *V. inaequalis* genome assembly of TE-rich regions for a better visualisation of genes hosted within this genomic environment and analysis of TE families, TE nesting and dating of transposition events.

Evaluation of the incidence of RIP on TE degeneracy and diversification of effector-encoding genes.

Within the *Leptosphaeria* species complex, generation of accurate phylogenies and dating of speciation times (coll. C.L. Schoch, NCBI).

Generation of an extensive repertoire of effectors in all species/isolates and elucidation of their origin and expansion/diversification mechanisms.

Validation of genomes annotation by transcriptomic analysis (microarray).

References

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FungIsochores
ANR-09-GENM-028
01.01.2010 - 01.07.2013

GEMO project: Genetic bases of pathogenicity and host specificity analysed through comparative and evolutionary genomics in the model fungus *Magnaporthe*.

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Introduction

The development of Next Generation Sequencing techniques allows comparative and evolutive genomics within a species. The GEMO project (Evolutionary Genomics of *Magnaporte oryzae)* encompasses the *de novo* sequencing of 9 *Magnaporthe* strains, 8 of which belong to the species *M. oryzae*, pathogenic on rice and other Monocotyledons, and one to *M. grisea*, pathogenic on *Digitaria*. The 8 *M. oryzae* strains were chosen with different host specificity. We are aiming the genomic fluidity, both at the interspecific level within the genus *Magnaporthe* and at the intraspecific level within the species *M. oryzae*. Thus we expect to determine the core- and pan-genome of the species *M. oryzae* and of the genus *Magnaporthe*. A finer evolutionary analysis will be carried out on Small Secreted Proteins (SSP) which are involved in pathogenicity and host-specificity. In particular, we also aim whether or not pathogenicity related genes are more targeted by genomic rearrangements and/or positive selection events.

Materials

- *De novo* sequencing of 9 *Magnaporthe* strains (Fig 1: Orange boxes):
  - ∗8 *M. oryzae* strains attacking different hosts: *Eleusine sp.* (1 strain), *Triticum sp.* (1strain), *Setaria sp.* (1 strain), and *Oryza sativa* (5 strains representative of worldwide genetic diversity).
  - ∗1 *M. grisea* pathogenic to *Digitaria* sp.
- 3 other genomes publicly available (Fig 1: Green boxes):
  - ∗*M. orizyae* pathogenic to rice.
  - ∗*M. poae*.
  - ∗*Gaeumannomyces graminis* f. sp. *tritici*

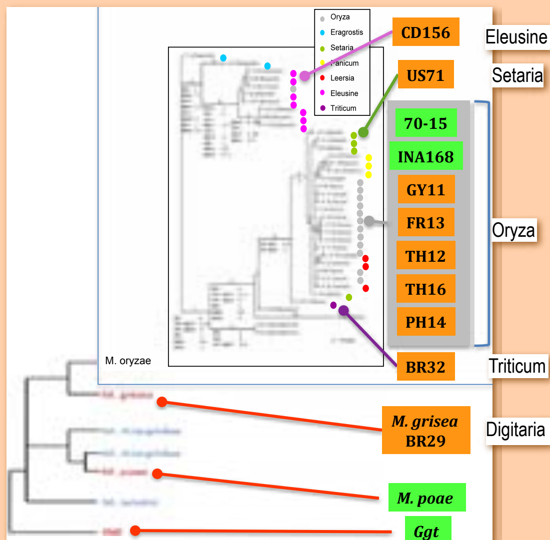


Figure 1: Schematic putative phylogeny of 6 representative members of the genus *Magnaporthe*, and of the host-specific lineages within the species *M. oryzae*, based on ribosomal and housekeepeing genes.

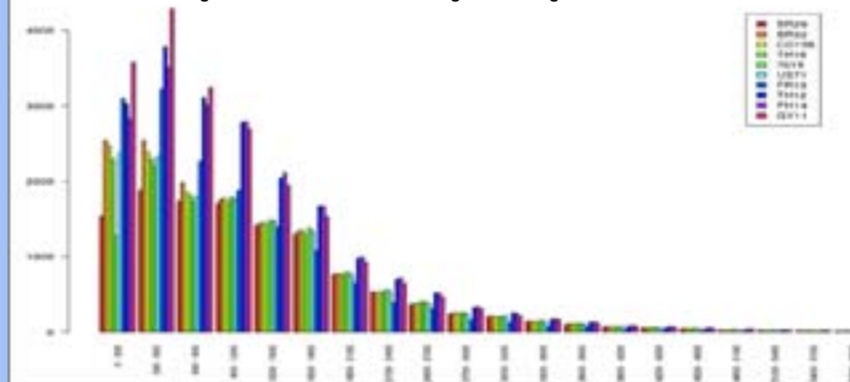
Methods - *De novo* sequencing and structural annotation

- *De novo* sequencing: Solexa + 454 Life Siences.
- Mixed assembly with Newbler 2.6 + scaffolding.
- *De novo* structural annotation: Eugene Pipeline trained with 300 curated genes from *M. oryzae* 70-15.

	70_15	BR29	BR32	CD156	FR13	GY11	PH14	TH12	TH16	US71
# Contigs	-	9,644	6,044	26,535	79,619	13,188	9,908	11,772	4,114	7,398
Scaffold N50	-	955	1,760	1,066	101	187	590	697	938	813
# Bases (Mb)	-	40.96	41.86	42.69	43.03	46.33	48.54	49.82	39.13	41.21
# CDS	12,757	12,292	14,349	14,067	14,900	20,477	19,474	19,866	13,571	13,803
% Truncated genes	0.0	0.1	9.3	5.5	35.5	27.7	12.5	17.7	8.6	4.8

Table 1 :Basic assembly statistics, and gene contents of the 10 genomes

Figure 2: Distribution of CDS length in all 10 genomes



The quality of the genome assemblies (Table 1) is good for 5 strains. For GY11, PH14, TH12 and FR13 the assembly seems more fragmented, as shown by higher numbers of contigs and lower N50 values. The number of predicted truncated genes is higher in these 4 genomes, which leads to more predicted CDS of shorter length (Table1, Fig 2).

Ongoing steps for characterizing the Genomic fluidity in the 10 genomes

- Search for ortholog families with OrthoMCL (in progress).
- *De novo* annotation of repetitive elements with REPET (in progress).
- Comparison of the gene contents (all genes, and SSP).
- Macro-synteny & micro-synteny analyses.
- Prediction of the protein localization.

We are aiming to show the most important mutagenic events causing the inter- and intraspecific divergence inside the *Magnaporthe* genus and the *M. oryzae* species.

Coming soon

- RNAseq analysis of *in planta* kinetics for 4 of the sequenced strains.
- Study other families of pathogenicity genes (genes involved in secondary metabolism).
- Functional annotation using ESTs from closely related species.
- Detection of single nucleotide polymorphism and search for signatures of positive selection in orthologs.

Pathogeny related genes

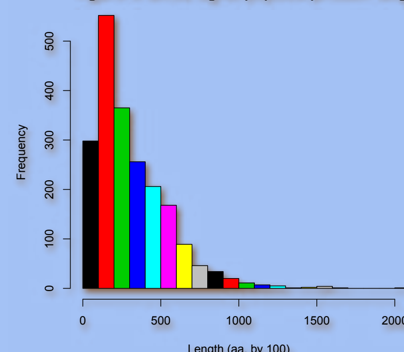
Detection of Small Secreted Proteins (SSP)

- Detection of peptide signals with SignalP 4.0 (done).
- Detection of Transmembrane Domains with TMHMM 2.0 (in progress).
- Prediction of the protein localization
- Cut-off maximum size of 300 aa.

Preliminary results - Signal Peptide detection:

➡High repeatability within the 9 genomes.
➡12-14% of the CDS are SP+ in each strain.
➡Median length of SP+ genes: ~260 aa (Fig3).

Figure 3: BR32 signal peptide protein length



- A particular attention will be given to the listing and characterization of the of these proteins for the 9 genomes as well as the reference strain *M. oryzae* 70-15.

We thank Couloux A and Cruaud C. from the Genoscope

GeneTOP (ANR-09-GENM-016, 01.01.2010 - 31.12.2013)

"Gene Targeting Optimisation in Plants"

Prog : Génomique et Biotechnologies végétales 2009

AGENCE NATIONALE DE LA RECHERCHE ANR

Coordinateur : INRA Versailles (F. Nogué)

Partenaires : CNRS Orsay (M-P. Doutriaux), CIRAD Montpellier (E. Guiderdoni), Biogemma Clermont Ferrand (W. Paul)

Aims of the project

The aim of our project is to optimize gene targeting to permit its routine use in crop plants. For this purpose three major steps of HR are investigated: (1) increase HR by inhibiting the illegitimate recombination (IR) pathways of gene repair; (2) improve the homologous recombination (HR) invasion step using the RAD51 function; (3) increase GT frequency by introducing double strand breaks at target sites.

The first task will be to obtain plants where IR is decreased as much as possible in order to increase RH and, as in fungi, to facilitate gene targeting. Those plants will be challenged for HR and GT. The results will be transferred to rice where mutants and RNAi techniques will be used in order to test the level of transferability of results obtained in model plants to crops.

The second task will be focused on the invasion step of HR. We have shown recently that the *Physcomitrella* RAD51 activity for GT is specific and cannot be replaced by the RAD51 protein of the flowering plant Arabidopsis. In the framework of this project we would like to analyse the specificity of the *Physcomitrella* RAD51 compared to the *Arabidopsis* protein in order to reveal information on critical domains of the RAD51 protein for gene targeting. Another aspect of this task will be to test the capacity of the *Physcomitrella* RAD51 proteins to stimulate GT in higher plants. For this purpose the moss RAD51 will be expressed, in *Arabidopsis* or rice and GT experiments, using the existing GT reporter genes in these two species, performed.

The feasibility of the use of the I-Sce1 meganuclease for the creation of landing pads for GT in maize has been proven recently. The third task will consist in the extension of this strategy to rice and in the generation of a set of landing pads in the two crop plants. The criteria for these landing pads will be that they are not inserted in a region containing a gene and that they permit a good and stable expression of the transgene.

At the end of the project results obtained from the tasks 1 and 2 will be used in order to increase the efficiency of targeted double-strand break-induced homologous recombination in maize and rice.

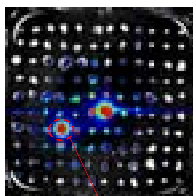
Results

Work package 1: Stimulation of HR and GT by IR inhibition

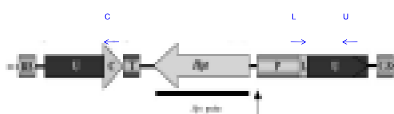
Ligase4 and *RAD1* are key genes for two important DNA repair process, NHEJ and SSA respectively. P. patens knock out mutants for *PpLIG4* and *PpRAD1* have been obtained and analysed for gene targeting. Interestingly knock out of these genes does not lead to an increase in gene targeting (98.7%, and 55.4% of the WT GT efficiency respectively for *Pplig4* and *Pprad1*). These results are in strong contrast with what is seen in fungi for example and furthermore show the importance of RAD1 for gene targeting in P. patens. Qualitative analysis of the gene targeting events obtained in the different genetic backgrounds shows that in wild type 2 types of integrations can be observed, (1) integration by TGR (Targeted Gene Replacement) resulting from HR between each end of a targeting construct and the targeted locus, (2) integration by TI (targeted insertion), integrating at one end of the targeted locus by HR accompanied by illegitimate recombination on the other end (5' or 3' TI). The proportions of these different types of integrations for the wild type and the *Pplig4* mutant are 80% of TGR, 10% of 5' TI and 10% of 3' TI. Interestingly these proportions for the *Pprad1* mutant are 90% of TGR, 5% of 5' TI and 5% of 3' TI.

RAD18 has been proposed to be involved in the choice between HR and IR. A gene disruption cassette has been constructed for the knock out of the *PpRAD18* gene. No *Pprad18* mutant has been obtained using this vector. This result, confirmed by A. Cuming (Leeds University, personal communication), shows that the *rad18* mutation is lethal in P. patens and is in contrast with what is observed in A. thaliana where the *rad18* mutation is viable. This result shows the importance of the RAD18 function in P. patens and leaves the question of its putative role in gene targeting open.

Arabidopsis T-DNA tagged mutants for the *LIG4*, *KU80*, *RAD1, RAD10* and *RAD18* genes have been isolated and characterized and double mutants *lig4*/*rad10* and *ku80*/*rad10* have been isolated. Mitotic HR efficiency will be measured in theses different genetic contexts tanks to a luciferase based HR substrate (Molinier et al., 2004). Moreover, gene-targeting efficiency will be estimated in these mutants and compared to the wild type plant. For this purpose two techniques of DNA delivery are currently developed. The first one is the classical *Agrobacterium*-mediated transformation by the *Arabidopsis* Floral-Dip method.



KanR rice lines transfered on NBS medium following spraying with luciferin. Calli were transformed with pUCLU (Molinier et al 2004) and fonctional LUC gene was restaured by homologous recombination.



The second one is based on direct transfer of the targeting construct in the *Arabidopsis* cell by protoplast PEG fusion. For this purpose a protocol for protoplasts isolation and regeneration from young *Arabidopsis* WS plantlets has been set up and PEG transformation experiments are in progress. Two strategies will be used for GT efficiency measurement. The first one will consist in the targeting of the *AtRAD51* gene (knock-out of this gene leads to sterility, an easy to score phenotype). For this purpose a construct containing 3 and 4 kb of the genomic region of the *RAD51* gene, separated by a positive selection marker hpt (hygR) and a negative selection marker, codA, which confers sensitivity to 5-fluoro -cytosine (5FC) at one end has been obtained. The second strategy is based on the knock-out of the APT gene which leads to resistance to the toxic adenine derivative, 2-fluoroadenine. A targeting construct, containing 1 and 1.2 kb of the genomic region of the *APT* gene, separated by a positive selection marker hpt (hygR) has been obtained.

