SFR QUASAV PHD STUDENT DAY

11TH EDITION

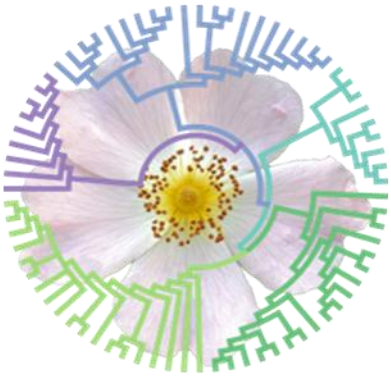
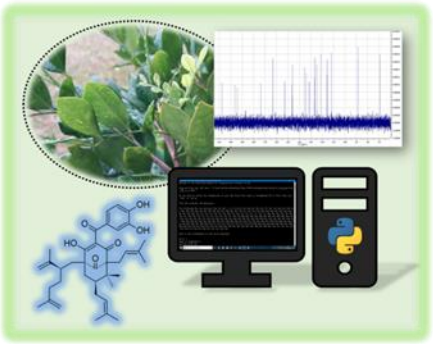
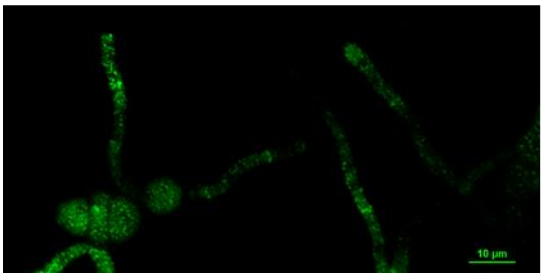
Tuesday, the October 9th

UFR Sciences – Building L - Amphitheater L003

08h45-17h00





PhD student work presentation seminar

Planning of the SFR QUASAV PhD student day

8h45 - 9h00 **Introducing talk of the PhD day**

9h00 - 9h25 **Lili Zang**, Institut de Recherche en Horticulture et Semences (IRHS) - SMS Team, Angers
Deciphering the nitrate signaling pathway leading to a reduction of primary root growth in Medicago truncatula

9h25 - 9h50 **Mathieu Marc**, Institut de Recherche en Horticulture et Semences (IRHS) - Respom team, Angers
Study of the molecular determinism of superficial scald in apple fruit

9h50 - 10h15 **Kevin Debray**, Institut de Recherche en Horticulture et Semences (IRHS) - GDO team, Angers
Deciphering species-level phylogenetic relationships in the evolutionary complex genus Rosa using an amplicon-sequencing approach

10h15 - 10h45 **Coffee break**

10h45 - 11h10 **Ellen Guitton**, Institut de Recherche en Horticulture et Semences (IRHS) - EcoFun team, Angers
Effects of secondary contacts with gene flow on epidemiological success of a fungal pathogen

11h10 - 11h35 **Justine Colou**, Institut de Recherche en Horticulture et Semences (IRHS) - FungiSem team, Angers
Implication of membrane protein complexes, the eisosomes, during the infectious process of Alternaria brassicicola

11h35 - 12h00 **Anne Schneider**, Institut de Recherche en Horticulture et Semences (IRHS) - Arch-E team, Angers
Understanding the role of sugars, together with hormones in the response of bud outgrowth to light intensity : an approach combining experiments and modeling.

12h00 - 14h00 **Lunch break**

14h00 - 14h25 **Martin Luquet**, Institute of Genetics, Environment and Plant Protection (IGEPP), Angers
Effects of cereal-legume intercropping on sugar consumption and parasitism by Aphidius parasitoids

14h25 - 14h50 **Antoine Bruguière**, Substances d'origine naturelle et analogue structuraux (SONAS), Angers
^{13}C-NMR dereplication of complex mixtures : Building a custom seach algorithm

14h50 - 15h20 **Coffee break**

15h20 - 15h45 **Estelle Billard**, Laboratoire de Biologie et de Pathologie Végétale (LBPV), Nantes
Cytokinins involvement in haustorium formation in parasitic plant Phelipanche ramosa

15h45 - 16h10 **Douae Ben Hdech**, Institut de Recherche en Horticulture et Semences (IRHS) - SMS team, Angers
Genetic variability of seedling heterotrophic growth depending on nitrate supply and temperature in Medicago truncatula

