



Structure Fédérative de Recherche 4207 QUASAV

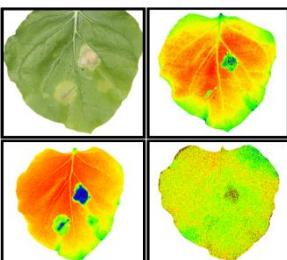
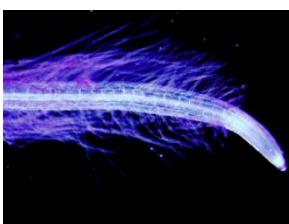
JOURNÉE DES DOCTORANTS DE LA SFR QUASAV

10^{EME} EDITION

Mardi 3 octobre

UFR Sciences –Bâtiment L - Amphi L003

08h45-17h30



Séminaire de présentation des travaux des doctorants

PROGRAMME

10è édition de la journée des doctorants de la SFR QUASAV

08h45 – 09h00 **Ouverture de la journée des doctorants**

09h00 – 09h30 **Alexia VILLE** - *Semisynthetic pathways starting from δ-garcinoic acid towards anti-inflammatory tocotrienols isolated from new-caledonian endemic plants*

09h30 – 10h00 **Ming WANG** - *Sucrose control the branch process by post-transcriptional regulation in Rosa hybrida*

10h00 – 10h30 **Elise RETHORE** - *Role of energy metabolism in acquired thermotolerance of Arabidopsis seedlings*

10h30 – 11h00 **Pause-café**

11h00 – 11h30 **Claude KOUTOUAN** - *Can the variation of secondary metabolite contents be part of carrot resistance to Alternaria dauci ?*

11h30 – 12h00 **Julia COURTIAL** - *Resistance and pathogenicity : how toxins are involved in the carrot-Alternaria dauci interaction*

12h00 – 12h30 **Javier Ernesto PITTI** - *Nanoencapsulation and synergistic effect of insecticides : An innovative strategy to improve treatment efficacy against pest insects while reducing doses*

12h30 – 14h00 **Pause déjeuner**

14h00 – 14h30 **Valerian MELINE** - *Combinatorial chlorophyll fluorescence imaging of plant pathogen interaction*

14h30 – 15h00 **Kay GULLY** - *Regulation of plant development and defense response by SCOOP, a new small endogenous peptide*

15h00 – 15h30 **Romain WARNEYS** - *Implication des mécanismes épigénétiques dans la potentialisation des défenses chez le pommier par un stimulateur de défense à fort potentiel de protection*

15h30 – 16h00 **Pause-café**

16h00 – 16h30 **Nicolas DACCORD** - *Apple genome and epigenome bioinformatic analysis*

16h30 – 17h00 **Anthony ROUAULT** - *Mise en œuvre d'une démarche participative d'éco-quali-conception appliquée aux systèmes de production viticoles*

17h00 **Délibération du Jury et remise de prix**

Semisynthetic pathways starting from δ-garcinoic acid towards anti-inflammatory tocotrienols isolated from new-caledonian endemic plants

Alexia VILLE, Guillaume VIAULT, Jean-Jacques HELESBEUX, Denis SERAPHIN

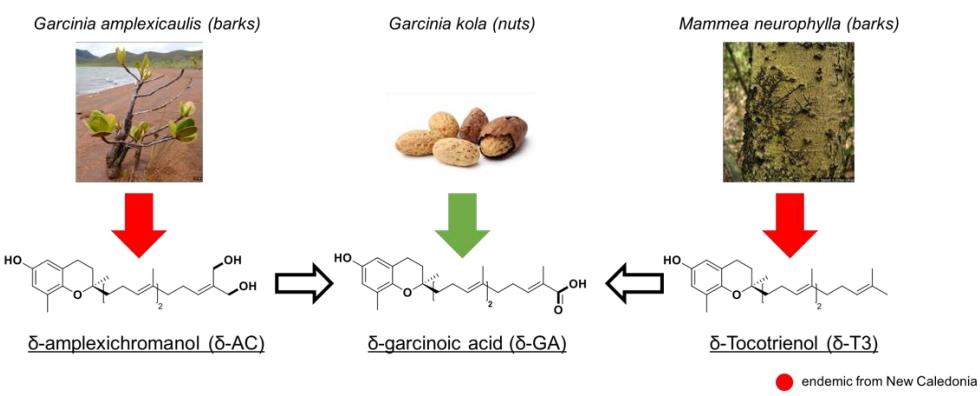
alexia.ville@univ-angers.fr

SONAS EA921, SFR QUASAV 4207, Université d'Angers, 42 Rue Georges Morel, 49070 Beauzoué

Vitamin E (VE) includes 8 chemically distinct chromanols named α, β, γ, δ-tocopherols (T) and tocotrienols (T3). They mainly differ by the number and the position of aromatic methyl groups and by the nature of the side chain, phytol for T and farnesyl for T3 [1]. Amongst the various bioactivities reported for this class of compounds, T3 derivatives showed hypocholesterolemic, anti-cancer and neuroprotective properties [1]. Besides VE, analogs oxidized on the terminal methyl groups of the side chain were also isolated from vegetal sources. In 2009, a phytochemical study of *Garcinia amplexicaulis* has allowed the isolation and characterization of δ-amplexichromanol (δ-AC) as a new and major secondary metabolite along with 15 other original oxidized T3 analogs and also known products such as δ-garcinoic acid (δ-GA) [2].

