

Spécialité: BIDAP - Biologie, Interactions, Diversité Adaptative des Plantes

Ecole Doctorale: GAIA - Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau

Titre: **Small RNA biology of plant-pathogen interactions in single and dual infections with viral and non-viral pathogens of cocoa plants**

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Encadrement: L'encadrement et la direction de la thèse seront assurés par Mikhail POOGGIN (DR2, INRA) qui coordonne au sein de l'équipe B2B2 (Biodiversité des badnavirus exogènes et endogènes) de BGPI. M. Pooggin a encadré de nombreux étudiants et quatre thésards à UniBasel. L'équipe B2B2 compte 9 personnes dont 6 permanents (trois chercheurs CIRAD, un directeur de recherche INRA (M.Pooggin) et deux techniciennes). Le/la thésard(e) travaillera en étroite collaboration avec ses deux encadrants et une technicienne. Le/la candidat(e) recevra une formation approfondie dans des techniques de pointe en biologie moléculaire végétale et phytopathologie moléculaire et sera, quand nécessaire, aidé(e) par le personnel technique de l'équipe pour la réalisation de ces expériences. Il/elle participera à des conférences scientifiques nationales et internationales pour compléter ses connaissances sur l'état de l'art en biologie végétale et phytopathologie moléculaire.

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Mots clés: Epigenetics; small RNA; gene silencing; RNAi; plant pathogens; virus; oomycete;

Profil candidat: Formation en Biologie Moléculaire, Bioinformatique; Phytopathologie ou Biologie Végétale

Training in molecular biology, bioinformatics, phytopathology or plant biology

Description de la problématique de recherche:

Multiple and recurrent pathogen infections pose serious constraints on cultivation and breeding of perennial plants such as cocoa. The epigenetic mechanisms based on small RNA-mediated RNA interference (RNAi) and gene silencing contribute to plant defences against pathogen invasions, but successful pathogens can suppress small RNA biogenesis and/or action. In mixed infections, synergistic counter-defence activities of viral and non-viral (bacterial, fungal, or oomycete) pathogens may result in severe disease outbreaks.

Cocoa plantations in Côte d'Ivoire, the largest cocoa producer in the world, are currently being co-invaded by two aggressive pathogens, the pararetrovirus *Cocoa swollen shoot virus* (CSSV) and the oomycete *Phytophthora megakarya*, both posing a major threat to cocoa cultivation with

socio-economic consequences. Dr. Neema and co-workers at BGPI are conducting a survey of the plantations in Côte d'Ivoire for the presence of these and other viral and non-viral pathogens with a main goal to investigate the genetic diversity of cocoa pathogens and the frequency of mixed infections with synergistic interactions between pathogens.

In the frame of the PhD project we will focus our study on small RNA biology of the plant interactions with CSSV and *P. megakarya* in individual and mixed infections of cocoa plants selected during the field survey as well as investigated under controlled greenhouse conditions by inoculating seedlings with the infectious clone of CSSV (Jacquot et al. 1999) and/or *P. megakaria* aggressive and mild strains (mastered at BGPI by Dr. Muller and Dr. Ducamp, respectively). For the greenhouse studies two cultivars of *Theobroma cacao* will be investigated, one lower-Amazonian Forastero (Amelonado genetic group), highly susceptible to the two pathogens, and one upper-Amazonian Forastero, tolerant to *P. megakarya*. The genome resources are available for a representative of the Amelonado genetic group (Motamayor et al. 2013). By deep sequencing and bioinformatic analysis of small RNA-ome and transcriptome of healthy versus singly and dually infected plants, we will investigate (i) the induction and evasion of the plant silencing-based antiviral defence, (ii) the role of small RNA silencing pathways in defence against the oomycete infection, and (iii) the existence of putative mobile small RNA effectors deployed by the oomycete to a plant cell to silence defense-related genes.

Small RNAs are known to be involved in plant antiviral defence, and episomal pararetroviruses are able to evade sRNA-directed methylation of viral DNA and transcriptional silencing as shown for six distinct pararetroviruses in persistently infected banana plants in a collaborative study by Dr. Pooggin, Dr. Chabannes and Dr. Iskra-Caruana at BGPI and University of Basel, Switzerland (Rajeswaran et al. 2014). However, little is known about antiviral defence mechanisms in cocoa, and small RNA biology of CSSV-plant interactions has not been investigated so far (and will be the focus of our study).

Emerging evidence suggests that small RNAs regulate plant-oomycete interactions (Burkhardt and Day 2016). Thus, *Phytophthora sojae* has evolved protein effectors that interfere with plant small RNA biogenesis (Qiao et al. 2015). However, the role of small RNAs in *P. megakarya*-cocoa interactions has not been investigated. Recent breakthrough studies of plant-fungus interactions revealed bi-directional cross-kingdom RNAi and gene silencing mediated by mobile small RNAs, in particular by plant microRNA and fungal small RNA effectors (Zhang et al. 2016; Wang et al. 2016). We will explore if mobile small RNA effectors are also involved in cocoa-*P. megakarya* interactions.