Work package 2: Stimulation of invasion step with RAD51

AtRAD51 has been shown to be unable to complement the function of PpRAD51 in GT. P. patens lines bearing chimerical fusion proteins between the PpRAD51 and AtRAD51 proteins have been produced and will be used to define the domains of the PpRAD51 protein essential for GT in P. patens.

In parallel, rice lines expressing the RAD51 genes from *Arabidopsis* or *Physcomitrella* have been isolated and construction of rice lines over-expressing the rice RAD51 or RAD54 (helper of RAD51) genes is in progress. These lines will be characterized and amplified in order to estimate the level of mitotic RH and the gene-targeting efficiency in these plants. The mitotic HR efficiency is measured with the luciferase (described previously) or GUS based HR substrates (Molinier et al., 2004) that have been adapted to rice. Preliminary results show that overexpression of the *Arabidopsis* or *Physcomitrella* RAD51 genes increases the level of mitotic RH but overexpression of the OsRAD51 or OsRAD54 genes decreases the level of mitotic RH.

Work package 3: Stimulation of HR and GT in the context of a double strand break (DSB)

DUT1 is an essential enzyme that protects DNA against uracil incorporation and its knock down leads to the accumulation of DSBs (Dubois et al., 2011). Knock downs of the *AtDUT1* gene have been obtained for the *Arabidopsis lig4*, *ku80* and *rad10* mutant lines and will be tested for their capacity of GT.

In order to induce a DSB at a specific locus in the *Arabidopsis* genome the recently described TALEN strategy (Cormak et al, 2011) will be tested. The TALEN is a modular endonuclease able to specifically induce a double strand break in a targeted sequence. Co-transformation with this TALEN and the APT targeting construct (see above) should enhance GT efficiency. Cloning of a TALEN designed for recognition of the APT gene is in progress.

Rice lines bearing a landing-pad containing an I-Sce1 endonuclease recognition site have been constructed. The rice landing pad construct allows, thanks to the reconstruction of a functional GFP gene with a targeting construct, the measurement of the efficiency of GT and will be also combined to the RAD51 overexpressor lines.

For maize, a pre-landing-pad (pBIOS2108) vector containing two I-Sce1 recognitions sites flanking a BAR and a GFP gene has been constructed and validated. Maize plants (46 lines) bearing a single copy of pBIOS2108 have been isolated and characterized. Flanking sequences of pBIOS2108 and stability of expression of the BAR and GFP genes will be characterized.

Conclusions and perspectives

Work package 1: The pattern of GT integration in the rad1 context will be further analysed as this work could give us important keys concerning the quality of GT. A new gene disruption cassette will be constructed in order to obtain a leaky mutant for the *PpRAD18* gene. Mitotic HR and GT efficiency will be estimated in the characterized *Arabidopsis lig4*, *ku80*, *rad10* and *lig4/rad10* and *ku80/RAD10* mutants using the RAD51 or the APT based targeting constructs.

Work package 2: P. patens lines with chimerical fusion proteins between the PpRAD51 and AtRAD51 proteins will be tested for their efficiency of GT in order to define important domain for GT.

Rice lines overexpressing RAD51 will be tested for GT efficiency using a targeting construct containing genomic regions of the rice *APT* gene, separated by a positive selection marker hpt (hygR).

Work package 3: Mitotic HR and GT efficiency will be estimated in the characterized *Arabidopsis lig4*, *ku80* and *rad10* mutants knocked down for the *AtDUT1* gene.

TALENs designed for recognition of the *Arabidopsis APT* gene will be tested to estimate their impact on GT efficiency in *Arabidopsis lig4*, *ku80*, *rad10* mutants.

Wild type and RAD51 overexpressor rice lines containing a landing-pad with an I-Sce1 site will be tested for gene targeting efficiency.

The maize plants showing an insertion of pBIOS2108 in a region of the genome where there is no gene and with a stable and high level of expression of the BAR and GFP genes will be selected and crossed to a maize line expressing the I-Sce1 gene. The progeny from this cross will be screened to find plant with a functional landing pad (excision of the BAR and GFP genes with one residual I-Sce1 site). Targeted integration of a new transgene at the landing pad locus will be possible thanks to the presence of the unique ISce1 restriction site. These lines will constitute innovative and useful landing pads for the targeted integration of transgenes in maize.

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The IMMUNIT-Ae project

Genetic diversity and mechanisms of resistance to *Aphanomyces euteiches* in legumes.

ANR 2010 – Génomique et biotechnologies végétales

Coordinator : Christophe JACQUET **(Université Paul Sabatier , Toulouse , LRSV)**

Partners : Marie-Laure PILET-NAYEL **(INRA Rennes APBV)** ; Nathalie CHANTRET **(INRA Montpellier DIAPC)** ; Sandrine BALZERGUE **(INRA Evry URGV)**.

Collaboration : Nevin YOUNG **(University of Minnesota)**.

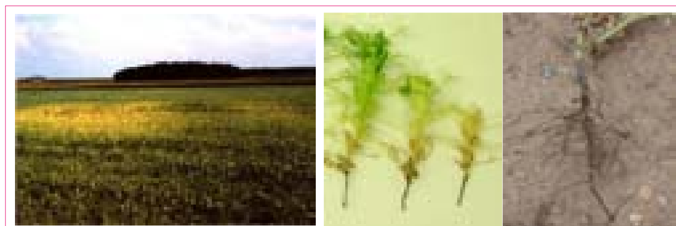
Objectives

As no chemical method of control is available against *A. euteiches* (*Ae*), the **development of resistant varieties** is a major objective to manage the disease in pea and other legumes. However breeding pea varieties with resistance to *Ae* remains difficult for several biological and genetic reasons.

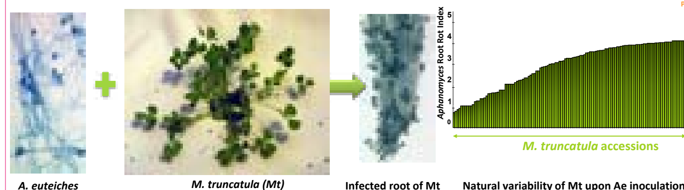
To accelerate this genetic improvement and better understand the molecular and genetic components involved in **quantitative resistance** to *Ae*, this project aims at

i) using the large genomic and genetic data and tools available for the **model legume** *Medicago truncatula* (*Mt*) to **identify genes and unravel the diversity of resistance loci** on Mt genome

ii) exploiting the **synteny** of *Mt* with other legumes to « translate » Mt genetic data to crop legumes and identify **new genetic markers** that will improve further legume breeding programs.



Aphanomyces euteiches is the most damaging pathogen of pea in France and Europe. This soilborne oomycete is responsible for pea root rot and seedling damping off.



The model legume *Medicago truncatula* is a host for *A. euteiches* . This legume species displays a high variability upon *Ae* inoculation that can be exploited to understand and improve resistance to this pathogen.

Cited References : Djebali N, et al. (2009). Mol. Plant. Microbe Interact. 22(9):1043-1055.
Pilet-Nayel ML, et al. (2009). Phytopathology 99(2):203-208.

Strategies and preliminary results

Previous genetic analyses performed on two different populations of recombinant inbred lines (RILs) screened with two strains of *Ae* identified one major QTL of resistance in each population, named ***AER1*** (Pilet-Nayel et al., 2009) and ***prAe1*** (Djebali et al., 2009). AER1 (440 kb) is **dominant** while prAe1 (150 kb) is **recessive**, but the two QTL are **both detected on the same genome region** , on the distal part of the Mt chromosome 3.

The first task of the project is i) to **clone the gene(s)** that are involved in these QTLs of resistance and ii) to understand the ***prAe1*-associated molecular mechanisms**. Size reduction of both QTL are in progress through the phenotyping/genotyping of **new recombinant lines** with **newly designed molecular markers**. **Microarray experiments** performed on two NILs, only different in the QTL alleles, showed the key role of jasmonic acid pathway and cell wall remodelling and strengthening mechanisms in the resistance phenotype.

The second task of the project aimed at detecting the diversity of Mt genome loci involved in the quantitative resistance to *Ae*. Two strategies are used : a **genome wide association mapping** (GWAM) and a complementary **nested association mapping** (NAM). Two hundreds Mt accessions were screened with four Ae strains in the first year. We are now receiving SNP data from the **Medicago Hapmap American project** and GWAM will start as soon as these files will be transformed in a compatible format for this approach.

The third task is to understand **structural and functional conservation of loci** and mechanisms of resistance between model legume and various cultivated legumes including pea. **Molecular markers have been developed** from ESTs identified from infected pea or Mt and their **co-localisation with previously identified QTL** is in progress.

Perspectives

This project should lead to the identification of **novel resistance molecular mechanisms** and the design of new molecular markers that will facilitate the production of crop legumes more resistant to Ae.

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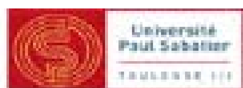


MS-DMind : multiscale data “minding” for molecular process related to biotic and abiotic stresses: pilot study with the nsLTP superfamily of proteins

Genomique, Edition 2008

Manuel Ruiz[1]
Cécile Fleury[1], Marie-Françoise Gautier[1], Jean-François Dufayard[1], Franck Molina[2], Frédéric de Lamotte[1]

Aims

Understanding biological processes requires managing many complex data sets. MS-DMind project aims at integrating the different ”-omics” spaces corresponding to the different domains of knowledge (genomics, transcriptomics, proteomics, metabolomics, structural biology, molecular dynamics, cellular biology, molecular/biological functions, interactomics and phenomics) in order to allow inferring reliable hypotheses from the data.
The non specific Lipid Transfer Proteins (nsLTPs) show large variations in their sequences, biological roles, quaternary associations and the nature of bound hydrophobic ligands. Besides, they are involved in a large number of biological processes relative to plant development and defense. However they share a conserved eight-cysteine-residue pattern which plays an important role in the structural scaffold. Thus, because its members show a high evolutionary divergence but a conserved common fold, the nsLTP superfamily constitutes a very interesting case of study to validate a method designed for the investigation of protein structure-function relationships.

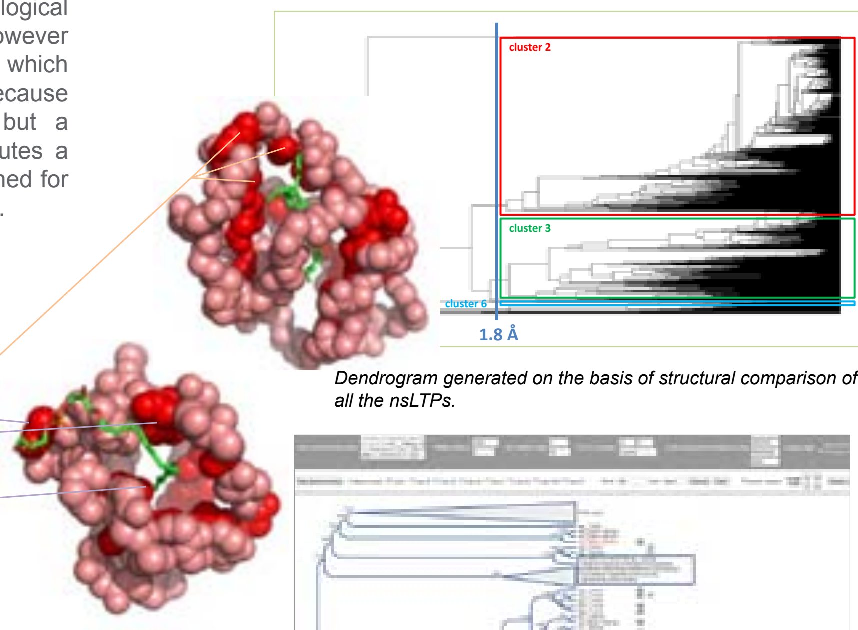
```
           1          10         20         30         40         50         60         70
Cluster 2 ---------- ---------- ---------- ---------- ---------- ---------A ---------- -----I----
Cluster 3 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Cluster 6 ---------- ---------- ---------- ---------- -Q---G---- ------G--G ---------- -----G----

          80         90         100        110        120        130        140        150
Cluster 2 ---S------ --C------- ---------G ---------- ----Q--VA- --SAIAPCIS YAR--G---- ----------
Cluster 3 ---------- ---------- ---------- ---------- -A--C--QA- --SQLAVCAS AIL--S---- ----------
Cluster 6 ---G------ ---------- ---------E ------C--- -V--P--QL- --NRLLACRA YAV--P---- ----------

          160        170        180        190        200        210        220        230
Cluster 2 ---------Q ---------G --------S- GP-SAGCCSG VRSLNNAAR- T--T-AD--R -RA---ACNC LKN----AA-
Cluster 3 ---------- ---------G --------A- KP-SGECCGN LRAQQ----- ---------- -GC---FCQY AKD---PTY-
Cluster 6 ---------G ---------A --------G- DP-SAECCSA LSSIS----- ---------Q -GC---ACSA IS--------

          240        250        260        270        280        290        300        310
Cluster 2 --A-G----- --V----S-- ---------- --G------- L--------- -----N---- AGNAASIPSK CGVSI-----
Cluster 3 --G------- -------Q-- ---------- --Y------- I--------- -----R---- SPHARDTLTS CGLAV-----
Cluster 6 ---------- ---------- ---------- ---------- ---------- ---------- -I-MNSLPSR CHLSQ-----

          320        330        340        350        360        370        380        390
Cluster 2 P--------- ----Y----- -------T-- ------I--- S--T-----S --T--D---- --C------S -RV-N-----
Cluster 3 P--------- ----H----- -------C-- ---------- ---------- ---------- ---------- ----------
Cluster 6 I--------- ----N----- -------C-- ------S--- ---A------ ---------- ---------- ----------

          400        410        420        430
Cluster 2 ---------- ---------- ---------- -------
Cluster 3 ---------- ---------- ---------- -------
Cluster 6 ---------- ---------- ---------- -------
```

Structure-based sequence alignment of the reference proteins of the 3 main structural clusters.