In the course of the Austrian interdisciplinary network project “Drugs from Nature Targeting Inflammation” (DNTI: 2007-2014), an *in silico* pharmacophore based virtual screening of T3 derivatives isolated from *G. amplexicaulis* was performed [3]. The parallel profiling of this family against various anti-inflammatory targets clearly revealed T3 analogs as potential 5-lipoxygenase (5-LO) inhibitors. This preliminary result has been confirmed with *in vitro* assays on purified 5-LO and polymorphonuclear leucocytes (PMNL) for both δ-AC and δ-GA.

We therefore decided to study the structure-activity relationships (SAR) to fully understand the mode of action of this class of metabolites and to improve the anti-inflammatory activity of the two lead compounds. To do so, we had to develop semisynthetic strategies that will require great amounts of δ-AC. Even though this secondary metabolite is isolated with a high yield (1.6%) from the barks of *G. amplexicaulis*, our main concern is that this tree is endemic from New Caledonia [2]. We then had to think at an alternative to access large amounts of δ-AC. As δ-GA is obtained with a high yield from *Garcinia kola* nuts (1%), a renewable source easily available in African groceries in France, this prompted us to use this compound as a precursor of δ-AC (Scheme 1). The same issues exist with the sourcing of δ-T3, that was also needed for our SAR studies. Indeed, the plant with the highest contents of δ-T3 is endemic from New Caledonia (*Mammea neurophylla*, 0.5% yield) [4]. Therefore, we also explored semisynthetic strategies to prepare this derivative from δ-GA (Scheme 1).



Scheme 1. General strategy to access T3 analogs from δ-GA

Keywords: Vitamin E, δ-tocotrienol, δ-amplexichromanol, δ-garcinoic acid, semisynthesis, extraction.

References: [1] A. Theriault et al. Clin. Biochem. (1999) 32: 309-319. [2] A. Lavaud, thèse de doctorat n° 2012ANGE0051, Université d'Angers (2012), Métabolites de Clusiaceae : une action sur l'endothélium vasculaire ? l'exemple de *Garcinia amplexicaulis*, 365 pages. [3] K. Alsabil et al. Planta Med (2016) 82: 1110–1116. [4] B. T. Dang et al. Fitoterapia (2014) 96: 65-75.

Sucrose control the branch process by post-transcriptional regulation in *Rosa hybrida*

Ming WANG¹, Laurent Ogé¹, Maria Dolores PEREZ GARCIA¹, Latifa HAMAMA², Soulaiman SAKR¹

ming.wang@inra.fr

¹ Agrocampus-Ouest, Unité Mixte de Recherche 1345 IRHS (Institut de Recherche en Horticulture et Semences), Angers F-49000, France

² University of Angers, Unité Mixte de Recherche 1345 IRHS (Institut de Recherche en Horticulture et Semences), Angers F-49000, France

The shoot apical meristem would differentiate into a branch if they meet the appropriate conditions and stimuli. In this process, some candidate genes were found to play critical roles in the past decade years. The experiment from many researchers indicated that *BRANCHED1* (*BRC1*) and its homologue genes in other plant species act as an integrator of branching signals within axillary buds (Aguilar-Martinez et al., 2007). Some researchers discovered that apical dominance strongly correlates with sugar availability and not apically supplied auxin (Mason et al., 2014). Further research indicated that sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in *Rosa hybrida*, and it influence the expression of *RhBRC1* (Barbier et al., 2015). In this experiment, 12 PUF protein members are isolated in *Rosa hybrida* genomic sequence. PUF protein is an RNA-binding proteins, might bind the conserved UGUR (where R represents a purine) tetranucleotide motif flanked downstream by an AU-rich sequence in the mRNA (Tam et al., 2014). And we found that the *RhBRC1*, *RhMAX2* and *RhD14* contained this motif in their 3'UTR. In this research, we indicated that the *RhPUF4*, a member of PUF protein family, transcripts in the bud which treated by sucrose and this transcription was stimulated by sucrose concentration. Moreover, this stimulation is inhibited by auxin which is the inhibitor of bud outgrowth. Therefore, the *RhPUF4* might involve in the branching regulation by influence the stability of *RhBRC1* mRNA, and this regulation process was under the control of sucrose in Rose. Based on this research, it is showed that the sugar might regulate the Rose branching by influencing the stability of *RhBRC1* mRNA.