16h10 **Jury deliberation**

Deciphering the nitrate signaling pathway leading to a reduction of primary root growth in *Medicago truncatula*

Lili Zang, Marie-Christine Morère-Le Paven, Thibault Clochard, Anis M.Limami,Françoise Montrichard
lili.zang@etud.univ-angers.fr
IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucouzé cedex, France

In the model legume*Medicago truncatula*, nitrate has been shown to inhibit primary root growth through the reduction of root cell elongation. Nitrate, as an essential nutrient, also acts as a signal molecule that is sensed and transduced through a nitrate transporter MtNPF6.8, with RNAi mutantsdeficient in MtNPF6.8 being insensitive to nitrate [1, 2]. We tested here whether reactive oxygen species (ROS) could be downstream mediators of the nitrate signal since ROS are able to transduce ABA signal in other contexts and also govern the primary root growth. Thus, we analyzed the distribution of ROS (H_2O_2, $O_2^{\bullet-}$, $^{\bullet}OH$) and peroxidase activity all along the primary root of seedlings sensitive or insensitive to nitrate usingdifferent genotypes of *M. truncatula*, three wild types and a *npf6.8RNAi*mutant grown with or without nitrate,to determine whether nitrate modifies ROS and peroxidase patterns. We found that nitrate modified the morphology of the root tip, induced an increase in H_2O_2, and a decrease in $O_2^{\bullet-}$and $^{\bullet}OH$ in seedlings sensitive to nitrate (R108, A17 and DZA315-16), but not in seedlings insensitive to nitrate (*npf6.8RNAi* mutant). These results suggest that ROS andperoxidases are downstream mediators in the nitrate signaling pathway. The origin of the change in ROS accumulation in response to nitrate was further investigated following the activity ofmajor enzymes (peroxidase, SOD, Nox) able to interferewith ROS accumulation.

Key words: *nitrate signal, primary root, ROS, H_2O_2, $O_2^{\bullet-}$, $^{\bullet}OH$, peroxidase, SOD, Nox*

References:

1.Morère-Le Paven, M. C., Viau, L., Hamon, A., Vandecasteele, C., Pellizzaro, A., Bourdin, C., ... and Legros, C. (2011). Characterization of a dual-affinity nitrate transporter MtNRT1. 3 in the model legume Medicago truncatula. Journal of Experimental Botany, 62(15), 5595-5605.

2.Pellizzaro, A., Clochard, T., Cukier, C., Bourdin, C., Juchaux, M., Montrichard, F., ... and Morère-Le Paven, M. C. (2014). The nitrate transporter MtNPF6. 8 (MtNRT1. 3) transports abscisic acid and mediates nitrate regulation of primary root growth in Medicago truncatula. Plant Physiology, 166(4), 2152-2165.

Study of the molecular determinism of superficial scald in apple fruit

Marc Mathieu, Hanteville S, Bruneau M, Poisson A.S, Guillou M.C, Renou J-P, Delaire M & Orsel M
mathieu.marc@inra.fr
IRHS, INRA, Agrocampus-Ouest, Université d'Angers, SFR 4207 QuaSaV, 42 rue Geoges Morel, 49071 Beaucouzé cedex, France

Superficial scald is a physiological disorder altering apple and pear fruit quality. Scald symptoms, brown patches on hypodermal fruit cell layers, appear during shelf-life after a long cold storage period (4 months) [1]. Incidence and severity of superficial scald depend on the genotype (cultivar intrinsic susceptibility), the environment (growing season and pre-harvest climate) and fruits management (maturity at harvest, post-harvest treatments and storage conditions) [2]. The main studied hypothesis to explain scald symptoms development is the oxidation of α-farnesene, a volatile compound, which produce reactive oxygen species (ROS) harmful to cell [3]. However the underlying molecular mechanisms are still poorly understood. A critical factor is likely to be the balance between antioxidant activity and ROS production in response to cold stress [4]. Based on this rationale, our aim is to identify early markers genes at harvest to predict the development of superficial scald and to understand the molecular determinism of its development, with a focus on the transcriptomic response to cold stress and the oxidative balance during cold storage.