Results

Numerous proteins have been annotated as nsLTPs but we focused the study on the monodomain proteins which present the strict and only nsLTP domain. Eight hundreds mature amino acid sequences belonging to more than 100 plant species have thus been selected. They have been submitted to phylogenic analysis and classified according to sequence identity. For ten of them, three-dimensional structures were available in the Protein DataBank as they had been experimentally determined. Theoretical structures have been calculated for the other proteins, using homology modeling method.

A second classification has been established on the basis of the overall structural alignment of all proteins of the dataset. Using the evolutionary trace method, the observation of structurally equivalent positions allowed identifying either evolutionarily important residues potentially involved in the structural integrity or class-specific conserved residues that may present a functional importance. A functional annotation has been performed manually and the data have been organized and stored in a dedicated multi-scale information system. The comparative structure/function analysis is currently being carried out and is already bringing insights of the ligand binding mechanisms of the nsLTPs.



Dendrogram generated on the basis of structural comparison of all the nsLTPs.

Online phylogenetic tree viewer displaying functional annotations according to Gene Ontology (GO) and Plant Ontology (PO) terms.

Perspectives

In a short term, the developed method will be fully automated and the analysis pipeline will become available to the community. The method has been conceived to be generic enough to handle any protein family, but specific adaptations may need to be made for particular proteins; for example proteins which contain repeats in their sequences and/or 3D structures (alignment tools, structure prediction method). Lipid binding assays are being carried out in SysDiag laboratory and will bring the experimental support to our hypothesis.

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MAGIC-TomSNP

Valorisation of genetic and genomic resources of Tomato for the improvement of fruit quality

Edition 2009

Colloque Plant Genomics 2012

Objectives

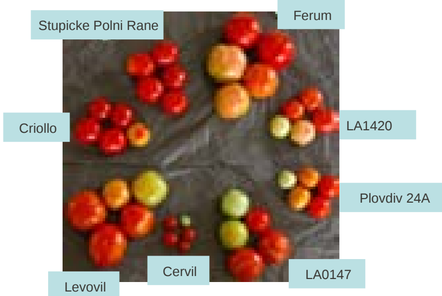
In this project we develop a set of genomic and plant resources necessary for QTL and association analyses in tomato, through:

1) Preparation of a multi-allelic advanced generation intercross (MAGIC) population, derived from the intercross of 8 divergent lines, to be used for genome wide scan and QTL mapping.
2) SNP discovery in the 8 lines used as parents of the MAGIC population, using the high throughput sequencing technology (Genome Analyser).
3) Development of a genotyping platform carrying 1536 SNP.
4) Use of the SNPplatform to characterise several plant resources characterized: the MAGIC population, a collection of cherry tomato accessions, and a collection of old and elite cultivars.
5) Develop a multi scale analysis of the parental lines and F1 hybrids through Digital Gene Expression, Proteomic characterization and metabolomic studies.

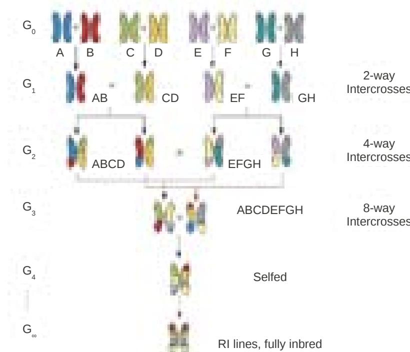
All together these resources and data produced will allow us to perform QTL analysis in a multi-allelic background, identify putative candidate genes based on their map location and gene/protein expression and set up the bases for genetic association studies and future innovative breeding approaches and gene discovery in tomato.

PLANT Materials

8 divergent tomato lines

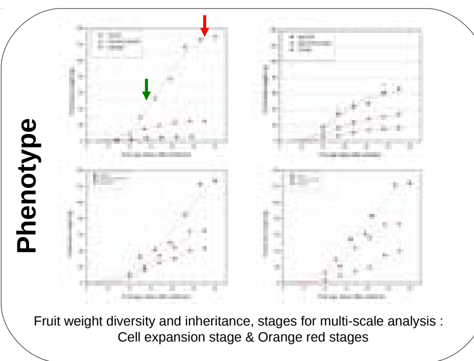


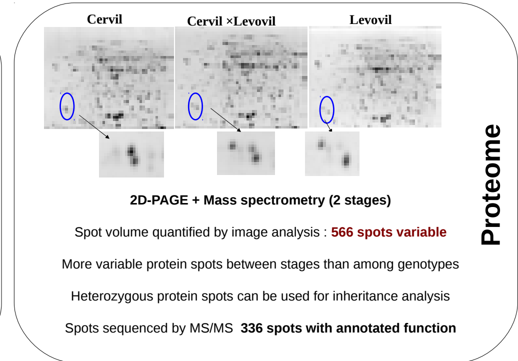
Multi-parent Advance Generation Inter-Cross

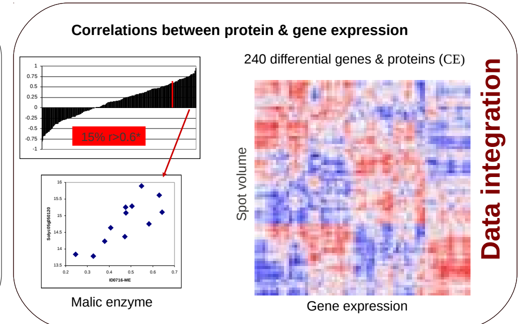


QTL mapping in a multi-allelic Population (400 familles)

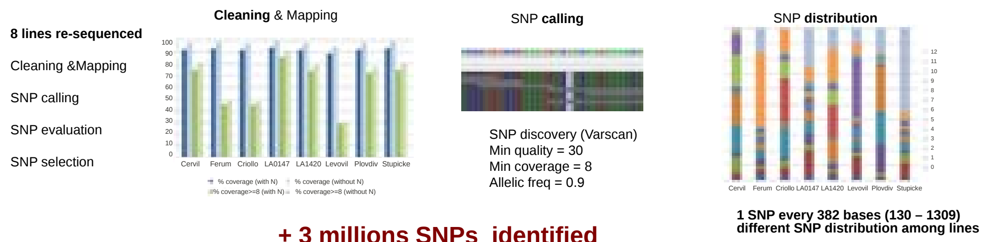
Integrated Multi-scale analysis of 8 parental lines & 4 F1







SNP discovery through Genome re-sequencing



+ 3 millions SNPs identified

Conclusions and perspectives

A Multi-parent Advance Generation Inter-Cross population containing 400 families has been developed in tomato.
The MAGIC population is already growing and will be phenotyped and genotyped in 2012:
More than 3 millions polymorphic SNPs were detected by re-sequencing 8 accessions.
Those SNPs constitute a precious tool for subsequent QTLs and genome wide association analysis.
A Platform carrying 1536 selected tomato SNPs is under development and will be used to genotype the MAGIC population and a collection of old and elite tomato cultivars.
The integrated multi-scale analysis of the 8 parental lines and 4 hybrids revealed a wide range of variation with more than 560/1,400 protein spots and 4,000/23,000 genes variable.

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EPGV
Evry

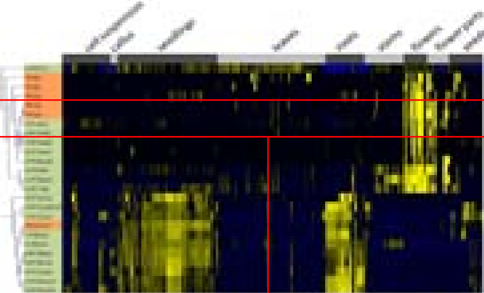
Thanks to Jiaxin Xu, Laura Pascual, Nelly Desplat, Jean Paul Bouchet, Yolande Carretero (GAFL), Dominique Brunel, Marie Christine Lepaslier, Maria Tchoumakov (EPGV)

METAMAP: (ANR-07-BLAN-0359-CSD 7). Metabolic mapping of gene families: a new strategy for the discovery of overlooked pathways.

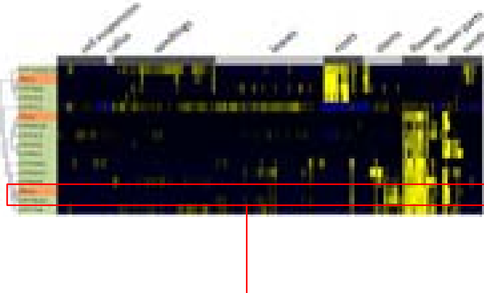
Jean-François Ginglinger[1], Jürgen Elthing[1], Marc Fischer[2], Vincent Compagnon[1], Hubert Schaller[1], Francis Karst[2] and Danièle Werck-Reichhart[1]
[1]*Institut de Biologie Moléculaire des Plantes UPR2357 CNRS Université de Strasbourg*
[2]*UMR1131 INRA Santé de la Vigne et Qualité du Vin, Colmar*

Co-expression analysis

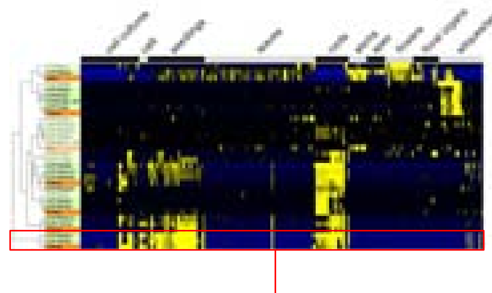
Monoterpenes



Sesquiterpenes



Triterpenes

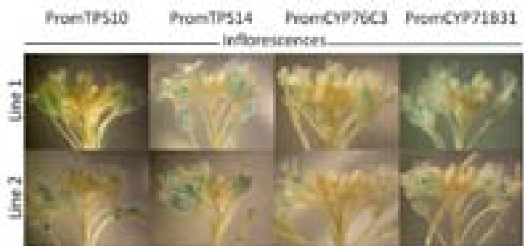


TPS = terpene synthase

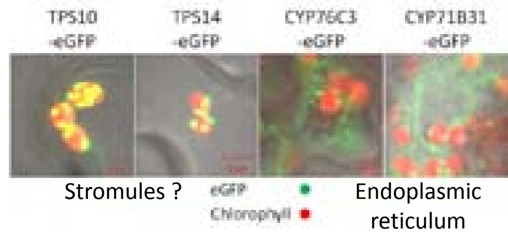
Expression heatmaps of the mono-, sesqui- and triterpene synthases and theirco-regulated P450s

In progress

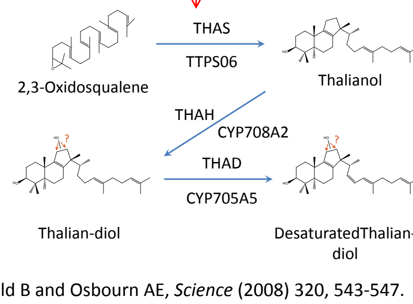
Expression of 4 co-regulated genes restricted to filaments



Subcellular localization



Stromules ? eGFP Chlorophyll Endoplasmic reticulum



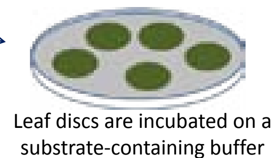
Field B and Osbourn AE, *Science* (2008) 320, 543-547.

Novel heterologous expression systems

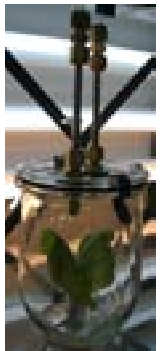
Engineered yeast for the production of monoterpenoids: modified to accumulate GPP and for stable expression of the P450 reductases ATR1 or ATR2. They can be further transformed for the expression of TPS and P450

Transient expression in *Nicotiana benthamiana*

The headspace volatile compounds are trapped and analyzed.

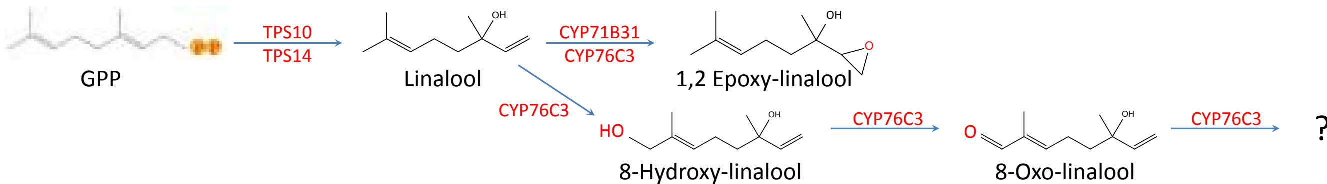


Leaf discs are incubated on a substrate-containing buffer



Comparison reveals the influence of the expression system on the products resulting from TPS activity

Novel pathways revealed by these analyses



Role of oxidized monoterpenoids *in vivo*

Flower headspace and profiles of soluble metabolites of KO mutants of the genes versus wild-type are analyzed. Plant-insect interactions are examined by monitoring the electroantennogram of flower volatile fractions. The impact of KO mutation on plant-insect interaction is being investigated.