Keywords: PUF protein; sugar; *BRC1*; post-transcriptional regulation

References:

- Aguilar-Martínez, J. A., Poza-Carrión, C., & Cubas, P. (2007). *Arabidopsis BRANCHED1* acts as an integrator of branching signals within axillary buds. *The Plant Cell*, 19(2), 458-472.
- Barbier, F., Péron, T., Lecerf, M., Perez-Garcia, M. D., Barrière, Q., Rolčík, J., & Roman, H. (2015). Sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in *Rosa hybrida*. *Journal of experimental botany*, 66(9), 2569-2582.
- Mason, M. G., Ross, J. J., Babst, B. A., Wienclaw, B. N., & Beveridge, C. A. (2014). Sugar demand, not auxin, is the initial regulator of apical dominance. *Proceedings of the National Academy of Sciences*, 111(16), 6092-6097.
- Tam, P. P., Barrette-Ng, I. H., Simon, D. M., Tam, M. W., Ang, A. L., & Muench, D. G. (2010). The Puf family of RNA-binding proteins in plants: phylogeny, structural modeling, activity and subcellular localization. *BMC plant biology*, 10(1), 44.

Role of energy metabolism in acquired thermotolerance of *Arabidopsis* seedlings

E. Réthoré, A. Benamar, M. Neveu, M.-H. Avelange-Macherel & D. Macherel

elise.rethore@etud.univ-angers.fr

UMR IRHS, INRA, Agrocampus Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucouzé, France

In the context of global warming, the frequency and severity of heat waves are likely to intensify, with major consequences on both cultivated and wild species. To counteract the impact of heat stress on membrane and protein stability, plants exhibit a heat stress response, which includes the induction of heat shock proteins and antioxidant systems. In this context, mitochondria and chloroplasts are likely to play an important role as energy transducing and signalling organelles. We aim to decipher the contribution of energy metabolism as a component of the heat acclimation mechanisms that allow plants to survive otherwise lethal temperatures. Using a robust system, whereby *Arabidopsis* seedlings are grown in liquid medium under conditions that cause developmental arrest at the cotyledon stage (Benamar *et al.*, 2013), we have shown that a priming treatment (2h at 38°C) enables seedling survival when, 24h later, they are given a 2h noxious heat shock at 43°C. Measurement of respiration and photosynthesis showed that these activities were partly protected by the priming treatment during and after heat shock. Full recovery was observed one day later in primed seedlings, while damage was irreversible in non-primed seedlings. The preservation of energy transduction thus appears to be essential for heat acclimation. Ongoing experiments compare the multi-level omics (transcriptome, proteome and metabolome) heat stress responses of primed and control seedlings to build an integrative picture of the acclimation process.

Keywords: acclimation; heat stress; energy metabolism; physiology; “omics”; cell biology

Reference:

*Benamar, A., Pierart, A., Baecker, V., Avelange-Macherel, M.-H., Rolland, A., Gaudichon, S., Gioia, L. di and Macherel, D. (2013) Simple system using natural mineral water for high-throughput phenotyping of *Arabidopsis thaliana* seedlings in liquid culture. Int. J. High Throughput Screen., 1–15.*

Can the variation of secondary metabolite contents be part of carrot resistance to *Alternaria dauci* ?

Koutouan C.^a, Yovanopoulos C.^a, Piquet M.^a, Hugueney P.^b, Halter D.^b, Claudel P.^b, Baltenweck R.^b, Hamama L.^a, Suel A.^a, Huet S.^a, Peltier D.^a, Briard M.^a and Le Clerc V.^a

claude.koutouan@inra.fr

^a UMR 1345 - Institut de Recherche en Horticulture et Semences (IRHS), Agrocampus Ouest, INRA, SFR Quasav, University Angers, France

^b UMR 1131, Métabolisme Secondaire de la Vigne, INRA-Université de Strasbourg, France

Combining different complementary mechanisms of resistance (i.e. different modes of action against the pathogen) through a QTL pyramiding strategy should be more efficient to develop durable resistant varieties than relying on a single mechanism. Based on various approaches we showed that different patterns of carrot resistance to *Alternaria dauci*, the fungus responsible for the most damaging leaf disease on this species, may exist (Boedo et al. 2010; Mickael Lecomte 2013; Mickaël Lecomte et al. 2014; Le Clerc et al. 2015). Some evidences support that some secondary metabolites could be involved in those different mechanisms of resistance. Indeed, laboratory experiments showed that jasmonic acid biosynthesis pathway could be involved in carrot resistance to *A. dauci* (Mickaël Lecomte 2013). Beside this, the inhibitory effect of falcarindiol on the development of *Alternaria dauci* was highlighted (Mickaël Lecomte et al. 2012). We also detected differential accumulation of other secondary metabolites between resistant and susceptible cultivars in various growing conditions. To strengthen our assumptions that these secondary metabolites may be involved in carrot resistance to *A. dauci*, we analyzed the variation of these different types of secondary metabolites in segregating populations after *Alternaria dauci* infestation in field conditions. Some of them were identified as good candidates for metabolite QTL (mQTL) analyses and their co-localization with previously detected resistance QTLs was investigated. Their potential implication in carrot resistance mechanisms to *A. dauci* will be discussed.

Keywords: Carrot, secondary metabolites, *Alternaria dauci*, resistance, QTLs.