Thématique: The PhD project will address fundamental open questions on how cultivated perennial plants cope with mixed infections with viral and non-viral pathogens and on the role of small RNAs in the plant defence and the pathogen counter-defence mechanisms. The knowledge gained in this project will help improve the breeding programs of cocoa and other perennials by accounting for biotic stress-related epigenetic mechanisms.

Domaine: Phytopathologie, Biologie Végétale, Biologie Moléculaire, Bioinformatique

Objectif: The main objective of the PhD project is to employ next generation sequencing and bioinformatics to investigate small RNA-mediated epigenetics mechanisms underlying cocoa interactions with aggressive oomycete (*Phytophthora megakarya*) and viral (*Cocoa swollen shoot virus*) pathogens, currently spreading in the cocoa plantations in Cote d'Ivoire and causing severe disease outbreaks.

Contexte:

Over the last ten years, the biology and genetics of cocoa-pathogen interactions and cocoa disease epidemics in Africa have been a focus of research investigations at BGPI. Thus, Dr. Muller, a CIRAD researcher in the BGPI team B2B2, has been investigating the genetic diversity of the pararetrovirus CSSV in Togo and Ghana (Oro et al. 2012; Abrokwah et al. 2016), while Dr. Ducamp, a CIRAD researcher in the BGPI team BECphy has been involved in collection and characterization of strains of *P. megakarya* (Mfegue et al. 2012) and studies on susceptibility vs resistance of cocoa cultivars to *Phytophthora palmivora* (Thevenin et al. 2012) and cocoa transcriptome responses to biotic and abiotic stress (Argout et al. 2008). Currently, Dr. Muller and Dr. Ducamp together with Dr. Neema, a SupAgro researcher in the BGPI team BECphy and the director of BGPI, are involved in the survey of cocoa pathogens in Cote d'Ivoire, where *P. megakarya* and CSSV emerge as the most aggressive and economically important pathogens.

RNA silencing is a sequence-specific epigenetic mechanism mediated by small RNAs, which regulates gene expression and defends against invasive nucleic acids such as transposons, transgenes and viruses in most eukaryotes including plants, insects, fungi and oomycetes. In plants, RNA silencing is a main antiviral defense mechanism, but it also contributes to defence against bacterial, fungal and oomycete pathogens by regulating plant innate immunity responses to pathogen-associated molecular patterns (PAMPs) and pathogen effector proteins. Emerging evidence from studies of plant-fungus interactions indicates that small RNAs are mobile between organisms and can mediate cross-kingdom RNA interference and gene silencing. Dr. Pooggin and co-workers at the University of Basel have been investigating RNA silencing-based defence mechanisms and small RNA biology for many years. Using the model plant *Arabidopsis thaliana*, they have characterized genetic requirement for the biogenesis and action of viral small RNAs in antiviral defence (Blevins et al. 2006; Blevins et al. 2011; Aregger et al. 2012). They have successfully used next generation sequencing and bioinformatics to characterize small RNA populations (sRNA-ome) in different cultivated plant species including annuals (rice, tomato) and perennials (grapevine, banana) infected with different viruses including pararetroviruses (Seguin et al. 2014; Rajeswaran et al. 2014; Rajeswaran et al. 2014b; Fuentes et al. 2016). They have developed new bioinformatic algorithms and tools to characterize small RNAs (Seguin et al. 2014; Seguin et al. 2016). More recently, Pooggin's group began to the cross-talk between RNA silencing and innate immunity in defence against viral and non-viral pathogens (Zvereva and Pooggin 2012) and discovered that virus-infected plants can become more susceptible to secondary infections with bacterial pathogens (Zvereva et al. 2016).

After joining BGPI as an INRA research director, Dr. Pooggin has initiated a collaboration with Dr. Muller, Dr. Ducamp and Dr. Neema on small RNA biology of cocoa interactions with viral and nonviral pathogens in the frame of the PERsRNA project (submitted to ANR). This project aims to investigate small RNAs involved in response and adaptation to environmental constraints in 11 perennial plants, including cocoa, banana, coffee, olive, date palm, oil palm, eucalyptus, oak, apple, grapevine, and rubber tree. The PhD project will be part of the PERsRNA project.