Prospective

METAMAP confirmed the efficiency of the predictive strategy to reveal new metabolic pathways and allowed the development of new methods and tools. It reveals the role of two P450 families in the biosynthesis of semiochemicals. As oxygenated linalool derivatives are major components of aromatic grape berries, it sets the stage of further investigations of this family that shows exceptional "blooming" in grapevine in relation to wine aroma.

Publications

1. Ehlting et al. (2008) An extensive (co-)expression analysis tool for the cytochrome P450 superfamily in *Arabidopsis thaliana* BMC Plant Biol. 8:47.
2. Ehlting et al. (2009) Genome-wide approaches in Natural products reserach *In* Plant-derived Natural Products: Synthesis, Function, and Application. Anne E. Osbourn and Virginia Lanzotti edts, Springer Dordrecht, 475-503.
3. Schaller H (2010) Sterol and Steroid Biosynthesis and Metabolism in Plants and Microorganisms *In* Comprehensive Natural Products II Chemistry and Biology; Mander, L., Lui, H.-W, Eds.; Elsevier: Oxford, Vol. No.1, 755 - 787.
4. Bouvier-Navé et al. (2010) Involvement of the phospholipid sterol acyltransferase1 in plant sterol homeostasis and leaf senescence. Plant Physiol. 152, 107-19.
5. Fischer et al. (2011) Metabolic engineering of monoterpene synthesis in yeast. Biotechnol. Bioeng., in press.
6. Fischer et al. (2011) Impact of *Quillaja saponaria* saponins on grapevine ecosystem organisms. Antonie Van Leeuwenhoek, in press.
7. Fischer et al. (2011) Identification of a lysine residue important for catalytic activity of yeast farnesyl diphosphate synthase. The Protein Journal, submitted.

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Olivier Coriton, *UMR 118 Amélioration des Plantes et Biotechnologies Végétales (APBV), INRA, AgroCampus Rennes, Rennes*

Objectives

Viticulture is an important component of French agriculture. *Muscadinia rotundifolia* is closely related to the *Vitis* genus and it is a source of resistance to several pathogens that is currently being used as a genitor in breeding programmes at INRA. A better knowledge of the similarities between the genomes of *Vitis* and *Muscadinia* would greatly optimise the use of the latter for breeding. The MUSCARES project aims at the development of resources for the analysis and exploitation of the syntheny between *V. vinifera* and *M. rotundifolia*. The objectives of the project are:

1. Development of markers for genetic mapping in *M. rotundifolia*.
2. Genetic mapping in *M. rotundifolia* and development of a comparative genomic map with *V. vinifera*.
3. Study of microsyntheny between *M. rotundifolia* and *V. vinifera* in two regions involved into disease resistance and study of the mechanisms of introgression.
4. Constitution of a collection of *M. rotundifolia* NBS-LRR resistance genes and preliminary testing of candidate genes.

Results

Anchoring a *M. rotundifolia* BAC library to the *V. vinifera* genome sequence and detailed analysis of regions rich in NBS-LRR genes

M. rotundifolia BAC library: 55000 clones, 75 kb average size (7X cover)
86540 BAC-end sequences (BES)
7141 BAC clones aligned in silico to the 12X version of the grapevine genome sequence

***M. rotundifolia* cv Regale Genetic Map**

Sophie Blanc (SVQV), Didier Merdinoglu (SVQV)
Sabine Wiedemann-Merdinoglu (SVQV)

Conclusions and perspectives

•Synteny between *Vitis* and *Muscadinia* is well conserved. KASPar genotyping of the 384 SNPs on the Regale S1 and Fry x Trayshed populations (in progress) will result in a more saturated integrated genetic map allowing a better comparison of both species.
•Automatic annotation of sequences from *M. rotundifolia* BAC clones from LG18 revealed the presence of NBS-LRR genes. Manual annotation and cloning is in progress.

NEWNAM

Creation of a new resource for gene validation in wheat: a NAM population

Génomique Végétale, édition 2008

Coordinator: Sébastien PRAUD (*Biogemma, Chappes)*
Partners: Marie-Reine PERRETANT, Yves LANDEAU, Gilles CHARMET (*INRA GDEC Clermont-Ferrand*) ; Jean-Bruno BEAUFUME (*Limagrain Europe, Verneuil l'Etang*)

Project objectives

The project proposes to create a new high resolution mapping powerful resource in winter bread wheat for further use in genetic studies and breeding purposes. This Nested Association Mapping population will allow a phenotype-driven integrated research:

•introgression of a wide allelic diversity into a common background genotype

↳ better estimates of allelic effects;

↳ plant materials available for direct use in breeding programs (gene stacking, pyramiding, recurrent selection…).

•Population valuable as theoretically free of structure that is the main source of false positive association.

•Population adapted to high density haplotype imputation from low density marker information

↳ efficient cost savings.

Methods and results

•Selection of the founders and the common parent, considering:

(i) a maximal genetic diversity between the lines and the pivotal line,

(ii) improved frequency of interesting alleles for new agronomic traits of interest (drought tolerance, disease resistance, heat tolerance),

(iii) the adaptation of the material to our agronomical environments and for application in breeding programs.

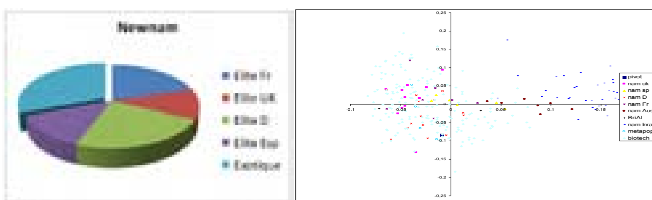


Figure3 : Représentations selon les axes 1 et 2 issus de l'ACP

•Mix of exotic lines, elite cultivars from France, UK and Germany and elite material from other countries (Spain, Australia, western Europe). The pivotal line is a winter elite French line.

•80 connected populations developed, each containing between 100 and 200 lines.

•RILs developed for most of the population and DH lines derived from the original F1 cross for a third of the population.

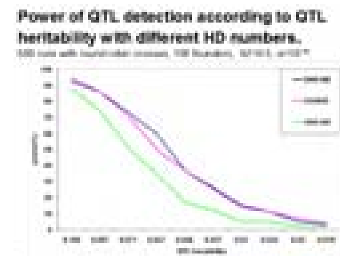
•In parallel, simulation tests and adaptation of available methods and programs to wheat association studies.

•Comparison of the strengths and the weaknesses of this design:

↳ DH part of the population will be good for the inference,

↳ SSD part will increase the resolution.

An optimal number of founder lines is 80-100 for a total progeny of 2,500-5,000.



•The last step of the project is to go on with the genotyping of the parents (densely) and of the population.

↳ DArT genotyping of the parents has already been produced;

↳ part of the population and the parents will be genotyped pretty soon with the 90K array produced by Illumina in the International Consortium.

Conclusions and perspectives

The population is now fully available and ready to be used; it should provide nearly the same power as a large association panel without the spurious associations caused by population structure and at lower cost of genotyping.

A FSOV project has been submitted this year in order to finalize the genotyping of the population, reduce the number of lines to adapt the material to field experiments, and start the phenotyping.

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NUE-MAIZE :

Improving nitrogen use efficiency in maize through functional validation of candidate genes

Réseau de Génomique Végétale , Génoplante 2010 : édition 2009

AGENCE NATIONALE DE LA RECHERCHE
ANR

Coordinator : INRA (Bertrand HIREL)/Département APE. INRA Centre de Versailles Grignon. UMR1318.
Partner : BIOGEMMA (Jacques ROUSTER).

Introduction

The concept of the project NUE-MAIZE is to link the function of genes to agronomic traits for selecting maize varieties adapted to a reduced nitrogen (N) fertilization. Improving N use efficiency (NUE) in maize (which is one of the main plants grown both in France and worldwide) while maintaining an acceptable performance is vital to reduce the excessive use of fertilizer that is harmful to the environment.

Project Objectives

We have developed a multidisciplinary approach including molecular genetics, genomic studies, whole plant physiology and agronomy to identifying key genes involved in regulating NUE including, N absorption, N assimilation and N remobilization.

1) The results already obtained from transcriptome studies have allowed the identification of new structural and regulatory candidate genes putatively involved in the control of NUE .

2) We will validate their function by mutagenesis and genetic engineering.

3) The identification of genes and loci involved in NUE will be used as a basis for developing new transgenic maize high yielding varieties adapted to reduced N fertilization.

Methodology and Results

A) Identification of NUE candidate genes

Maize genes whose expression is altered in leaves and roots as function of N nutrition were identified in two previous GENOPLANTE projects conducted in collaboration with INRA and BIOGEMMA.

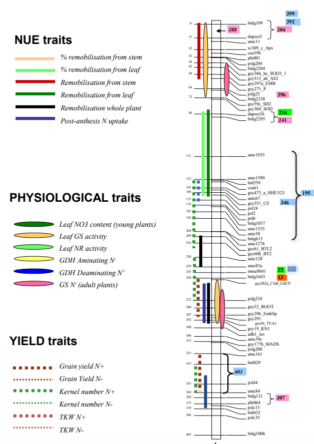

Figure 1. Example of colocalization between QTLs for yield, UE agronomic and physiological traits on chromosome 1 of maize. The candidate genes (represented by a number on the right side of the chromosome) were identified by transcriptome analysis and mapped on the maize genetic map

Bioinformatics studies were then performed to define their function and identify colocalizations with quantitative trait loci (QTLs) involved in the control of plant performance and NUE (Figure 1). We used data from genetic mapping and QTL location available at INRA in BIOGEMMA.

B) Functional validation of candidate genes

We have selected a number of genes for functional validation through mutagenesis (12 candidate genes) and by genetic engineering (18 candidate genes). These genes are involved in a number of metabolic and signalling functions, or have no known biological function. Eighteen molecular constructs over-expressing constitutively the 18 candidate genes were produced.

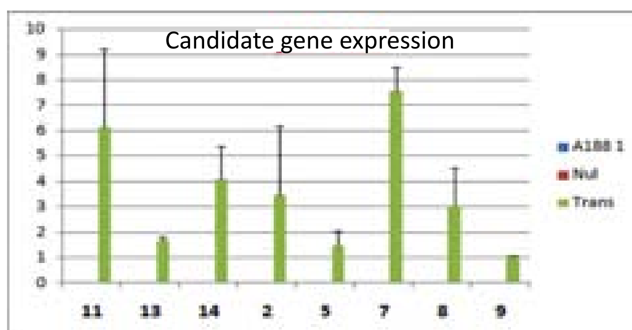

Figure2. Overexpression analysis of a candidate gene in maize. The number on the X axis correspond to different independent transgenic lines.

C) Functional studies : Mutants and Transgenics

A minimum of 10 independent single copy transgenic events were produced for 13 constructs and are being produced for the remaining five constructs. The integrity of the transgene and its expression (quantification of transcripts of the transgene by qRT-PCR) were also determined (Figure 2). Until now, homozygous lines of transgenic maize for four candidate genes were selected to produce hybrid seeds and conduct field trials in 2012 to determine if grain yield is improved. Mutants for nine candidate genes were identified. The introgression of the mutation to obtain a homogeneous genetic background is under way for three genes and was initiated for six other candidate genes.

Conclusions and perspectives

The project made good progress towards identifying candidate genes that can be used to improve NUE. Their functional validation in currently being performed using transgenic plants and mutants. Detailed phenotypic characterization of transgenic plants with increased yield and mutants in which the yield is reduced will be performed using transcriptomics and metabolomics in order to understand why yield is modified.

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biogemma INRA **Partners**

Gene flow from oilseed rape to wild radish

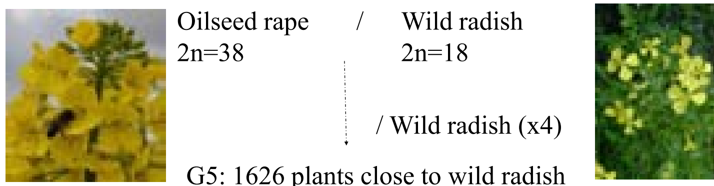
A.M. Chèvre[P1], E.Jenczewski[P2], K. Adamczyk[P3], D. Poulain [P4], M. Leflon [P5], H. Darmency[P6], J. Lecomte [P7]

(P1) INRA, UMR 1349 IGEPP, Le Rheu cedex, France, (P2) INRA , UMR1318 IJPB, Versailles, France, (P3) UR MIA, Jouy, France, INRA, (P4)Agrocampus Ouest, CNRS CERHIO, Rennes, France, (P5) CETIOM, Grignon, France, (P6) UMR AgroEcologie, Dijon, France, (P7) Université Paris Sud, Orsay, France

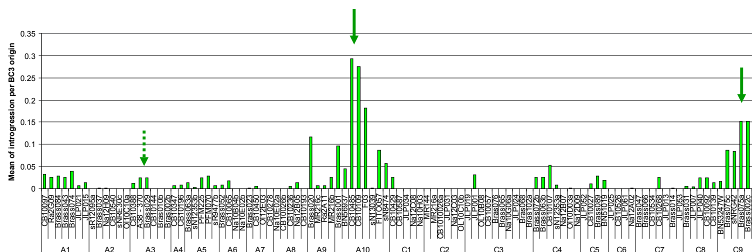
Introduction

Gene flow between a crop and its weeds is one of the critical aspects of environmental risk associated to genetically modified plants. For oilseed rape (*Brassica napus*, AACC, 2n=38), which is a natural allotetraploid between *B.rapa* (AA, 2n=20) and *B.oleracea* (CC, 2n=18), our previous studies showed that interspecific hybrids can form at a very low frequency with wild radish (*Raphanus raphanistrum*, 2n=18). In this project we assessed the following questions: What is the selective value of these hybrids? Does the initial location of a (trans)gene in the oilseed rape genome play a role on its transfer into wild radish genome? Is it possible to identify oilseed rape genetic markers into spontaneous wild radish populations? Is it possible to establish a model from the data? Two strategies were used.