'Granny Smith', a susceptible cultivar, was studied over four years (2014, 2015, 2016 and 2017). Fruits harvested earlier and stored over several months in different cold conditions developed 38% of scald symptoms in 2014, 100% in 2015, 61% 2016 and 97% 2017. Global transcriptomic analyses of fruit peels at harvest to compare contrasted phenotypes after cold storage (2014 *vs* 2015 and 2014 *vs* 2016) revealed a significant overrepresentation of genes involved in abiotic stress responses up regulated in 2014, including 5 different heat shock proteins (HSP). HSPs are known to prevent protein aggregation, misfolding and to protect plasma membrane of degradation during several stresses [5]. Pre-harvest induction of these HSPs in 2014 could have reduced the impact of stress induced by cold storage and therefore reduced superficial scald symptoms. Moreover, in 2017, scald incidence was reduced by 33% when fruits were cold acclimated for 8 days at 8°C before the classic long cold storage (2°C). These results suggest that an abiotic stress (cold/heat) before long cold storage can reduce superficial scald incidence and severity and HSPs expression could be used as predictive markers. Proteomic and biochemical analyses (antioxidant activities, lipids peroxidation, H_2O_2) are in progress to support these results.

Keys words: *Malus x domestica, superficial scald, oxidative stress, transcriptome analysis, α-farnesene*

References: *[1] Lurie and Watkins, 2012, Postharvest Biology and Technology, 65, 44–60; [2] Whitaker, 2013, Acta Hortic., 989, 47-60; [3] Whitaker, 2004, HortScience, 39, 933–937; [4] Kochhar et al., 2003 J. Amer. Soc. Hort. Sci. 128(6):910-916; [5] Wang et al., 2004, Trends Plant Sci. 9(5):244-52.*

Deciphering species-level phylogenetic relationships in the evolutionary complex genus *Rosa* using an amplicon-sequencing approach

Kevin Debray[1], Jordan Marie-Magdelaine[1], Jérémy Clotault[1], Tatiana Thouroude[1], Gilles Michel[1], Aurélie Bérard[2], Marie-Christine Le Paslier[2], Fabrice Foucher[1], and Valéry Malécot[1]

kevin.debray@inra.fr

[1]*IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, Beaucouzé, France*

[2]*INRA, US 1279 EPGV, Université Paris-Saclay, Evry, France*

The genus *Rosa* comprises around 150 species well distributed throughout the temperate regions of Northern Hemisphere. More than half of rose species are polyploid ranging from 3x to 10x. Uneven chromosome numbers are sometimes encountered as well as asymmetrical meiosis and multiploid species. Hybridization and polyploidization are evolutionary processes that shield from deleterious mutations and increase adaptability to new environmental constraints. In the genus *Rosa*, these 2 processes are regarded as relatively recent and were certainly an asset to withstand paleoclimatic changes and adapt to very different ecosystems, from the Tibetan plateau to the British coasts through the arid valleys of Baja California in Mexico. However, due to homogeneity in morphology, associated with hybridization and polyploidization, classifying the genus *Rosa* using morphological and molecular data is a real challenge for taxonomists. In the past years, several attempts to reconstruct a phylogeny for the genus have been undertaken mostly using plastid sequences despite their maternal inheritance only reflects part of the evolutionary processes. In 2018, more than 20 roses species genomes have been released including the high-quality reference genome sequence of *R*. 'Old Blush'. These genomes give an unrecorded access to sequence variations in the genus *Rosa*. By taking advantage of this tremendously increase in genomic data, our objective is to develop a robust phylogenetic hypothesis able to reflect the complex evolutionary patterns that shaped the genus *Rosa*. We first mined all available genomic data from rose species to build a phylogenomics dataset of amplifiable orthologous single-copy nuclear *loci* containing sufficient phylogenetic signal at deep, medium, and shallow levels of the phylogenetic tree. Then, we chose an amplicon-sequencing approach to target these *loci* on a broader sample of ~120 rose species enabling us to get polymorphic nuclear sequences for each individual. Polymorphic nuclear sequences within one individual are more likely to reveal hybridization and polyploidization events than plastid sequences only. After building a backbone species tree consisting of putative diploid progenitors, we tried to graft alleles obtained from hybrids and polyploids to detect traces of auto-, allo-, and no polyploidizations events. The resulting network best represents evolutionary history of the genus *Rosa* than bifurcating trees. With this knowledge, we hope to study traits evolution and better characterize this gene-pool for breeding, while less than 10 species have contributed to create the thousands presently cultivated roses.