References:

- Boedo, C., R. Berruyer, M. Lecomte, S. Bersihand, M. Briard, V. Le Clerc, P. Simoneau, and P. Poupart. 2010. "Evaluation of Different Methods for the Characterization of Carrot Resistance to the *Alternaria* Leaf Blight Pathogen (*Alternaria Dauci*) Revealed Two Qualitatively Different Resistances." *Plant Pathology* 59 (2): 368–75.
- Le Clerc, Valérie, Sabrina Marques, Anita Suel, Sébastien Huet, Latifa Hamama, Linda Voisine, E. Auperpin, et al. 2015. "QTL Mapping of Carrot Resistance to Leaf Blight with Connected Populations: Stability across Years and Consequences for Breeding." *Theoretical and Applied Genetics* 128 (11). Springer Berlin Heidelberg: 2177–87.
- Lecomte, Mickael. 2013. "Analyse Des Mécanismes de Défense de La Carotte (*Daucus Carota*) Face Au Champignon Pathogène *Alternaria Dauci*, Responsable de L'alternariose Ou Brûlure Foliaire." University of Angers, France.
- Lecomte, Mickaël, Romain Berruyer, Latifa Hamama, Cora Boedo, Piétrick Hudhomme, Stéphanie Bersihand, Joseph Arul, et al. 2012. "Inhibitory Effects of the Carrot Metabolites 6-Methoxymellein and Falcarindiol on Development of the Fungal Leaf Blight Pathogen *Alternaria Dauci*." *Physiological and Molecular Plant Pathology* 80.
- Lecomte, Mickaël, Latifa Hamama, Linda Voisine, Julia Gatto, Jean-Jacques Hélesbeux, Denis Séraphin, Luis M. Peña-Rodríguez, et al. 2014. "Partial Resistance of Carrot to *Alternaria Dauci* Correlates with In Vitro Cultured Carrot Cell Resistance to Fungal Exudates." Edited by Richard A. Wilson. *PLoS ONE* 9 (7).

Acknowledgements: The authors would like to thank IRHS-IMORPHEN team involved in tunnel experiments and Stéphan Plas (Invénio) for field experiment. This work was financed by the Région Pays de La Loire (RFI Objectif Végétal) and by The Ministry of Agriculture (FranceAgriMer).

Resistance and pathogenicity : how toxins are involved in the carrot- *Alternaria dauci* interaction

Julia Courtial, Latifa Hamama, Yann Renaux, Mickael Lecomte, Esteban Guichard, Jean Jacques Hellesbeux, Pascal Richomme, Mathilde Briard, Romain Berruyer et Pascal Poupart.

Julia.courtial@etud.univ-angers.fr

UMR 1345 IRHS, Université d'Angers, INRA, Agro-campus ouest, SFR QUASAV, 42 Rue Georges Morel, 49070 Beaucouzé, France.

Leaf blight caused by *Alternaria dauci* is the most damaging foliar disease on carrots, impeding the mechanistic harvest. Fungicides remain the most effective way to control this disease. However, those molecules are expensive, affect environment and could be insufficient. Only partially resistant cultivars are known and marketed but their resistance levels are not sufficient yet, and plant quantitative pathogen resistance mechanisms are poorly characterized. We chose to investigate such mechanisms in the *Alternaria dauci* – carrot interaction. Previously, several converging experimental results were obtained, showing that fungal toxins take a central place in this interaction (1). Toxicity tests performed on carrot cells revealed that only the organic phase of *A. dauci* exudates is toxic (1). Moreover, a correlation between carrot plant resistance to *A. dauci* and carrot cells resistance to fungal exudates was found. These results incited us to identify and characterize *A. dauci* toxin(s) involved in pathogenicity, to decipher their biosynthesis pathway(s) and their regulations. Using HPLC profiles of organic phase exudates from different fungal strains, we uncovered a great variation in toxins production between fungal strains. Furthermore, a new correlation, between aggressiveness of these stains and some fungal exudate's molecules quantities was found, suggesting that toxins production plays a major role in the *A. dauci* – carrot interaction. We performed extraction and purification of one of the candidate molecules and we resolved its chemical structure. A toxicity test of this molecule has been achieved with a new protocol of survival quantification. The next step in this project will be the elucidation of its biosynthesis pathway. To this end, the *A. dauci* transcriptome data will be used to select genes possibly involved in its biosynthesis pathway.

Keywords: Phytotoxin, quantitative disease resistance, aggressiveness, *Alternaria Leaf Blight*

References: (1) Lecomte, M., Hamama, L., Voisine, L., Gatto, J., Hellesbeux, J.-J., Séraphin, D., Peña-Rodriguez, L. M., Richomme, P., Boedo, C., Yovanopoulos, C., Gyomlai, M., Briard, M., Simoneau, P., Poupart, P., & Berruyer, R. (2014). Partial resistance of carrot to *Alternaria dauci* correlates with in vitro cultured carrot cell resistance to fungal exudates. *PLoS ONE* 9(7): e101008.