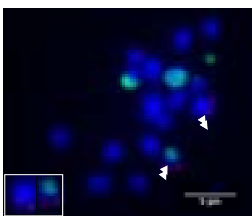
From F1 interspecific hybrids to introgressed wild radish

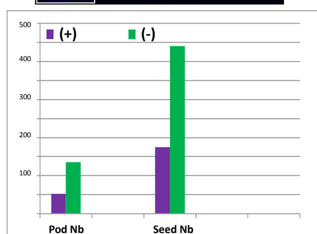


- Selection of 307 plants in G5 with 2n ~18
- Identification of 105 molecular markers specific of oilseed rape, covering 67% of the genome.
- 50% of the plants carried at least one oilseed rape specific marker and only 1% of plants showed a complete chromosome of oilseed rape in addition. Different sizes of oilseed rape genomic regions were observed per plant but the frequency of introgression varied according to the initial location of the oilseed rape marker; loci located on A10 and C9 were more frequently introgressed.



→ Further generations of plants carrying introgression with loci located initially on A3, A10 and C9 were analyzed

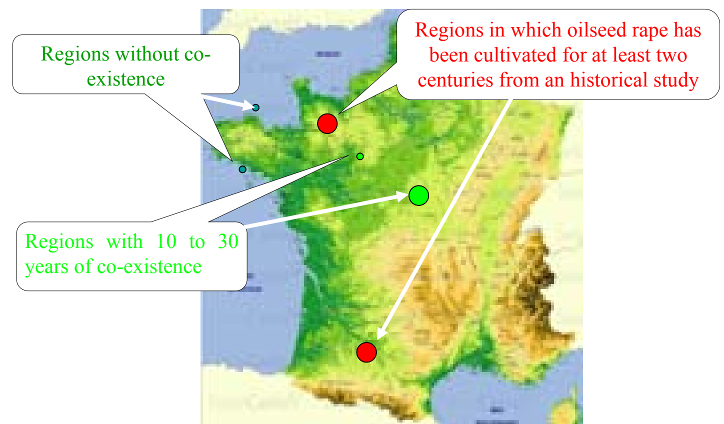




Molecular markers and specific BAC of the regions allowed genetic and physical characterization of the introgressions. Ex: A3 loci carried by a specific *B. rapa* BAC (in red) were located on two different wild radish chromosomes, one of them carrying rDNA (in green).

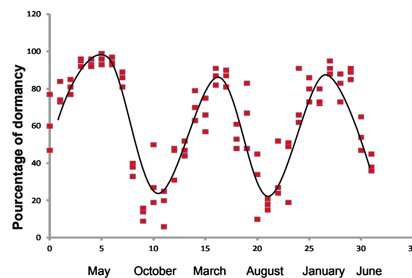
All the introgressed plants showed a transmission rate lower than expected. All introgressions had a fitness cost (ex: A3 introgression).

In spontaneous wild radish populations with different history of coexistence with oilseed rape



137 wild radish populations were collected (2365 plants) and analyzed with 60 molecular markers evenly distributed across oilseed rape genome and absent in wild radish.

Some markers potentially specific of oilseed rape were found only in wild radish populations having a long history of co-existence with oilseed rape. Even if introgression is confirmed, the extended time that a wild radish population has coexisted with oilseed rape had no dramatic effect on its morphological and reproductive traits.



Complementary information on life history traits of wild radish were acquired (ex: seed dormancy)

A stochastic simulation model to predict the fate of an advantageous transgene in a population of wild radish after hybridization with a GM oilseed rape crop was developed. Three descriptors of the invasion by the transgene were defined: the probability of invasion, the number of rotations before invasion onset and the invasion speed. We showed that the large uncertainty on input parameters led to unpredictability for the fate of the transgene in the wild radish population.

Conclusion

We showed for the first time that oilseed rape genes can be introduced into wild radish genome but the stable introgressions observed are complex and the fitness of the plants is often affected as the transmission rate was lower than expected; growth and reproduction were reduced. When working with natural wild radish populations, it is difficult to get the molecular proof that oilseed rape genomic regions are introduced into wild radish genome as both species have a common ancestor. However, using our experimental data, it was possible to establish models which simulate what can happen with gene flow between the two species.

PHENOBLE

Development and utilization of new generation phenotyping tools to analyse genetic determinants of nitrogen fertilisers use efficiency in bread wheat

Programme génomique et biotechnologies végétales, Edition 2010

ARVALIS-Institut du végétal
INRA / UBP UMR GDEC Clermont Ferrand, INRA / UBX UMR BFP Bordeaux, INRA / CIRAD / SupAgro UMR AGAP Montpellier, INRA/ INP UMR AGIR Toulouse, INRA / UA UMR EMMAH Avignon, BIOGEMMA

Objectifs du projet

PHENOBLE proposes to validate, adapt and improve new phenotyping tools by evaluating a collection of wheat elite lines under field conditions differing in terms of nitrogen regimes. This project aims to decipher the genetic factors involved in genotype x environment interactions regarding nitrogen uptake through the development of two innovative phenotyping methods: (1) based on non destructive and rapid automated field platforms for monitoring in the field kinetics of growth and development, (2) based on the join study of the metabolome and transcriptome as ways to prospect the biochemical responses of the plant.

Méthologie et Résultats

The PHENOBLE first field season was conducted in 2011 on several cultivars to produce original results in several areas:

(1) Demonstration of the use of new technologies (metabolomics) and tools (spectrometer, pictures, ASD Labspec® spectrometer) to accurately and precisely phenotype wheat in the field

(2) Identification of new parameters that will be partly predictive of yield in the tested conditions

(3) Improvement of the current methodologies for phenotyping cultivars

(4) Fully equipment of one field location with sensors and devices adapted to the characterization of the whole plant based on several criteria

(5) Creation of database merging all phenotypic informations (agronomics, spectrum, pictures, metabolomics, transcriptomics…).

(6) Dense genotyping of an association panel with SNP that will be available in the public domain in order to identify clear associations between molecular markers and new phenotypes.

Based on the experience of the first year, we will apply in 2012 and 2013 the different tools on a thousand plots to screen the phenotypic variability for the new traits of a set of 200 elite lines.

The database will permit statistical approaches at several steps: estimation of measurements precision level, phenotypic correction of data in case of heterogeneity in the environments studied, computation of complex traits, determination of integrative environmental data, correlations between classical agronomical traits and the new data set and whole genome association studies.

Conclusions et perspectives

PHENOBLE will be the first project to complement automated high throughput in field phenotyping with metabolic/transcriptomic phenotyping. Association genetics derived from PHENOBLE will accelerate the understanding of physiological processes that lead to yield performance and stability under nitrogen stressed conditions.

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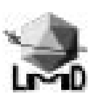


PHYTOSOL-2: **Cloning a candidate gene for a broad-spectrum resistance QTL to *Phytophthora* blight in pepper**

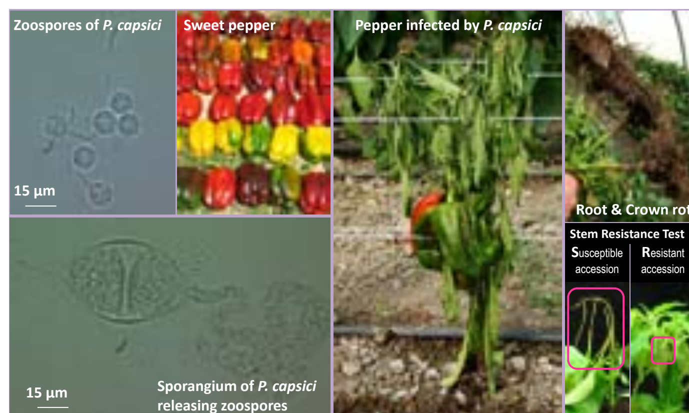
ANR-07-GPLA-008 Edition 2007

AGENCE NATIONALE DE LA RECHERCHE ANR

[1] Lefebvre V (Coordinator), Vandecasteele C, Cantet M, Mallard S, Bouchet JP, Aarrouf J, Blattes-Massire A, Bachellez A, [2] Bergès H, Vautrin S, Prat E, [3] Bendahmane A, Troadec C.

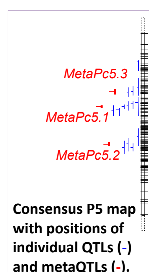
Project aims

Root rot and blight caused by ***Phytophthora capsici*** is one of the most damaging diseases of **pepper**. No *R* gene has yet been reported. Literature reported several accessions displaying partial resistance under polygenic control. A major effect QTL named *Phy-P5* has been systematically detected on chromosome P5. We aim to **determine the molecular basis of the QTL *Phy-P5***, by cloning the responsible gene.



Strategy & Results

- A **meta-analysis** of 14 individual QTLs from literature and INRA maps permitted the identification of a **cluster of 3 metaQTLs**. *MetaPc5.1,* including *Phy-P5,* confers a **broad-spectrum** resistance to at least 12 isolates collected worldwide (Mallard *et al*. 2012).

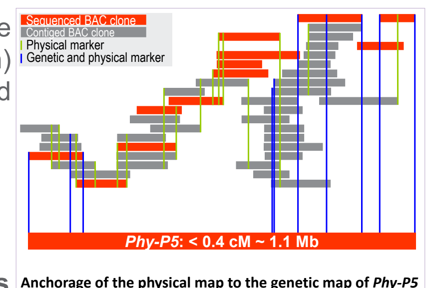


Consensus P5 map with positions of individual QTLs (-) and metaQTLs (-).

- A **fine mapping** based on a recombinant population of **>14,000 plants** delimited *Phy-P5* to **<0.4 cM**. The physical map was constructed by chromosome walking based on two BAC libraries, and anchored to the fine genetic map.

- **12 BAC** clones (the minimum tiling path) were sequenced and assembled.

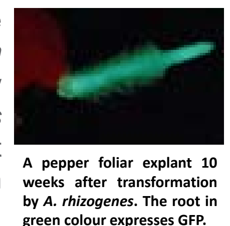
- **The reference sequence of *Phy-P5*** is constituted of **a scaffold of 10 contigs covering 1.1 Mb.**



Anchorage of the physical map to the genetic map of *Phy-P5*

- An automatic and manual gene **annotation** identified **less than 10 ORFs**. **>90%** of the sequence **are transposable elements.** One ORF shows homology with a **transcript differentially expressed in *P. capsici*-infected peppers**, and exhibits **SNPs between resistant and susceptible lines**.

- As pepper is recalcitrant to stable transformation by *Agrobacterium tumefaciens*, we developed a new system to produce *A. rhizogenes* transformed hairy roots. Then, we set up a resistance assay to *P. capsici* on transformed roots (Aarrouf et al 2012).



A pepper foliar explant 10 weeks after transformation by *A. rhizogenes*. The root in green colour expresses GFP.

Conclusions and Prospects

- Pepper has a large genome (~3 Gb), *Phy-P5* belongs to a complex locus **rich in repeat sequences** and with a **low recombination rate**, that hinder the cloning strategy.

- We are now **validating the candidate gene** by *A. rhizogenes* transformation. We will also transform susceptible tomato lines by *A. tumefaciens*. Transformed materials will be assessed for *P. capsici* resistance.

- Identifying the responsible gene should facilitate **marker-assisted selection**, and the study of the **molecular crosstalk between plant and pathogen**.

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(2) **UR 1258 CNRGV**, Castanet-Tolosan.
(3) **UMR 1165 URGV**, Evry.

Mallard *et al*. 2012. A key QTL cluster conserved among pepper accessions and displaying a broad-spectrum resistance to *Phytophthora capsici*: a promising step towards durability. *Submitted*.
Aarrouf *et al*. 2012. *Agrobacterium rhizogenes*-dependent production of transformed roots from foliar explants of pepper (*Capsicum annuum*): a new and efficient tool for functional analysis of genes. *Plant Cell Rep* 31:391-401.

PT-FLAX : Phenotyping and TILLinG of flax EMS mutants

Genom BTV 2010

AGENCE NATIONALE DE LA RECHERCHE
ANR

Coordinateur : S. Hawkins

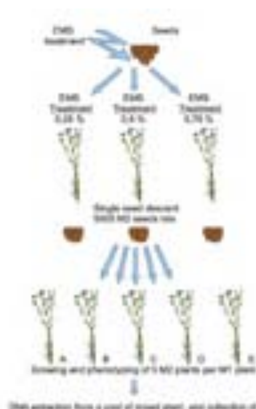
Partenaires : B. Thomasset, B. Chabbert, O van Wuytswinkel, X Guillot, R Tavernier, JP Trouvé

OBJECTIVES

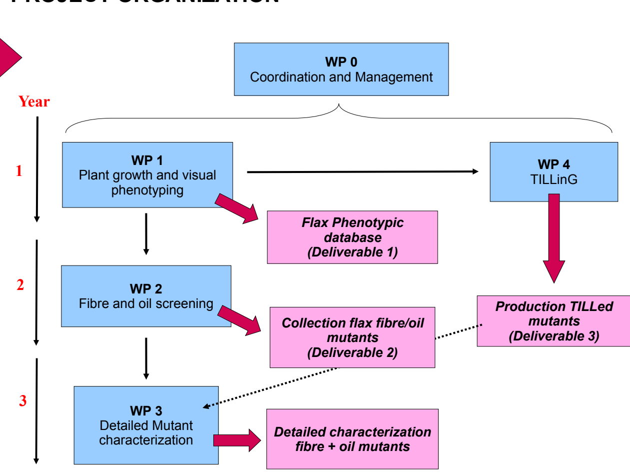
The scientific objectives of this project are 1) to provide an important new genomic resource (flax phenotypic database and TILLinG platform for flax EMS mutants) and 2) to identify and characterize in detail a certain number of flax 'fiber' and 'oil' mutants. This project will ultimately allow us to improve our knowledge about the genetic bases underlying fiber formation and oil biosynthesis in this economically-important species.