Key words: Phylogenomics, Single-copy orthologous loci, Polyploidy-hybrid system, Target amplicon sequencing, PCR multiplex, Species networks, Phylogenetic signal

Effects of secondary contacts with gene flow on epidemiological success of a fungal pathogen

Ellen Guitton, Valérie Caffier, Bruno Le Cam, Christophe Lemaire

ellen.guitton@inra.fr

IRHS, UMR 1345-INRA, ACO, Université d'Angers - 49071 Beaucouzé, France

Globalization favours the dispersal of organisms on large scales and can bring together divergent populations that were isolated geographically. These secondary contacts can lead to hybrid production and allow a better understanding of the speciation mechanisms. Indeed, during speciation, a reproductive isolation occurs due to genetics and/or environmental barriers and this isolation may be an indication of two species establishment. Venturia inaequalis is an ascomycete responsible for apple scab. During the domestication of apple tree, this ascomycete seems to have followed and adapted to its host and its new constraints. After five thousand years of geographical separation, this pathogen population (referred to as "agricultural" population) present on the cultivated apple Malus x domestica came into secondary gene flow contact and interbred with the ancestral population (referred to as "wild" population) present on the wild apple Malus sieversii in Kazakh mountains one hundred years ago. The first objective of my thesis project is to evaluate the putative impact of this secondary contact on the fitness of the pathogen populations and its impact on their capacity to infect cultivated and wild apple tree. A phenotyping of natural populations (agricultural, wild and hybrids strains) and in vitro hybrids successfully obtained between an agricultural and a wild strain have been made to evaluate their aggressiveness and virulence on M. x domestica and M. sieversii. Agricultural strains seem to be the only ones to infect Malus x domestica. On Malus sieversii, natural hybrids strains are significantly more aggressive than wild strains and therefore seem to be more adapted than wild strains. However, in natural environment, few hybrids were observed (only 10%), although both wild and agricultural populations were present together. This could be due to barriers that lead to reproductive isolation and the second objective of my thesis project is to identify putative reproductive barriers. A genotyping has been made on natural populations and in vitro hybrids with a 50K SNP chip. These results will allow the identification of genomic regions without gene flow whereas QTL cartography will measure their impact on the hybrid fitness. I will also check if there are some pre-zygotic barriers as mating choice preferences, with different crosses between the different populations (agricultural and wild strains). These results will allow to know if the production of hybrids between a wild and an agricultural population which were submitted to different selection pressures are a threat to cultivated and wild apple tree.

Key words : Epistasis, DMI, hybridation, Venturia inaequalis, cultivated and wild apple

Implication of membrane protein complexes, the eisosomes, during the infectious process of *Alternaria brassicicola*

Justine Colou[1], Ophélie Dubreu[1], Kévin Fontaine[1], Roxane Raulo[1], Florence Manero[2], Aurélia Rolland[1], Fabienne Simonneau[1], Guillaume N'Guyen[1], Anthony Kwasiborsky[1], Franck Bastide[1], Claire Campion[1], Philippe Simoneau[1], Thomas Guillemette[1]

justine.colou@etud.univ-angers.fr

[1]*Université d'Angers, INRA, Agrocampus Ouest, UMR 1345 IRHS, SFR 4207 QUASAV, Angers, France*

[2] *Université d'Angers, SFR4208 Interactions cellulaires et applications thérapeutiques, Angers, France*

Alternaria brassicicola is a fungal necrotroph responsible for the Brassicaceae dark spot disease (Belmas *et al.*, 2018). This fungus is a seed borne pathogen that only affect the aerial parts of host plants causing great damages with major incidence on yield and product quality. Because of its ability to colonize *Arabidopsis thaliana, A. brassicicola* is routinely used as a model necrotrophic pathogen.