Nanoencapsulation and synergistic effect of insecticides: An innovative strategy to improve treatment efficacy against pest insects while reducing doses

Javier Pitti Caballero¹, Guillaume Bastiat², Corinne Lefrançois¹, Bruno Lapié¹ & Véronique Marchais¹

javierernesto.pitticaballero@etud.univ-angers.fr

¹Laboratoire SiFCIR UPRES EA 2647/USC INRA 1330, Faculté des Sciences, 2 boulevard Lavoisier,
F-49045 Angers cedex, France

²Laboratoire MINT INSERM 1066 / CNRS 6021, IBS-CHU, 4 rue Larrey, 49933, Angers cedex 9, France

Increasing agricultural productivity is today a challenge with global issues such as climate change, soaring food and fuel prices. This can happen through improved use and management of agricultural biodiversity resources (such as seeds, pollination, and beneficial fauna). Increasing agricultural production has a number of implications, amongst which is ensuring that crops are safe, both during their production and consumption. Crop protection is a critical aspect of production and the over-use pesticides have impacts on crop-associated biodiversity as well as human health. In addition, side effects, like insecticide resistance, are currently reported. In such context, the French Ecophyto II 2025 program has been planned to reduce by 50% the use of pesticides in order to avoid side effects on the environment and non-target organisms but also to limit the development of resistance. The development of innovative nanotechnologies as the nanoencapsulation of insecticides can help farmers mitigate pest resistance and open exciting alternative approaches against pest insects. This insecticide nanocapsule system is a method used to obtain products with controlled release properties. It is designed to reduce mammalian toxicity, evaporative losses, phytotoxicity and also to extend the activity, protect insecticides from environmental degradation and reduce leaching. Therefore, our project is to develop a technique of nanoencapsulation of two insecticide molecules of different chemical families, with specific modes of action in the central nervous system of insects: a pyrethroid, deltamethrin used as synergistic agent and an oxadiazine, indoxacarb which is a pro-insecticide. The synergistic effect of this non-encapsulated mixture has already been evaluated, *in vitro*, on cockroache cells (DUM neurons) in the laboratory SiFCIR. Based on these findings, the first aim of this approach has been to perform *in vivo* toxicity studies on cockroaches (*Periplaneta americana*) to determine the lethal dose 50 (LD₅₀) for each insecticide used in order to determine the best ratio of insecticides mixture necessary to obtain the synergistic effect. The second step has been to develop lipid nanocapsules (LNCs) containing the synergistic agent, deltamethrin. Our toxicity studies reveal a synergistic effect between the LNCdeltamethrin and indoxacarb on whole insects. Finally, the third step of the project is to study the kinetics of release of nanoencapsulated deltamethrin and to determine the effective lowest doses of both deltamethrin and indoxacarb to be used in the final formulation.

Keywords: Insecticide, Nanoencapsulation, Synergistic effect, Crop protection

Combinatorial chlorophyll fluorescence imaging of plant pathogen interaction

Valerian Méline^a, Lydie Ledroit^b, Etienne Belin^c, Tristan Boureau^d

valerian.meline@inra.fr

^a EMERSYS, SFR 4207 QUASAV, IRHS, UMR1345, INRA, F-49071 Beaucouzé, France

^b IMHORPHEN, SFR 4207 QUASAV, IRHS, UMR1345, INRA, F-49071 Beaucouzé, France

^c IMHORPHEN, SFR 4207 QUASAV, F-49045, LARIS, Université d'Angers, F-49000 Angers, France.

^d IMHORPHEN, SFR 4207 QUASAV, IRHS, UMR1345, University of Angers, F-49045 Angers, France.

In the basic research in plant phytopathology, the study of plant microorganism interactions is frequently confronted with phenotypes which can't be discriminated with human eye or even with visible imaging (RGB).

Chlorophyll fluorescence imaging has been increasingly used to quantify the impact of different pathogens on plants (Mahlein, 2016). Many conventional chlorophyll fluorescence parameters such as FvFm and NPQ have been identified that have physiological interpretation and are used to image the impact of stresses on plants (Zabala *et al*, 2015; Zhou *et al*, 2015; Rousseau *et al*, 2013). However they do not necessarily provide the best diagnostic signatures of the disease. The aim of the present work was to develop specific parameters which aim at increasing the precision of the phenotyping to better discriminate impacts originating from different stresses on plant tissues.

Leaves of *Nicotiana benthamiana* were inoculated with various mutants of *Xanthomonas* which tend to lead to chlorotic or necrotic phenotypes. These leaves were imaged using all conventional chlorophyll fluorescence parameters available. Although these classical parameters allowed a visualization of the different phenotypes, they can't specifically illustrate each of them. We use different combinatorial imaging technics such as linear combination (Pineda *et al*, 2008) or indices development (Huang *et al*, 2014) to elaborate new parameters which improve the specificity and sensibility of the chlorophyll fluorescence imaging.

Using these new parameters we are now able to visualize and quantify specifically each phenotype. The application of these approaches to other imaging techniques like hyperspectral imaging could allow the discrimination of mutants which show identical profiles with chlorophyll fluorescence imaging.