STRATEGY

We have previously generated a collection of 5,000 (M2) EMS flax mutants. In this project (2010-2012), forward- (phenotyping) and reverse (TILLinG)-genetic approaches will be used to identify mutants showing modified cell wall structure and oil/seed composition. Initial analyses (phenotyping of 1,000 M2 families in 2009) has allowed us to i) identify a number of potentially-interesting mutants (see below) and ii) identify potentially weak points in our screening strategy. These initial results demonstrate the feasibility of the project.



PROJECT ORGANIZATION



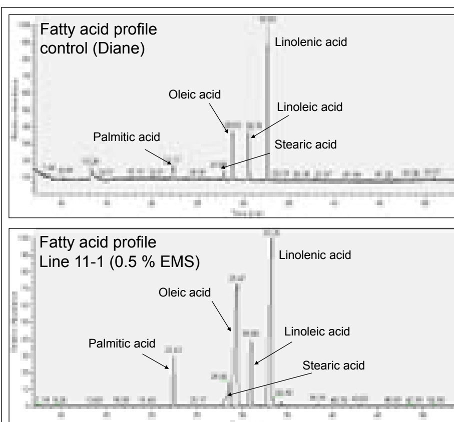
'Dwarf' mutant

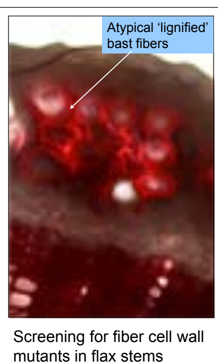
'Pigmentation' mutant

'Capsule (fruit)' mutant

'Branching' mutant

WP1 Visual phenotyping of flax mutants





Screening for fiber cell wall mutants in flax stems

WP2 Screening for flax fiber and oil mutants

WP3 : Detailed mutant characterization: 'Fiber' and 'oil' mutants will be selected for further detailed characterization. Different microscopic (histochemistry, immunolocalisation), physical (x-ray spectroscopy) and chemical analyses (polysaccharide, lignin) will be used to characterize cell wall structure in fiber mutants. Different biochemical and chemical (GC-MS/MS, LC-MS/MS) will be used to characterize lipid metabolism in oil mutants. Flax-specific microarrays (Nimblegen) will be used to analyze the mutant transcriptomes.

WP4 : TILLinG: DNA pools will be prepared from different M2 families and screened (TILLinG, HRM, NGS) in order to identify mutants of pre-selected key genes involved in cell wall formation/development and oil biosyntheisis.

Perspectives : Positional cloning and/or direct sequencing approaches will ultimately allow us to identify those genes responsible for the observed phenotypes. This information will be used in flax breeding programmes to improve quality.

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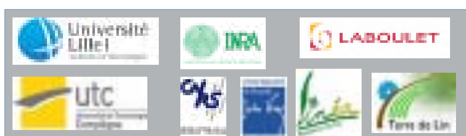

ANR-08-GENM-014

Signaling Peptides and Cytoskeleton Regulators Involved in Plant Disease Susceptibility

Partner 1: Bruno Favery, Isabelle Baurès, Isabelle Damiani, Michaël Quentin Equipe IPN, UMR INRA-UNSA-CNRS IBSV, Sophia Antipolis, France
Partner 2: Harald Keller, Natalia Rodiuc, Laetitia Zurletto Equipe IPO, UMR INRA-UNSA-CNRS IBSV, Sophia Antipolis, France
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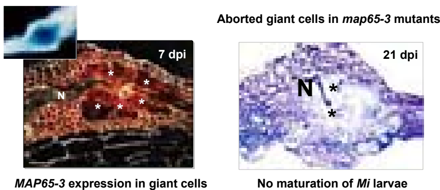
Contact favery@sophia.inra.fr

Summary: The present project aims at understanding genetic reprogramming of the host during disease development by the root-knot nematode *Meloidogyne incognita (Mi)*, the oomycete *Hyaloperonospora arabidopsidis (Hpa)*, and the soilborne bacterium *Ralstonia solanacearum (Rs)*. We found that several plant genes were essential for the compatible interaction with at least two of these pathogens. Among them were genes coding for components of the perception complex of signaling peptides such as Clavata 3 and phytosulphokines (PSKs), which appeared being central for pathogen infection. Successful invasion by all three pathogens required the Microtubule-Associated Protein AtMAP65-3, which is a key player in the organization of microtubule arrays. This project proposes to elucidate the molecular mechanisms underlying Clavata-, PSK-, and MAP65-3- dependent susceptibility. We generated different tools (such as knock-out and knock-down lines, overexpressors, reporter lines, EMS mutants, Y2H interaction libraries) that are exploited to dissect the disease susceptibility signaling pathways. The novelty of this project resides in the coordinated effort to understand the mechanisms underlying disease development caused by three pathogens with different life styles and colonization strategies. A better characterization of these mechanisms may allow elaborating novel disease control strategies.

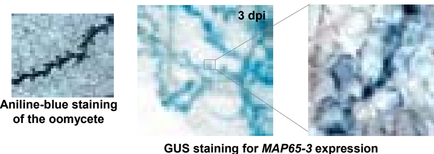
WP1: MAP65-3

Essential for nematode-induced giant cell ontogenesis



MAP65-3 expression in giant cells — No maturation of *Mi* larvae

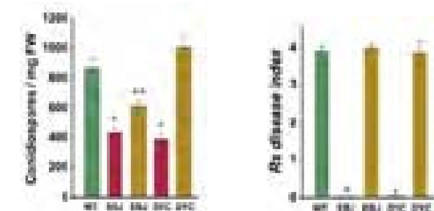
Locally expressed in cells with *Hpa* haustoria



Aniline-blue staining of the oomycete

GUS staining for *MAP65-3* expression

Mutants are more resistant to the oomycete & bacterium



Oomycete asexual reproduction are reduced by 50% in *map65-3* mutants (*dyc, ebj*) and restored in complemented mutant lines

no bacterial symptom in *map65-3* mutants

Constitutive and *Hpa*-inducible upregulation of defense genes restricted to *map65-3* shoots

Genome-wide transcriptome analysis of map65-3 inoculated or not with *Hpa* compared to WT

Among genes upregulated constitutively in map65-3: 41% are related to « response to abiotic or biotic stimulus » including SA- or JA/ET associated genes

PR1, PR2, PR5, PDF1.2, WRKY70...

Identification of MAP65-3 signaling components involved in the pathogen response

Characterization of MAP65-3 interacting proteins (MIPs)

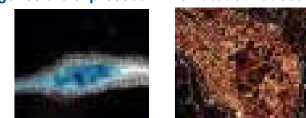
Yeast-2-hybrid (Y2H) screens of libraries obtained from uninfected tissues and nematode- or oomycete-infected tissues.

BUBs spindle assembly checkpoint proteins are MIPs

Surveillance mechanism ensuring chromosome – MT attachment

BUB3.1 on unattached kinetochores

BUBs genes are expressed in nematode-induced giant cells

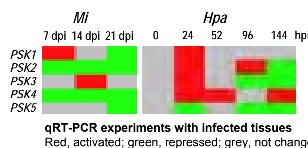


ProBUBR1:GUS expression in galls and giant cells

WP2: Phytosulfokines

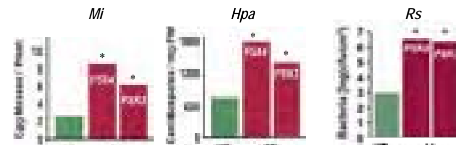
PSKs are tyrosine-sulfated peptides

AtPSK genes are differentially expressed during infections



qRT-PCR experiments with infected tissues
Red, activated; green, repressed; grey, not changed

PSK production correlates with disease susceptibility



PSK overexpression results in increased nematode, oomycete and bacterium susceptibility

The leucine-rich repeat PSK receptor kinase, AtPSKR1, is essential for full susceptibility to the 3 pathogens

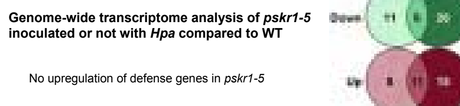
Four allelic *pskr1* mutants reveal similar phenotypes

-- See Natalia Rodiuc poster --

Patent — Rodiuc N, Marco Y, Favery B, Keller H (2011). "PLANTS RESISTANT TO PATHOGENS AND METHODS FOR PRODUCTION THEREOF (*Phytosulfokines and their receptor as novel breeding targets for plant resistance to diverse pests*)". Génoplante-Valor **WO/2012/017067**.

AtPSKR1-dependent disease development is independent of plant defense

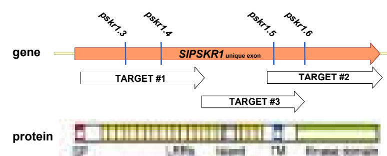
Genome-wide transcriptome analysis of *pskr1-5* inoculated or not with *Hpa* compared to WT



No upregulation of defense genes in *pskr1-5*

Prevalorisation – proof of concept in tomato

Identification of PSKR1 ortholog in tomato and of 4 missense mutations by TILLING



Characterization of AtPSKR1 interacting proteins

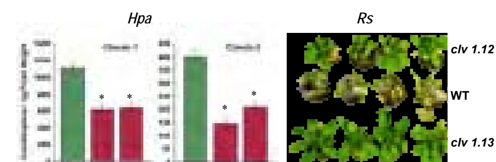
Yeast-2-hybrid (Y2H) screens of libraries obtained from uninfected tissues and nematode- or oomycete-infected tissues.

9 proteins interact with the kinase domain; 3 with the LRR domain, including one LRR-RLK protein

WP3: Clavata

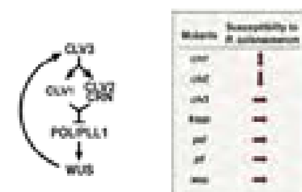
CLAVATAs regulate stem cell fate in the meristem

The leucine-rich repeat receptors, CLV1 and CLV2, are essential for susceptibility to the oomycete & the bacterium

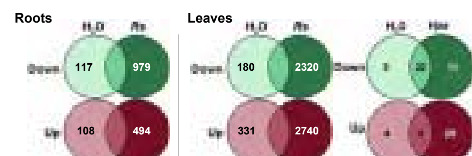


Complementation of *clv1* with a *proCLV1:CLV* gene fusion restores the susceptibility phenotype

Major components of the CLV signaling pathway do not participate in susceptibility to *Rs*.



Comparative whole-genome transcript profiling of *Rs*- and *Hpa*-inoculated WT and *clv1* or *clv2* tissues



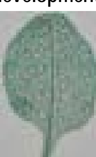
Upregulation of several transcription factors in *clv1* and *clv2* mutants

Very little overlap in the reprogramming of gene expression in roots and leaves during disease development

Expression of *CLV1* during infection and plant development

Subcellular localization with *ProCLV1:CLV1:YFP*

Spatio-temporal expression with *ProCLV1:GFP:GUS*



CLV1 expression in vascular tissues

Characterization of CLV1, CLV2 interacting proteins

Screen of Y2H library obtained from bacteria-infected tissues

CLV1 interacting proteins identified not validated using FRET-FLIM

Caillaud *et al.*, (2009). Spindle Assembly Checkpoint Protein Dynamics Reveal Conserved and Unsuspected Roles in Plant Cell Division. **PloS ONE**, 4 : e6757
Rodiuc et al. (2012). Evolutionarily distant pathogens recruit the Arabidopsis phytosulfokine signaling pathway to establish disease. **Cell Host & Microbe**, *submitted.*
Hanemian et al. *CLV1*, an *Arabidopsis thaliana* gene required for full susceptibility to the bacterial pathogen, *Ralstonia solanacearum*. *In preparation.*

SingleMeiosis : Identification of the whole set of meiotic recombination events in a single meiosis of *Arabidopsis thaliana*

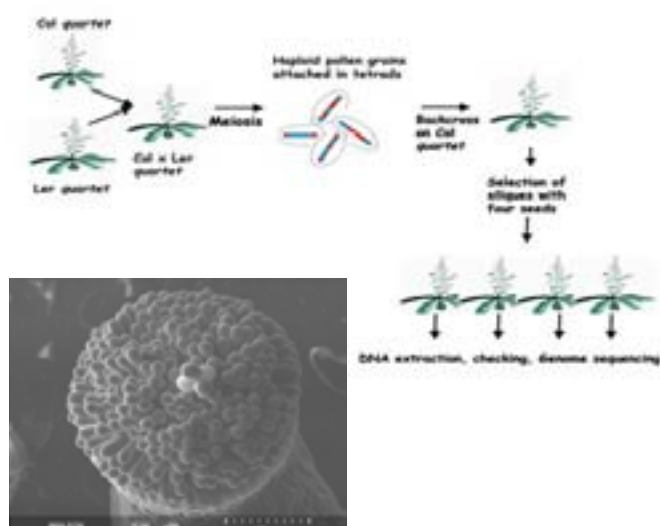
Plant Genomics 2009

ANR — Agence Nationale de la Recherche

Coordinateur: **Christine Mézard**, IJPB, INRA Versailles
Partenaires: **Matthieu Falque et Olivier Martin**, GVM, INRA/CNRS/Univ Paris-Sud/ AgroParisTech, **Marie-Christine Le Paslier et Dominique Brunel**, EPGV, INRA, Evry

Objectifs du projet

In a single meiosis of the yeast *Saccharomyces cerevisiae*, it has been shown that at least 1% of the genome is concerned by meiotic recombination events (Mancera et al., 2008; Chen et al., 2008). The aim of this project is to better understand the constraints on the localization of recombination events in meiosis in plants , using the model plant *Arabidopsis thaliana*. We want to establish for the first time the number, the precise localization and the properties of all the recombination events (crossovers (CO) and simple gene conversions now called non-crossing over (NCO)) that happen during a single meiosis in a higher eukaryote. The understanding of meiotic mechanisms is a key step towards the control of sexual reproduction and the introduction of traits of interest into plants. We will generate four plants issued of sister spores from a single meiosis (Figure 1). To obtain this combination, we make use of the *quartet* mutation that allows the recovering of the four pollen grains issued from a single meiosis because they stay attached until the end of gametogenesis (Preuss et al., 1994). Such a tetrad of spores resulting from a meiosis of a F1 hybrid plant is used to backcross on one parent of the hybrid. The four seeds are sowed and the DNA extracted from the corresponding plant. The DNA is then sequenced using the Illumina Genome Analyzer GAII.