Recently, we showed that some eisosomal protein coding genes were overexpressed during osmotic and hydric stresses. Those stresses correspond to the main constraints encountered by the fungus during the seed colonization process. The eisosomes are membrane microdomains whose function is still unclear (Wather *et al.*, 2006). Now, we are trying to decipher the potential involvement of eisosome in pathogenicity using a reverse genetic approach by generating and characterizing mutants deficient for key eisosomal protein encoding genes (*Δpil1*, *Δnce102*, *Δlsp1* and *Δpil1Δlsp1*). In parallel, these proteins have been fused with GFP to study the monitoring of their cellular location during the plant infection and following the exposure to different stresses.

Key words: pathogen/host interaction, eisosomes, seeds, necrotroph.

References:

- Belmas, Elodie, Martial Briand, Anthony Kwasiborski, Justine Colou, Guillaume N'Guyen, Béatrice Iacomi, Philippe Grappin, Claire Campion, Philippe Simoneau, Matthieu Barret, and Thomas Guillemette. "Genome Sequence of the Necrotrophic Plant Pathogen Alternaria Brassicicola Abra43." Genome Announcements 6, no. 6 (2018). doi:10.1128/genomea.01559-17.
- Walther, Tobias C., Jason H. Brickner, Pablo S. Aguilar, Sebastián Bernales, Carlos Pantoja, et Peter Walter. 2006. « Eisosomes mark static sites of endocytosis ». Nature 439 (7079): 998-1003. doi:10.1038/nature04472.

Understanding the role of sugars, together with hormones in the response of bud outgrowth to light intensity: an approach combining experiments and modeling

A. Schneider [1], L. Ledroit [1], MD. Perez-Garcia [1], N. Brouard [1], F. Boudon [2], C. Godin [3], S. Sakr [1], J. Bertheloot [1]

anne.schneider@inra.fr

[1]IRHS, Université d'Angers, INRA, Agrocampus-Ouest, SFR 4207 QUASAV, 49071 Beaucouzé, France

[2]UMR AGAP, CIRAD, INRIA, INRA, Montpellier, France

[3]Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, Inria, F-69342, Lyon, France

Branching is a major agronomic variable that determines yield and quality. Branching is highly sensitive to environment, so that understanding the underlying mechanisms is a key to develop new crop management strategies and varieties. The few studies on the impact of light intensity on bud outgrowth at the plant scale have highlighted a major role of cytokinins. However, this result is valid for specific light conditions (Roman et al., 2016; Corot et al., 2017). Moreover, recent data suggest that sugars may also play a role (Mason et al., 2014; Barbier et al., 2015 ; Kebrom et al., 2017).

The objective of my PhD thesis is to give a model of bud outgrowth regulation accounting for the effect of several light intensity regimes, and the quantitative contribution of sugars in this regulation. We made biological experiments on different systems (whole plants and decapitated plants) under different light regimes.

Bud outgrowth was stimulated at continuous high light compared to continuous low light, and over stimulated by a temporary light restriction before the outgrowth period in accordance with Demotes-Mainard et al. (2013). We demonstrated that the stimulation by high light was correlated to high cytokinin contents in the stem. A temporary restriction of light before bud outgrowth resulted, in addition, in a starch accumulation in the stem. That suggests that an excess of sugars is involved in the over stimulation of bud outgrowth after a temporary light restriction. This assumption was confirmed by feeding plants under constant high light intensity with an external supply of sucrose, which led to bud outgrowth stimulation. Net photosynthesis and areas of mature leaves were not higher after the temporary light restriction compared to the continuous high light regime. On the contrary, measurements of organ size dynamics revealed a lower growth rate of organs on the primary axis during the outgrowth period after the temporary light restriction. This lower growth rate may be responsible for starch accumulation and higher sugar availability for bud outgrowth.

Next step is to integrate in a computer model at the plant scale the knowledge about sugars and hormones role in bud outgrowth regulation by light intensity. This model will represent a good tool to test assumptions about the underlying mechanisms by confronting biological and virtual experiments.

Key words: bud outgrowth, branching, sugars, light intensity, plant model

References:

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Mason, M. G., Ross, J. J., Babst, B. A., Wienclaw, B. N. & Beveridge, C. A. Sugar demand, not auxin, is the initial regulator of apical dominance. Proceedings of the National Academy of Sciences 111, 6092–6097 (2014).

Roman, H. et al. Cytokinins are initial targets of light in the control of bud outgrowth. Plant physiology 172, 489–509 (2016).