Keywords: chlorophyll fluorescence, imaging technics, plant pathogen interaction

References:

- Mahlein, A-K. 2016. Plant Disease Detection by imaging sensors. *Plant Disease*. Vol 100 No 2. 241:251
- Zabala, M. Littlejohn, G. Jayaraman, S. Studholme, D. Bailey, T. Lawson, T. Tillich, M. Licht, D. Bolter, B. Delfino, L. Truman, W. Mansfield, J. Smirnoff, N. Grant, M. 2015. Chloroplasts play a central role in plant defense and are targeted by pathogen effectors. *Nature Plants* 1, 15074
- Zhou, J. Zeng, L. Liu, J. Xing D. 2015. Manipulation of the Xanthophyll Cycle Increases Plant Susceptibility to Sclerotinia sclerotiorum. *PLoS Pathog* 11(5): e1004878. doi:10.1371/journal.ppat.1004878
- Rousseau, C. Belin, E. Bove, E. Rousseau, D. Fabre, F. Berruyer, R. Guillaumès, J. Manceau, R. Jacques, MA. and Boureau, T. 2013. High throughput quantitative phenotyping of plant resistance using chlorophyll fluorescence image analysis. *Plant Methods*. 9:17
- Pineda, M. Soukupova, J. Matous, K. Nedbal, L. Baron, M. Conventional and combinatorial chlorophyll fluorescence imaging of tobamovirus-infected plants . 2008. *Photosynthetica*. 46 (3): 441:451
- Huang, W. Qingsong, G. Juhua, Luo. Dongyan.Zhang, 2014. New optimized spectral indices for identifying and monitoring winter wheat diseases. *IEEE*. Vol 7. No 6

Regulation of plant development and defense response by SCOOP, a new small endogenous peptide

Kay Gully^{1*}, Sandra Pelletier^{1*}, Marie-Charlotte Guillou¹, Marina Ferrand², Sophie Aligon¹, Emilie Vergne¹, Mathilde Fagard², Philippe Grappin¹, Jean-Pierre Renou¹, Etienne Bucher^{1‡}, Sébastien Aubourg^{1‡}.

kay.gully@inra.fr

¹ IRHS (*Institut de Recherche en Horticulture et Semences*), UMR 1345, INRA, Agrocampus-Ouest, Université d'Angers, SFR 4207 QuaSaV, Beaucozé F-49071, France.

² IJPB (*Institut Jean-Pierre Bourgin*), UMR 1318, INRA, AgroParistech, ERL CNRS 3559, Université Paris-Saclay, RD10, Versailles F-78026, France.

* These authors contributed equally to this work

‡ Co-corresponding authors

In order to counter constant pathogen aggressions, plants have developed sophisticated detection and response systems that decipher pathogen signals and induce specific defenses. As one of the defense signals, small secreted endogenous peptides play an important role either by directly interacting with pathogens or through their function in development and cell-to-cell communication. In Arabidopsis, we have identified a new small gene family of 12 members with similar intron-exon structure (2 or 3 exons) encoding proteins with length ranging from 72 to 117 amino acids. We termed the gene family *PROSCOOP* and have identified orthologues in eight Brassicaceae genomes. RNAseq data gave us insights into the conditions and organs in which *PROSCOOP* gene family members are transcribed. Multiple-alignment allowed us to identify two significant conserved motives within the gene family. We show here that a small conserved peptide (SCOOP, 13 amino acids) presumably derived from the *PROSCOOP* precursor protein can induce defense responses (production of reactive oxygen species, callose deposition and inhibition of seedling growth) in Arabidopsis. Moreover we show that the transcription of the early defense marker FRK1 is induced by SCOOP. Interestingly we observed that exogenous SCOOP peptide could affect root growth and that a knock out mutant of *PROSCOOP* showed increased root growth indicating a role in root development and therefore a possible dual role of this new peptide.

Keywords: *Arabidopsis; DAMP; PTI; small endogenous peptide; root development*

Implication des mécanismes épigénétiques dans la potentialisation des défenses chez le pommier par un stimulateur de défense à fort potentiel de protection

Romain WARNEYS, Matthieu Gaucher, Etienne Bucher, Marie-Noëlle Brisset & Alexandre Degrave

romain.warneys@agrocampus-ouest.fr

UMR 1345 IRHS Angers Equipe Respom

La France est le troisième pays producteur de pommes dans l'union européenne, ce secteur présente une activité économique d'importance. Depuis la mise en place du plan Ecophyto qui vise à réduire de moitié l'utilisation de produits phytosanitaires en agriculture, les pomiculteurs sont dans la nécessité de mettre en œuvre de nouveaux systèmes de culture plus respectueux de l'environnement. Les stimulateurs de défenses des plantes (SDP) représentent une nouvelle génération d'intrants qui pourraient participer à la diminution de l'usage des pesticides. Plusieurs types de stimulateurs se distinguent, les SDP à effet direct qui induisent après application une réponse de défense de la plante et les SDP à effet potentialisateur qui vont conduire la plante à pouvoir réagir de manière plus rapide lorsqu'elle sera soumise à une attaque. Parmi les stimulateurs de défense des plantes, le Bion WG50 est un analogue fonctionnel d'acide salicylique qui a démontré une importante efficacité de protection vis-à-vis des trois principaux bioagresseurs du pommier.