Résultats

From more than 1000 crosses performed during the first 18 month of the project, only a few tens of fruits with four seeds have been obtained. When we checked the DNA extracted from the corresponding plants using a set of 11 microsatellites distributed on the 5 pairs of chromosomes, only 4 set of four plants corresponded to true products of a single meiosis. The others were due to multiple independent fertilizations with different tetrads even if pistils were cautiously isolated after fertilization.

Genomic DNA obtained from the first two tetrads the, two parents (Columbia and Landsberg *erecta*) and the F1 were sequenced using GAIIx in pair-ends 101 bp.
10 crossovers were scored in the first tetrad and 9 in the second tetrad.

Conclusions et perspectives

10 additional tetrads will be sequenced. Bioinformatic analyses will be performed to detect non crossover events Then we will analyze in details the localization of iwith genomic features

Authors

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Frank Gauthier, Matthieu Falque, Olivier Martin
Maria Tchoumakov, Marie-Christine Le Paslier, Dominique Brunel

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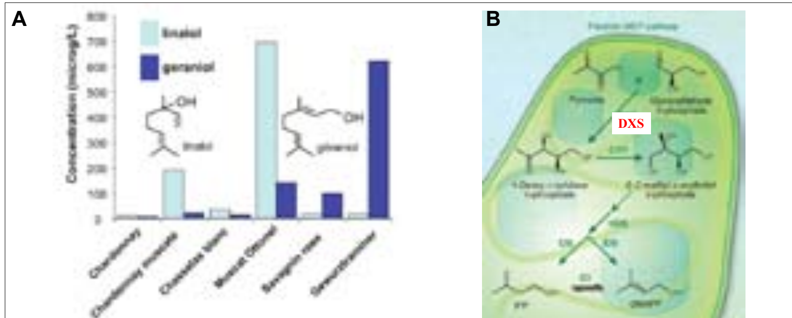
Functional genomics of aroma compounds biosynthesis in grape berries

VITAROMA (ANR-09-GENM-023, 2010-2013)

Partner 1: Philippe Hugueney (Coordinator), Raymonde Baltenweck, Gisèle Butterlin, Patricia Claudel, Eric Duchêne, Marc Fischer, Andrea Ilg, Nathalie Jaegli, Sophie Meyer, UMR 1131 SVQV, INRA/Univ. de Strasbourg, Colmar
Partner 2: Eric Gomès, Stéphane Decroocq, Serge Delrot, Sabine Guillaumie, Claudine Trossat-Magnin, UMR INRA/Univ. Bordeaux 1287 EGFV, ISVV, Bordeaux
Partner 3: Philippe Darriet, Maxime Brette, Monique Pons : USC 1219 INRA/Univ. Bordeaux EA 4577 Œnologie, ISVV, Bordeaux

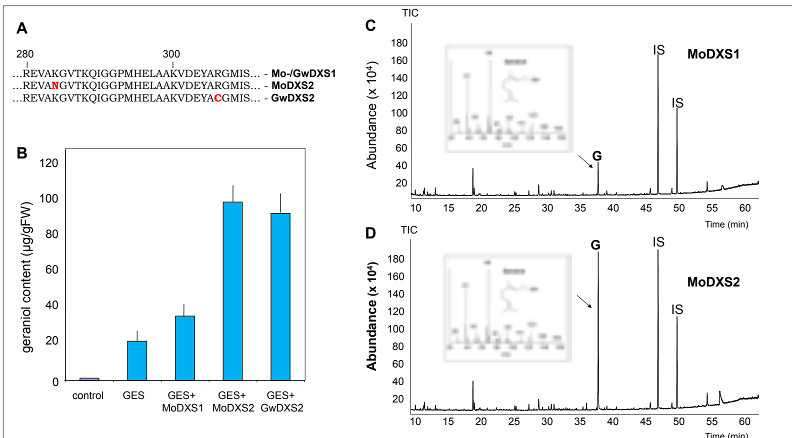
Introduction

Despite the major importance of grape aromas, either as positive attributes of wine typicity or as detrimental off-flavours, very few genes and enzymes involved in aroma compounds biosynthesis have been characterized in grapevine. The aim of this project is therefore to combine genetic, transcriptomic and metabolomic approaches to identify candidate genes potentially involved in aroma compounds biosynthesis in grape berries, with an emphasis on **monoterpenols** (left panels) and **pyrazines** (right panels). The function of these candidates will be characterized, in order to identify their role in aroma biosynthesis in grape berry.



The origin of aromatic grape varieties

Terpenes, such as linalool and geraniol are important aroma of wines. High linalool and geraniol content is responsible for the typical flavours of aromatic wines, such as muscats and gewurztraminer (**A**). Muscat and gewurztraminer can therefore be considered as **natural terpene overproducers**. Genetic analysis of the aromatic character in pointed out the DXS gene as a candidate for this overproducer phenotype (Duchêne et al., 2008). 1-deoxy-D-xylulose 5-phosphate synthase (DXS) catalyzes the first step in the plastid-localized pathway of terpene biosynthesis (MEP-pathway) (**B**). DXS alleles from Muscat and Gewurztraminer have therefore been cloned and characterized.



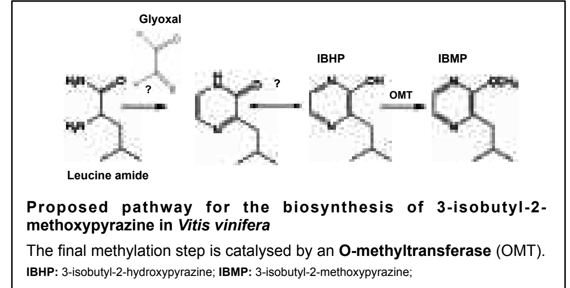
Characterization of DXS alleles responsible for enhanced terpene biosynthesis

(A) Muscat and Gewurztraminer are heterozygous at the DXS locus, sharing the DXS1 allele and having different DXS2 alleles. SNPs lead to an amino acid exchange in DXS2 (MoDXS2: K_{284}→N; GwDXS2: R_{306}→C).
(B): *In planta* geraniol biosynthesis, following transient co-expression of geraniol synthase from basil (GES) and DXS alleles in tobacco leaves. Untransformed tobacco leaves (control), leaves expressing GES alone and leaves co-expressing GES and DXS alleles were analysed using GC-MS, 4 days post *Agrobacterium*-mediated transformation. For each condition, geraniol amounts are means (± standard errors) of 9 independent experiments.
(C, D): Typical GC-MS analyses of extracts from tobacco leaves transiently co-transformed with GES and either MoDXS1 (**C**) or MoDXS2 (**D**).
Expression of the DXS alleles MoDXS2 (K_{284}→N) and GwDXS2 (R_{306}→C) lead to enhanced terpene biosynthesis in transformed plants. Due to the potential biotechnological applications of these genes to enhance the production of valuable terpenes in plants , these results are the subject of the European patent n°100138098-2403 Génoplante-Valor.

Perspectives

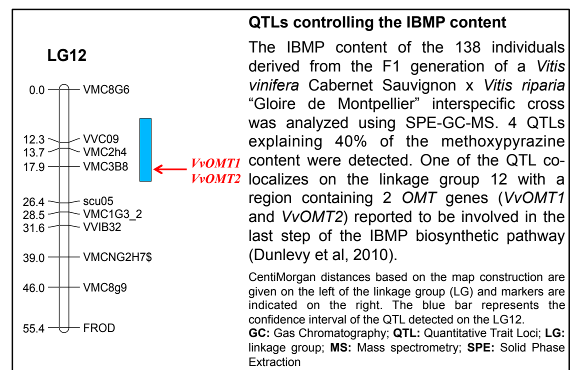
The VITAROMA project has already led to the characterization of several genes involved in the biosynthesis of major grape aroma, one of them being the subject of a European patent (Hugueney et al., 2010). Several additional candidate genes potentially involved in terpene or pyrazine biosynthesis are currently under investigation. In particular, genomic regions corresponding to 3 more QTLs are currently under analysis to identify other candidate genes for IBMP biosynthesis. Meanwhile, several alleles of *VvOMT1* and *VvOMT2* have been isolated from high and low IBMP producers, and the corresponding proteins are currently being functionally characterized by heterologous expression in *E. coli*. The functional characterization of the candidate genes involved in IBMP biosynthesis will be pursued and involves a close collaboration between the three partners of this project. Special emphasis will be placed on the characterization of the kinetic properties of the proteins encoded by various alleles of *VvOMT1* and *VvOMT2*.

Hugueney P, Duchêne E, Merdinoglu D (2010) 1-deoxy-D-xylulose 5-phosphate synthase alleles responsible for enhanced terpene biosynthesis. European patent n°100138098-2403 Génoplante-Valor.



Proposed pathway for the biosynthesis of 3-isobutyl-2-methoxypyrazine in *Vitis vinifera*

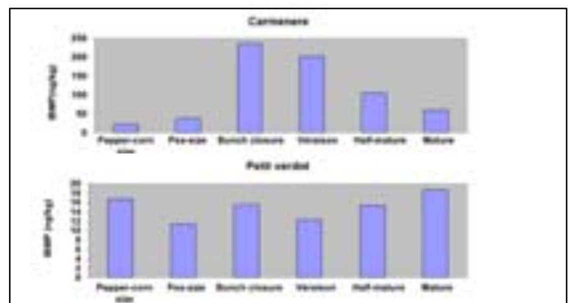
The final methylation step is catalysed by an **O-methyltransferase** (OMT).
IBHP: 3-isobutyl-2-hydroxypyrazine; **IBMP:** 3-isobutyl-2-methoxypyrazine;



QTLs controlling the IBMP content

The IBMP content of the 138 individuals derived from the F1 generation of a *Vitis vinifera* Cabernet Sauvignon x *Vitis riparia* "Gloire de Montpellier" interspecific cross was analyzed using SPE-GC-MS. 4 QTLs explaining 40% of the methoxypyrazine content were detected. One of the QTL co-localizes on the linkage group 12 with a region containing 2 *OMT* genes (*VvOMT1* and *VvOMT2*) reported to be involved in the last step of the IBMP biosynthetic pathway (Dunlevy et al, 2010).

CentiMorgan distances based on the map construction are given on the left of the linkage group (LG) and markers are indicated on the right. The blue bar represents the confidence interval of the QTL detected on the LG12.
GC: Gas Chromatography; **QTL:** Quantitative Trait Loci; **LG:** linkage group; **MS:** Mass spectrometry; **SPE:** Solid Phase Extraction



IBMP content in whole berries of two highly contrasted genotypes throughout development

The amount of IBMP is always greater in the Carmenere berries than in Petit Verdot ones.
Carmenere IBMP levels increased until "bunch closure" stage and then declined toward mature stage.
IBMP concentrations are expressed as ng/kg of fresh weight.

Gene Name	Gene Expression Values (FPKM)			
	Bunch closure stage		Half mature stage	
	Carmenere	Petit Verdot	Carmenere	Petit Verdot
VvOMT1	44.1	28.2	1.9	0.5
VvOMT2	9.1	6.6	0.3	0.1

Expression pattern of VvOMT1 and VvOMT2 genes

The comparative transcriptomic berry analysis (RNA-seq) was performed on Carmenere (strong IBMP producer) and Petit Verdot (almost no IBMP production). Two stages of harvest were compared: bunch closure and half-mature stages. The timing of the expression of VvOMT1 and VvOMT2 was associated with the period of IBMP accumulation in berries. At the bunch closure stage, expression of both genes was highest in Carmenere samples than in Petit Verdot ones especially for *VvOMT1*.
Gene expression value are expressed as fragments per kilobase of exon per million fragments mapped (FPKM)

ANR GENOPLANTE ISVV INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN BORDEAUX AQUITAINE UNIVERSITÉ DE BORDEAUX UNIVERSITÉ DE STRASBOURG INRA

WHEATPERFORMANCE

Genetic control of Yield components in Wheat

Génomique Végétale, édition 2007

Coordinator : Sébastien PRAUD (*Biogemma, Chappes*)
Partners:
Jérôme SALSE, Christine GIROUSSE, Vincent ALLARD, Gilles CHARMET (*INRA GDEC Clermont-Ferrand*) ;
Katia BEAUCHENE (*Arvalis - institut du végétal*);
Mickaël THROUDE (*Biogemma, Chappes*).