Effects of cereal-legume intercropping on sugar consumption and parasitism by *Aphidius* parasitoids

LUQUET Martin[1,2], PENALVER-CRUZ Ainara[3], SATOUR Pascale[4], ANTON Sylvia[1,5], CORTESERO Anne-Marie[1,6], LAVANDERO Blas[3], JALOUX Bruno[1,2]

martin.luquet@agrocampus-ouest.fr

[1] *Institute of Genetics, Environment and Plant Protection (IGEPP)*
[2] *Agrocampus Ouest, Angers*
[3] *Biological Control Laboratory, University of Talca, Chile*
[4] *Research Institute on Horticulture and Seeds (IRHS), University of Angers*
[5] *INRA, Angers*
[6] *University of Rennes 1*

Most parasitoid wasps rely on plant-derived food sources such as nectar, to ensure their survival and dispersal. However, in conventional single crop farming, flower resources are scarce and often restricted to the border of fields. Lack of such resources leads to a lower abundance and performance of parasitoids, especially in the centre of the fields. There has recently been a growing interest in field diversification, for various purposes, and notably as a way to provide food sources for parasitoids. For instance, intercropping allows mixing crops that do not produce such resources (e.g. wheat) with crops such as faba bean, which produces extrafloral nectar on which parasitoids can feed as demonstrated in laboratory bioassays. Nectar provision in intercropped fields may then allow increased food consumption by parasitoids, leading to better parasitoid performances and parasitism, thus increasing pest population control. Here, we studied sugar uptake and parasitism patterns of *Aphidius* aphid parasitoids in wheat single crops and wheat-faba bean intercrops. We tested the hypotheses that a) *Aphidius* parasitoids feed more in intercrops than in single crops and b) increased nectar consumption in intercropped fields leads to higher aphid parasitism. Parasitoid captures and parasitism surveys were carried out in an organic field network around Angers, France. Several sampling points were made at different positions in the field (center, border) to consider nectar uptake outside the field. Feeding history of field-caught parasitoids was inferred from their sugar profile, using HPLC. *Aphidius* feeding patterns were then linked to estimated parasitism rates.

Key words: parasitoids, intercropping, conservation biological control, complementary resources, nutritional ecology

^{13}C-NMR DEREPLICATION of COMPLEX MIXTURES: BUILDING A CUSTOM SEARCH ALGORITHM

A. Bruguière[1], S. Derbré[1], J. Dietsch[2], F. Saubion[3], P. Richomme[1]
antoine.bruguiere@univ-angers.fr
[1]*EA921 SONAS/SFR4207 QUASAV, Université d'Angers, France.* [2]*JEOL Europe SAS, Croissy sur Seine, France*
[3]*EA2645 LERIA, Université d'Angers, France.*

Isolation and identification of natural products (NPs) is often a long and difficult task that can unfortunately lead to the characterization of already known compounds, resulting in a considerable waste of time. Over the past few years, so-called "dereplication techniques" have emerged, allowing to quickly identify the major compounds in a mixture with, ideally but depending on the extract or fraction complexity, a minimum of purification/separation steps. Dereplications are usually conducted using mass spectrometry (MS) and nuclear magnetic resonance (NMR) analyses which require appropriate databases (DB). Looking for a specific type of compound, biologically active polycyclic polyprenylated acylphloroglucinols (PPAPs), a dereplication analysis using ^{13}C-NMR was conducted in order to quickly target them inside plant extracts. Even if ^{13}C-NMR seemed to be the most adequate method for this type of compound, the available published methods gave mixed results. Thus, one goal of my thesis was to develop a custom search algorithm (Figure 1), which allowed us to improve the matching process as desired. This algorithm not only compares chemical shift in the extract with the ones of every molecule in the database, giving them a score depending on the percentage of matched signals. Indeed, different filters were added, greatly narrowing the search. These filters include a matching by carbon type and a molecular weight range filter through optional DEPT-135 and MS data input. This new algorithm was tested on plant extracts from the *Garcinia* genus and led to the identification of major compounds with a better accuracy than the method previously published [1]. Those results showed that the functionalities implemented in the algorithm allowed a satisfying dereplication process using only the data from one extract or one enriched fraction.