Le but de ce projet de thèse est de (i) comprendre le mode d'action de ce stimulateur de défense des plantes (SDP) et déterminer quels sont les mécanismes mis en place par la plante permettant d'induire un état de résistance vis-à-vis des trois principaux bioagresseurs du pommier, (ii) identifier les gènes présentant une expression potentialisée et déterminer si ces gènes sont soumis à des modifications épigénétiques et (iii) déterminer la durée de mémorisation du traitement par la plante. Afin de déterminer quels sont les mécanismes activés suite à l'application du SDP plusieurs méthodes d'analyses globales ont été réalisées. Ces analyses ont permis de mettre en évidence l'induction et la potentialisation de nombreux gènes de défenses ainsi que la production de composés organiques volatiles connus pour être des répulsifs d'insectes ravageurs. Des analyses de la structure chromatiniennes sont en cours, le but étant de corrélérer les modifications épigénétiques avec l'expression potentialisée des gènes de défense. Enfin le dernier aspect de ce projet de thèse est de déterminer la durée de mémorisation par la plante d'un traitement SDP. Pour ce faire nous nous plaçons à deux échelles de temps : un temps court allant d'une semaine à trois semaines entre l'application du produit et le stress secondaire et un temps long correspondant à une génération. Cette mémorisation sera caractérisée via les marqueurs épigénétiques, transcriptionnels et protéiques précédemment identifiés.

Mots clés: Acibenzolar-S-methyl, Priming, Epigénétique, Defense.

Références:

Dugé de Bernonville T, Marolleau B, Staub J, Gaucher M and Brisset MN. (2014). Using molecular tools to decipher the complex world of plant resistance inducers: an apple case study. *J. Agr. Food Chem.* 62, 11403-11411.

Jaskiewicz M, Conrath U, Peterhänsel C (2011) Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO reports*, 12(1): 50-55.

Apple genome and epigenome bioinformatic analysis

Nicolas Daccord, Claudine Devauchelle, Etienne Bucher

nicolas.daccord@inra.fr

Institut de Recherche en Horticulture et Semences (IRHS), UMR1345 (INRA/Agrocampus-ouest / Université d'Angers), 42 rue Georges Morel – CS 60057, 49071 Beauzoué cedex – France

Accurate sequence information and genome assemblies are critical for studies on genomic and epigenomic variations. Although partial genome information was already available for apple (Velasco et al. 2010), the assembled sequence is fragmented and lacks repeated regions. First, using second- (Illumina) and third-generation sequencing and optical mapping technologies (PacBio and BioNano), we have generated a high quality genome assembly of a 'Golden Delicious' doubled haploid tree (Daccord, Celton et al. 2017). Our de novo assembly resulted in a genome of 649.7 Mb, with a N50 of 5.6 Mb. Seventeen pseudo-chromosomes were constructed and validated using a high density integrated genetic linkage map (Di Pierro, E. A. et al. 2016) and linkage disequilibrium analysis. Using similarities, transcript resources and the EuGene predictor/combiner (Foissac et al. 2008), 45,115 protein coding genes were predicted and tagged, when possible, by putative function.

In a second time, to understand the potential role of epigenetic marks on fruit development, we constructed genome-wide DNA methylation maps that compared different tissues and two isogenic apple lines that produce large or small fruits. General correlations between methylation patterns in promoters and gene expression were established. Moreover, this led to the identification of differentially methylated regions that are associated with genes involved in fruit development.

Keywords: *apple, genome annotation, genome assembly, DNA methylation*

References:

- Daccord, N., Celton, J. M. et al. (2017). High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. *Nature genetics*, 49(7), 1099-1106.
- Di Pierro, E. A. et al. (2016). A high-density, multi-parental SNP genetic map on apple validates a new mapping approach for outcrossing species. *Horticulture research*, 3, 16057.
- Foissac, S. et al. (2008). Genome annotation in plants and fungi: EuGene as a model platform. *Current Bioinformatics*, 3(2), 87-97.
- Velasco, R. et al. (2010). The genome of the domesticated apple (*Malus [times] domestica* Borkh.). *Nature genetics*, 42(10), 833-839.

Mise en œuvre d'une démarche participative d'éco-quali-conception appliquée aux systèmes de production viticoles

Anthony Rouault^{1,2}, Christel Renaud-Gentie¹, Aurélie Perrin¹, Frédérique Jourjon¹

a.rouault@groupe-esra.com

¹Unité de Recherche GRAPPE, Univ Bretagne Loire, Ecole Supérieure d'Agricultures (ESA)-INRA, 55 rue Rabelais, BP. 30748, 49007 Angers Cedex, France; ²ADEME, SAF, 20, Avenue du Grésillé, 49 000 Angers, France

La viticulture doit aujourd'hui faire face à des exigences croissantes de qualité de la part des consommateurs mais aussi à des enjeux environnementaux grandissants. Ces derniers imposent une réduction des intrants phytosanitaires mais aussi une re-conception des itinéraires techniques (ITK) viticoles afin de diminuer leur impact global sur l'environnement. L'Analyse du Cycle de Vie (ACV) s'avère une méthode pertinente pour identifier les pratiques viticoles les plus impactantes et ainsi aider au changement de pratiques (Renaud-Gentie, 2015). Elle peut être couplée à des démarches d'évaluation de la qualité (Beauchet, 2016). Enfin, plusieurs travaux soulignent l'intérêt des démarches de recherche-intervention avec des groupes d'agriculteurs pour contribuer à l'émergence de systèmes agricoles durables (Mac Millan and Benton, 2014 ;Meybeck and Redfern, 2016). Ce travail de thèse vise à créer et tester une nouvelle démarche, l'"éco-quali-conception[®]", qui intègre les deux dimensions (environnement et qualité du raisin) dans un processus de co-conception conduit avec un groupe de viticulteurs.