Méthode:

The experiments with cocoa plants and pathogens will be conducted in the BGPI greenhouse under controlled conditions. Mature pods of susceptible and tolerant cultivars of *Theobroma cacao* will be obtained from French Guiana and the cocoa seeds will be germinated in soil. Young seedlings will be inoculated with the infectious clone of CSSV using an agroinfiltration method (Jacquot et al. 1999). Leaves of virus-infected and control mock-inoculated plants will then be spray-inoculated with aggressive and mild strains of *Phytophthora megakarya* (Mfegue et al. 2012) and the disease progression will be monitored in a time course manner. Leaf tissues will be collected at different time points for molecular analysis of viral and oomycete infections. Total RNA and total DNA will be prepared, validated using small RNA bolt hybridization, Northern and Southern blotting methods, following the protocols established in Pooggin lab (Rajeswaran et al. 2014) and then used for next generation sequencing (NGS)-based profiling of small RNA-ome, transcriptome and degradome using Illumina technology at FASTERIS AG (Switzerland). For NGS we will select a total of 24 samples from the two cocoa cultivars, including three biological replicates for each mock-inoculated healthy (n=6), virus-infected (n=6), aggressive (or mild) oomycete-infected plants (n=6), and virus- and aggressive (or mild) oomycete co-infected plants (n=6). From the field survey we will select a total of 8 samples including two biological replicates for healthy (n=2), virus-infected (n=2), oomycete-infected (n=2), and dually (virus+oomycete)-infected (n=2) plants. In the greenhouse experiment, we will generate a reference transcriptome by stranded mRNA-seq of pooled total RNA samples (n=12) for each of the two cultivars and profile the degradome for the pooled total RNA samples (n=3) under each condition (mock, virus, oomycete, and virus+oomycete). The bioinformatic analysis of the NGS data will be conducted using available tools and algorithms as established in Pooggin lab for small RNA-omics (Sequin et al. 2014; Sequin et al. 2016) and at BGPI for transcriptomics. The results of the bioinformatic analysis will be validated in wet lab by small and long RNA blot hybridization analyses and quantitative RT-PCR.

Résultat attendu:

We expect to obtain a comprehensive genome-wide resource comprising the profiles of small RNA-ome and transcriptome of cocoa plants in response to single and dual infections with the viral and oomycete pathogens. In-depth analysis of alterations in small RNA and transcriptome profiles will allow us to gain knowledge on (i) the induction and evasion of the RNA silencing-based antiviral defence in CSSV-infected cocoa plants, (ii) the role of small RNA silencing pathways in defence against the oomycete infection, and (iii) the existence of putative mobile

small RNA effectors deployed by the oomycete to a plant cell to silence defense-related genes. We will also learn if viral infection alters susceptibility of cocoa plants to secondary infection with oomycete pathogens. The field survey in Cote d'Ivoire will reveal the genetic diversity of viral and non-viral pathogens in cocoa plantations and the incidence of mixed infections with synergistically increased disease severity.

Référence bibliographique

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Conditions scientifiques matérielles (conditions de sécurité spécifiques) et financières du projet de recherche

The PhD project is part of the ANR project PERsRNA "Perennial plant small RNAs involved in response and adaptation to environmental constraints" (2016-2019) which insures the financial support of PhD student's salary (for three years) and bench fees (15K per year for three years).

The student will have access to all the facilities at BGPI (including wet lab, greenhouse, and bioinformatics platform) and the equipment for advanced molecular biology, genetics and phytopathology required for implementation of the project.

Ouverture Internationale

Besides the access to a national scientific network of the PERsRNA consortium that engages 28 researchers (from CIRAD, IRNA, CNRS, SupAgro and IRD) studying perennial plant species in South France, the student will have an opportunity to participate in the activities of the COST Action FA1407 DIVAS (2014-2018) "Empowering high throughput sequencing for the study and the diagnostic of plant viruses" (<http://www.cost-divas.eu/>) that engages 145 scientists from 40 countries. Dr. Pooggin is a substitute member of the Management Committee, and, in the frame of this Action, he runs a research project "Next generation sequencing for the diagnosis and diversity studies of viral pathogens in Swiss agriculture".

Collaborations envisagées

The PhD project will be performed in close collaboration with Dr. Claire NEEMA, Dr. Emmanuelle MULLER and Dr. Michel DUCAMP at the BGPI. We also envisage collaborations with Dr. Mattheiu CHABANNES and Dr. Marie-Line CARUANA at BGPI, who are involved together with Dr. Pooggin in investigation of small RNA biology of banana-pathogen interactions in the frame of PERsRNA project, and Dr. Julie LECLERCQ, a CIRAD researcher at AGAP and the coordinator of PERsRNA project, who is studying stress-related microRNAs in rubber tree using the degradome sequencing approach.

The project is not confidential and the results obtained will be disseminated through presentations at national and international scientific conferences and publications in international peer-reviewed journals.

Début de la thèse: 1 octobre 2017