Key words: *^{13}C-NMR dereplication; Algorithm;* Garcinia

References: [1] *J. Hubert et al J. Nat. Prod. 2015, **78**, 1609–1617*

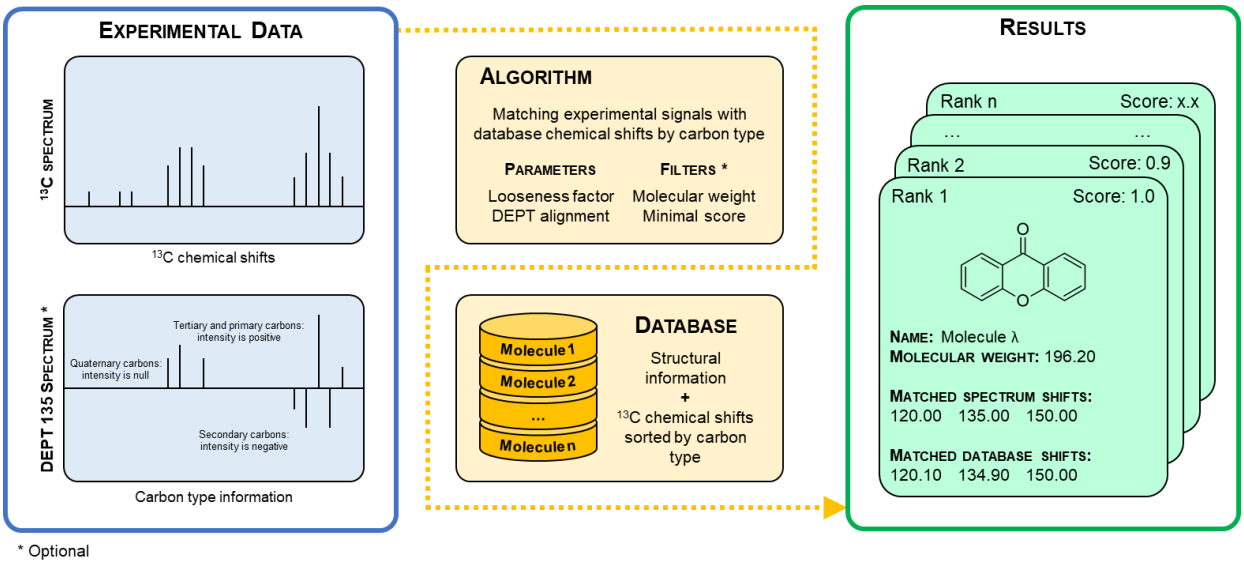

Figure 1. General process of the ^{13}C-NMR dereplication analysis

CYTOKININS INVOLVEMENT IN HAUSTORIUM FORMATION IN PARASITIC PLANT *PHELIPANCHE RAMOSA*

Estelle Billard, Grégory Montiel, Philippe Simier
estelle.billard@univ-nantes.fr
Laboratoire de Biologie et de Pathologie Végétale, EA1157, Université de Nantes, 2 rue de la Houssinière, PB 92208, 44322 Nantes Cedex 3, France

Broomrapes (*Orobanche* and *Phelipanche* genera) are obligatory root parasitic plants which are devoid of chlorophyll and thus rely on host plants for their water, carbohydrates and mineral supplies. Some of them, such as *Phelipanche ramosa*, are especially adapted to many agrosystems and became in few years a major issue for European legume and oleaginous cultures. The ability of Orobanchaceae to establish a parasitic relationship is based on the development of a specific invasive organ called haustorium which is required for invasion of the host root, connection to the vascular system and spoliation of host's resources. Haustorial induction is a well-described mechanism in hemiparasitic plants (*Striga sp*, *Triphysaria versicolor*), since two decades of studies shown that early steps of this process are triggered by phenolic compounds exuded by the root system of the host. However, this step is poorly understood in holoparasitic plants such as *P. ramosa*. Recently, Goyet *et al.*, (2017), showed that the induction of early haustorial structures (EHS) in *P. ramosa* can be achieved by cytokinins-like molecules contained in host exudates and which increased significantly the aggressiveness of the pathogen. The present research work focuses more accurately on the early molecular mechanisms leading to the development of EHS upon treatment with cytokinins. It aims on one hand to characterize host plant exudates and to identify cytokinins with haustorial activity by mass spectrometry, combined with the use of *Arabidopsis thaliana* mutant plants for cytokinin transporters or biosynthesis. On the other hand, in order to decipher cytokinin signaling during the establishment of the haustorium in *P. ramosa*, a second step is to clarify molecular function of cytokinin receptors by expressing them in a bacterial system developed by our partner the Laboratory of Growth Substances, University of Olomouc (Spíchal *et al.*, 2011). Finally, a functional validation method based on transformation system of *P. ramosa* calluses is developed and should confirm the involvement of keys regulator genes which have been identified in this process using a transcriptomic approach.