Deux sessions de trois ateliers d'éco-quali-conception[®] sont organisées avec deux groupes distincts de viticulteurs déjà engagés dans des démarches environnementales (Dephy et Agriconfiance). Ces ateliers s'inspirent des démarches de co-conception en agriculture (Meynard et al., 2012) et s'appuient sur des cas d'étude réels issus de viticulteurs du groupe. Les objectifs de la démarche sont de sensibiliser les viticulteurs aux démarches d'écoconception, de les faire réfléchir ensemble aux impacts environnementaux de leurs pratiques et d'aboutir à la co-conception d'ITK alternatifs plus performants sur le plan environnemental et en termes de potentiel de qualité du raisin.

La 1^e session d'ateliers menée en 2017 a permis de concevoir des ITK plus performants sur le plan environnemental que les cas d'études initiaux (36 à 59% de réduction d'impact). L'ACV a notamment permis d'identifier la réduction de dose de produits phytosanitaire (via Opti-dose ou panneaux récupérateurs), le changement de substance active (suppression des CMR) et l'éco-conduite comme leviers prioritaires. Une analyse réflexive a permis d'identifier la nécessité d'une intégration plus forte du lien pratiques-qualité pour la 2^e session d'ateliers prévue en 2018. Cette 1^e session a également permis de valider scientifiquement l'originalité de la démarche et sa contribution potentielle au changement de pratiques en viticulture.

Mots clés: viticulture, analyse du cycle de vie, itinéraire technique, éco-conception, démarche participative

Référence:

- Beauchet S. (2016). *Évaluation multicritère d'itinéraires techniques viticoles associant l'évaluation environnementale par Analyse du Cycle de Vie avec l'évaluation de la qualité du raisin. Contribution au choix des pratiques pour une amélioration des itinéraires techniques viticoles, thèse de doctorat, L'Université Bretagne Loire.*
- MacMillan, T.; Benton, T.G. (2014). "Agriculture: Engage farmers in research." *Nature* 509: 25-27.
- Meybeck, A. and Redfern, S. (2016). "Knowledge needs for sustainable food systems." *Knowledge and Information for Sustainable Food Systems*: 217.
- Meynard, J.-M., B. Dedieu (2012). "Re-design and co-design of farming systems. An overview of methods and practices." In: *Farming Systems Research into the 21st Century: The New Dynamic*. Springer Netherlands: 405-429.
- Renaud-Gentie C. (2015). *Eco-efficience des itinéraires techniques viticoles : intérêts et adaptations de l'Analyse du Cycle de Vie pour la prise en compte des spécificités de la viticulture de qualité, application aux itinéraires techniques de production de raisins de Chenin blanc pour vins blancs secs d'AOC en Moyenne Vallée de la Loire, thèse de doctorat, l'Université Nantes-Angers-Le Mans*, 250p.

Remerciements

Aux participants:

Alexia VILLE	alexia.ville@univ-angers.fr
Ming WANG	ming.wang@inra.fr
Elise RETHORE	elise.rethore@etud.univ-angers.fr
Claude KOUTOUAN	claude.koutouan@inra.fr
Julia COURTIAL	julia.courtial@etud.univ-angers.fr
Javier Ernesto PITTI	javierernesto.pitticaballero@etud.univ-angers.fr
Valerian MELINE	valerian.meline@inra.fr
Kay GULLY	kay.gully@inra.fr
Romain WARNEYS	romain.warneys@agrocampus-ouest.fr
Nicolas DACCORD	nicolas.daccord@inra.fr
Anthony ROUAULT	a.rouault@groupe-esa.com

Aux membres du jury :

Etienne Neethling (GRAPPE)	etienne.neethling@inra.fr
Alain Vian (IRHS 1)	alain.vian@univ-angers.fr
Alexandre Degrawe (IRHS 2)	alexandre.degrawe@agrocampus-ouest.fr
Yves Cadot (IRHS 3)	yves.cadot@inra.fr
Lucie Poulin (LBPV)	Lucie.Poulin@univ-nantes.fr
Joëlle Fustec (LEVA)	j.fustec@groupe-esa.com
Delphine Goven (SIFCIR)	delphine.goven@univ-angers.fr
Andreas Schinkovitz (SONAS 1)	andreas.schinkovitz@univ-angers.fr
Pascal Gentit (ANSES-BVO)	pascal.gentit@anses.fr
Sophie Herpin (EPHOR)	sophie.herpin@agrocampus-ouest.fr