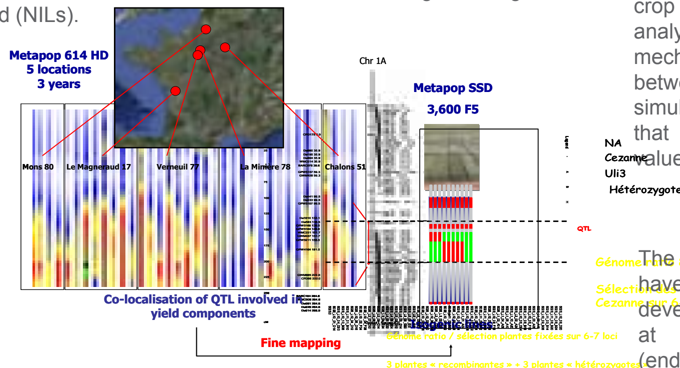
Project objectives

Our research program aimed at studying how yield potential can be fully achieved and how its stability can be improved. The main focuses were :

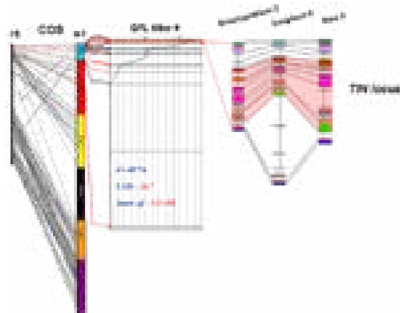
1) the genetic components of yield potential,
2) the influence on yield of major genes involved for varieties adaptation to their environment
3) the effect of heat stress on wheat kernel filling.

Methodology & results

The project advanced the fine mapping of 82 Meta-QTLs for reproductive organs development and photosynthetic apparatus as proxy for yield: the confidence intervals of QTL derived from elite diallel population and explaining a good part of agronomical traits as grain number/m², tiller number, TKW and biomass were reduced with SNP markers and 22 QTLs were validated in homogenous genetic background (NILs).



A major QTL for tillering (tin 1A) derived from a oligoculm line was precisely delimited to a region containing 10 genes, thanks to the syntenic relationships between sequenced plant genomes and wheat. Another minor tillering gene (tin 3A) has been precisely fine mapped.

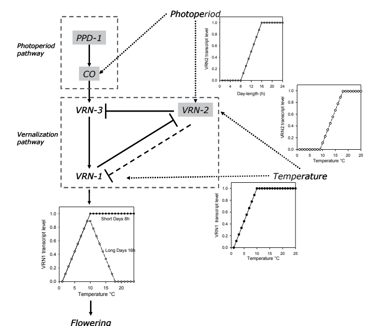


A mixed approach merging transcriptomic, synteny and classical genetics allowed a QTL for TKW to be delimited to a region of 78 genes and one promising gene selected for transgenic validation.

We obtained robust yield component and developmental rhythm phenotypic data from two connected panel : a Balance panel of 50 lines chosen to be well balanced in terms of major genes. Allele content has been evaluated in 10 contrasted locations in order to quantify their impact on yield achievement

A total of 300 elite lines have been phenotyped (3 locations * 2 years) for agronomics traits in the field and for photoperiodic response in controlled chambers. Using a huge amount SNP markers developed in others frameworks, we have been able to proceed genome wide association studies through mixed model approaches and we obtained an interesting heat that will accelerate discovery and characterization of regions and genes for yield components

In terms of modeling, we have in parallel enriched the Sirius crop model developed by INRA. Focusing on the Vrn1 genes, we have analyzed their effects on the vernalization requirements using an original mechanistic framework that gave new information on the interactions between the homeoalleles. This study delivered the first wheat simulation model based on gene actions, and a new module in Sirius that automatically converts vrn1 allelic combinations into parameters values for future simulations.



The last but not least, we have initiated the first developments of a protocol at a microscopic scale (endosperm, embryo and teguments cells volume and number). Firsts evidences of the most critical and susceptible phases to maintain the grain development under hydric or heat stresses have been precisely characterized. We now need to correlate that with data produced in field conditions and analyze the genetic variability for these parameters.

Conclusions & perspectives

The project achieved most of its goals and has generated a huge quantity of data that still need to be fully exploited.

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GAINSPEED

Genome sequence, Association mapping and NILs to SPEED up QTL tagging on wheat chromosome 3B

Génomique Végétale, Edition 2009

Coordinator: Sébastien FAURE (*BIOGEMMA*)

Partners:
Sébastien PRAUD, Nadine DURANTON, Jean-Philippe PICHON, Nathalie RIVIÈRE and Jorge DUARTE (*BIOGEMMA*), Catherine FEUILLET, Etienne PAUX, Pierre SOURDILLE, Charles PONCET, Bouzid CHAREF and Stéphane BÉNÉDIT (*INRA*)

Project objectives

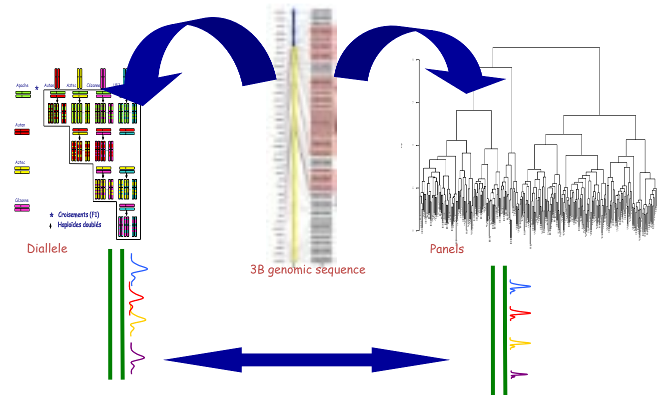
The main objectives of this project in hexaploid wheat are:

To develop an integrated approach based on association mapping and classical NILs mapping to speed up genetic mapping towards the cloning of QTLs of agronomic interest.

To use the genomic sequence produced in a companion project (3BSEQ) and the NimbleGen Sequence Capture technology to accelerate marker development.

To reduce the confidence interval (from 30cM to 1-5 cM) around four QTLs on chromosome 3B and lay the foundation for marker-assisted selection and QTL cloning.

More in general, this project aims at laying the foundation for faster and more efficient marker-assisted selection in wheat, drawing on all available resources and bringing together industrial objectives with fundamental research developed in a companion project.



Methods and Results

Marker saturation of the QTL intervals

201 SSR, 711 ISBP and 118 SNP tested for position in metapop and polymorphism in association panel

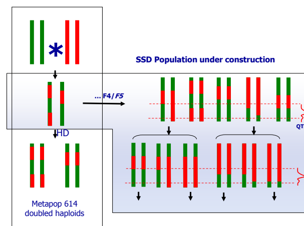
QTL28 (yield and yield components): 11 SSR, 5 ISBP and 22 SNP
QTL29 (flowering time): 1 SSR, 1 ISBP and 19 SNP
QTL30 (tillering) and QTL100 (pre-harvest sprouting): 1 SSR, 1 ISBP and 15 SNP

Association results point towards specific markers, but map resolution reveals several distinct peaks under each QTL. Ordered genomic sequences might provide the answer.

Using the markers thus mapped, 1949 scaffolds developped in the 3BSEQ project were identified as located under our QTL, representing 534Mb and containing 7950 automatically annotated genes that will be used as baits for SNP detection using Sequence Capture.
Moreover, using the 3BSEQ sequences, 55000 ISBP will be used as baits for ISBP-SNP identification by SureSelect capture.

NIL creation for QTL validation

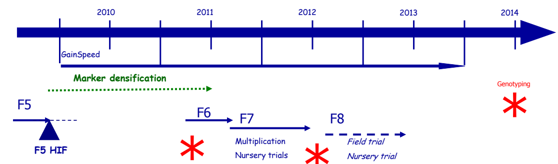
Markers flanking each QTL were used to screen F5 lines. The F6 seeds obtained from the selected F5 constitute the basis of the NILs (HIF) for the QTL validation



QTL28: followed in 3 crosses, with respectively 3, 5 and 7 HIF_S1 individuals selected. In each case, at least 2 homozygous lines for each allele could be indentified in the HIF_S2 generation.

QTL29: followed in 2 crosses, with 2 HIF_S2 families with both homozygous alleles selected for both crosses.

QTL30: followed in 2 crosses, with 3 HIF_S2 and 2 HIF_S2 respectively.



Conclusions and perspectives

The material for QTL validation is now in the seed multiplication phase, with a first validation in nursery expected in 2012-2013 for tillering and flowering time.
New technologies for sequence capture, next generation sequencing and high-throughput genotyping have allowed to reconsider the marker development phase of the project. Thus, a larger number of ISBPs will be screened for ISBP-SNP identification.
Although the resolution of the genetic map in the QTL detection population has been increased with the new markers, the structure of the population itself hampers further saturation. However, LD mapping using the association panel will enable us to order markers within clusters and to dissect the QTL regions. Moreover, with the NILs population developed for each QTL and the targeted markers, the foundations for positional cloning will be laid.

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WALLTALK - The Plant Cell Walls: Where Microbes Meet Plants

Program: Genoplante 2007 – Project: ANR-07-GPLA-014

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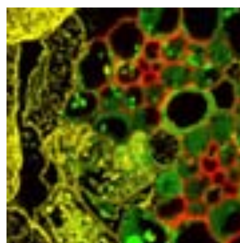
WALLTALK: Role of the plant cell wall in plant – microbe interactions

The first encounter between a pathogen and a plant cell takes place at the plant cell wall. Paradoxically, the extent to which the plant cell wall determines the success or failure of pathogen attack is still largely unknown. To address this issue, we performed an in-depth analysis of *wat1* (*walls are thin1*), an Arabidopsis mutant which is characterized by both a fiber cell wall defect and enhanced resistance to the soil-borne, vascular bacterial pathogen, *Ralstonia solanacearum* (*Rs*). Beyond the functional characterization of WAT1 in both the developmental and pathology context, the objectives of this project were i) to determine how plant cell walls are modified during the infection process with *Rs* and ii) to identify novel resistance mechanisms to *Rs*.

WP1: Identification of *Rs*-induced plant cell wall modifications

Dignonnet et al., in preparation.

✓ **Deciphering the route followed by *Rs* to colonize Arabidopsis** roots during a compatible interaction.
✓ Penetration by the **root apex**, propagation in the **intercellular spaces**, gain access to the **vascular vessels** via two **specific pericycle cells**, proliferation.
✓ Selective **degradation of pectin** thanks to an appropriate enzymatic arsenal.

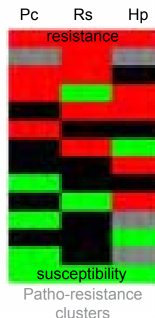


Rs: yellow; red: pectins; green: autofluorescence

WP2: Search for new cell wall mutants with an altered response to *Rs*

Rivière et al., in preparation.

✓ More resistant/susceptible lines to *Rs, H. arabidopsidis* (oomycete) and *P. cucumerina* (fungus) among 80 putative cell wall mutants (***pwm***): **patho-resistance clusters.**
✓ Cell wall composition and digestibility, plant biomass, seed production, and resistance to dehydratation were analyzed.
✓ **Uncoupling resistance to pathogens from trade-offs** by remodeling Arabidopsis cell wall architecture.



Patho-resistance clusters

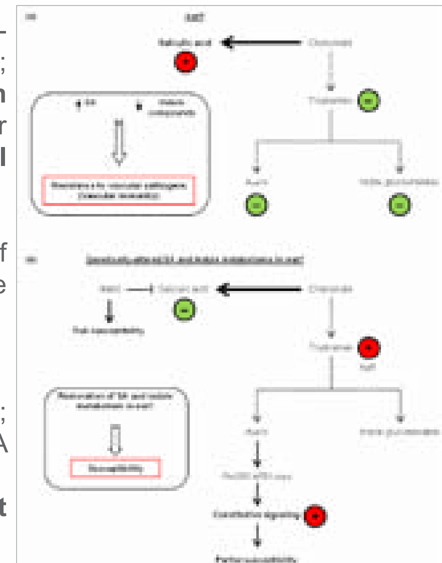
WP3: Detailed characterization of *wat1*

Denancé et al. The Plant Journal (in revision); Arrighi et al., in preparation; Ranocha et al., in preparation; Denancé et al., Plant Signaling & Behavior (2010); Ranocha et al., The Plant Journal (2010).

✓ WAT1: a **tonoplast-localized nodulin** protein; a novel type of **auxin transporter**; required for **secondary cell wall** formation in fiber stem.

✓ **Cross-regulation** of salicylic acid and indole metabolism in *wat1* **roots**
✓ **Vascular immunity**

✓ **VTR**: *wat1* suppressor; role in chloroplastic RNA splicing
✓ Highlight: **chloroplast** in **plant immunity**



Working model of *wat1*-mediated resistance to vascular pathogens.
(a) In *wat1-1* roots, a metabolomic reorientation occurs from the chorismate towards SA instead of indole metabolism. Levels in Trp, IAA and IGS are lower but content in SA is higher. That creates a new metabolomic balance which may constitute an environment hostile for pathogen colonization in xylem sap, resulting in the establishment of a vascular immunity in *wat1*.
(b) When the new hormonal balance is disrupted in *wat1* by a genetic approach, resistance to pathogens is lost, at least partially, indicating that the reduction of the susceptibility in *wat1* is due to alteration of the crosstalk between SA and indole metabolism.

Conclusions and Perspectives

The WALLTALK project provides the first description of Arabidopsis colonization by *Rs* coupled to the resulting cell wall modifications in plant roots. The identification of WAT1 as a vacuolar auxin transporter is a major discovery in the auxin biology field. The extinction of WAT1 results in the systematic resistance to all vascular pathogens, presumably via a novel mechanism involving the reorientation of indole metabolism in favor of salicylic acid, specifically in the root. Finally, the identification of a battery of new cell wall mutants, in addition to *VTR*, opens new avenues to uncover novel, previously unsuspected mechanisms involved in plant defense against pathogens.

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