Keywords : *haustorium, cytokinin, signaling, receptors, Phelipanche ramosa, parasitism*

References :

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Genetic variability of seedling heterotrophic growth depending on nitrate supply and temperature in *Medicago truncatula*

Douae BEN HDECH, Bénédicte ALIBERT, Daniel BEUCHER, Claudine LANDÉS, Catherine AUBRY, Anis M. LIMAMI, Béatrice TEULAT
douae.benhdech@inra.fr
IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucouzé cedex, France

Background: Heterotrophic growth is a key step in successful crop establishment. Its success depends on seed reserves, genotype and environmental conditions (nutrients, temperature). Climate change and development of sustainable agriculture involve major changes in cropping systems. With these modifications, the seedlings grow more and more under low temperatures with low nutrient availability, especially nitrate. The aim of this study is to characterize the genetic variability of heterotrophic growth in response to an absence of nitrate and to low temperature.

Results: To determine the impact of the nitrate absence, organs lengths and biomass allocation from seeds to seedling organs were measured on 192 accessions, which are representative of natural diversity of *Medicago truncatula.* Measurements were performed at the end of heterotrophic growth at optimal and low temperature, with (5 mM) and without nitrate in the growth medium. Global response throughout the core collection showed that the absence of nitrate differently impacted the organs growth and had a lower effect than the low temperature stress. Furthermore, a wide range of genetic variability was revealed whatever the growth conditions, which allowed to determine contrasted genotype behaviors for each trait. Then, thanks to a large-scale clustering analysis, we put forward genotype groups representative of the main genotypic behaviors of the core collection. Finally, in order to dissect our phenotypic traits, a study "Genome Wide Association Study" (GWAS) was conducted using Genome-wide Efficient Mixed Model Association algorithm (GEMMA) and multi-locus mixed model (MLMM). Both approaches identified SNPs across the whole genome by their significant association with heterotrophic growth.

Conclusions: This study identified genetic variability of the core collection, which has allowed us to firstly determine genotypic behaviors groups and secondly to identify loci underlying organs elongation and biomass use efficiency under abiotic stress.

Key words: *seedling establishment, nitrate supply, natural diversity, low temperature, legume, GWAS*

Thanks to:

The participants:

Lili ZANG	lili.zang@etud.univ-angers.fr
Mathieu MARC	mathieu.marc@inra.fr
Kévin DEBRAY	kevin.debray@inra.fr
Ellen GUITTON	ellen.guitton@inra.fr
Justine COLOU	justine.colou@etud.univ-angers.fr
Anne SCHNEIDER	anne.schneider@inra.fr
Martin LUQUET	martin.luquet@agrocampus-ouest.fr
Antoine BRUGUIERE	antoine.bruguiere@univ-angers.fr
Estelle BILLARD	estelle.billard@univ-nantes.fr
Douae BEN HDECH	douae.benhdech@inra.fr

The jury members:

Alain VIAN	alain.vian@univ-angers.fr.
Ronan SYMONEAUX	r.symoneaux@groupe-esa.com
Françoise MONTRICHARD	francoise.montrichard@univ-angers.fr
Jérémy LOTHIER	jeremy.lothier@univ-angers.fr
Bruno LE CAM	bruno.lecam@inra.fr
Séverine THOIRON	severine.thoiron@univ-nantes.fr
Joëlle FUSTEC	j.fustec@groupe-esa.com
Jérémy CAMON	jeremy.camon@univ-angers.fr
Séverine DERBRE	severine.derbre@univ-angers.fr
Séverine BOISARD	severine.boisard@univ-angers.fr
Mathieu ROLLAND	mathieu.rolland@anses.fr
René GUENON	rene.guenon@agrocampus-ouest.fr