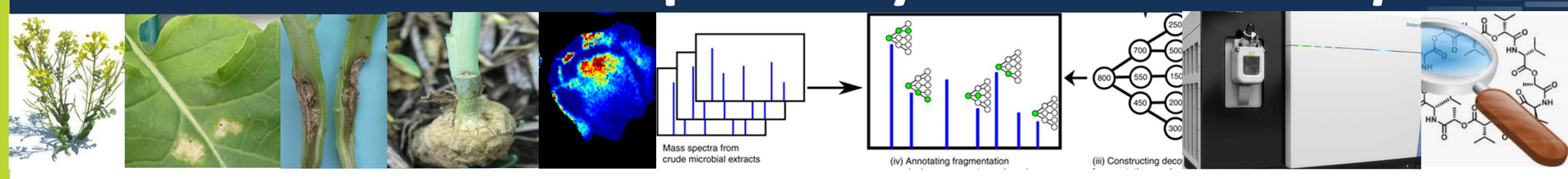
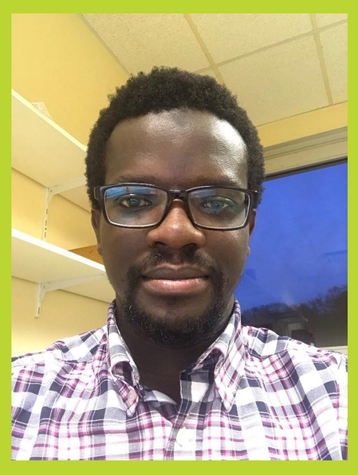
Multiomics-guided study and discovery of induced defense metabolic pathways in *Brassica napus*





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Team name

Resistance and Adaptation

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Keywords

Interaction pathogens - *B. napus,* Blackleg of crucifers, clubroot, specialized metabolites, response to pathogens, Regulation of metabolic pathways, Un/targeted metabolomic, Molecular Networks, Metabolic networks

Socio-economic context

Rapeseed (*Brassica napus*) is a major oleaginous crop in Europe. Plant breeders are interested by discovery genetic resistances to diseases that affect this crop. However, the genetic resistances used in breeding are usually breakdown in a few years due to fast evolution and adaptation of pathogens populations. Therefore it is necessary to increase our knowledge on plant cellular responses to pathogens.

Scientific context

Functional genomic approaches in *Arabidopsis thaliana* have led to advanced knowledge of metabolic processes involved in plant defence. However metabolic pathways involved in the biosynthesis of specialized metabolites are mostly taxon-specific, and thus many of them remains partially understood in *B. napus*. For example Pedras et *al.* (2010) reported about 40 *Brassica*-specific indole phytoalexins, but the biosynthetic pathways have been identified for only 3 of them (see Fig. 1). This lack of knowledge is a limit to the integrative study of pathogen responses in *Brassica* species.

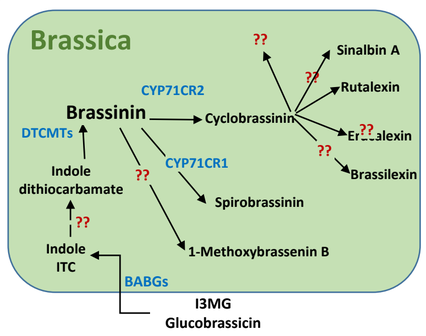


Fig. 1 | Putative pathway involved in the biosynthesis of a selection of indole phytoalexins in *Brassica* species. Only a few enzymes have been identified up to now (in blue by Klein et *al*. 2017).

Objectives

My thesis project aims to answer the following question : **How metabolic pathways involved in response to pathogens affecting *B. napus* are regulated *?*** To that purpose, multi-omics approaches will be conducted to analyse metabolic responses in a series of plant genotypes challenged by infection with *Leptosphaeria maculans* (causal agent of blackleg) and *Plasmodiophora brassicae* (causal agent of clubroot).

(1) Metabolomic approaches will be focused on the identification of an extensive diversity of metabolites regulated during plant infection (glucosinolates (GLS), flavonoids and indole phytoalexins). Data from Mass Spectrometry Imaging and LC-HRMS will highlight a series of compounds, and those data will be correlated with transcriptomic data.

(2) Transcriptomics and genomics approaches will be focused on the identification of candidate genes potentially involved in the regulation of *Brassica* specialized metabolism (inferred from annotation and gene/metabolite co-expression networks).

(3) Functional validation of candidate genes through heterologous expression in *Arabidopsis* and Yeast.

Results

Here, I present my preliminary metabolomic results on the response to *L. maculans* in infected rapeseed stems.

<u>LC-MS development for the detection and identification of known and unknown indole phytoalexins in *Brassica* tissues.</u>

The use of LC-HRMS allowed to highlight (see Fig. 2) the accumulation in infected stems of many of the phytoalexins previously reported by Pedras et *al.* (2010). This approach also document a series of additional putative indole phytoalexins, which have never been reported previously, and which now require additional structural investigations.

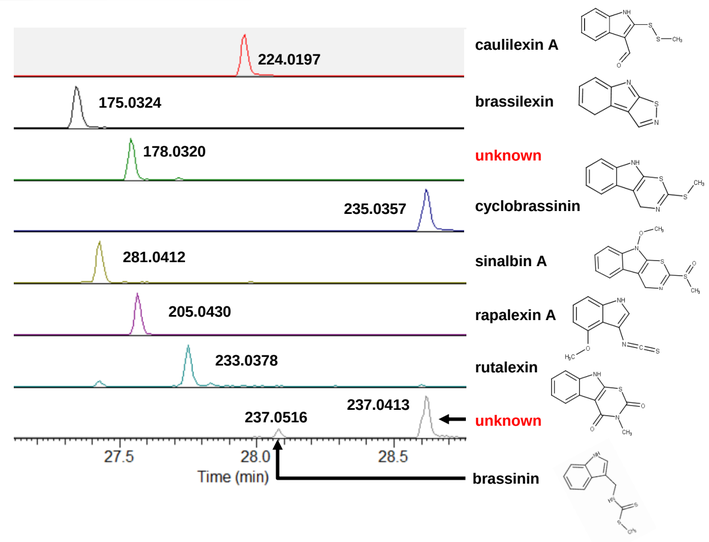


Fig. 2 | Illustration of an LC-HRMS analysis for a series of phytoalexins and putative phytoalexins (indicated as 'unknown') that are specifically accumulated in *B. napus* stems following infection by *L. maculans*. Putative identification of molecules is based on exact m/z values, indicated on the figure.

<u>Molecular network for exploring the identification of unkown signal from mass spectrometry.</u>

Further identification of unkown compounds was investigated using a molecular network approach : Networks are builded from systematic fragmentation LC-HRMSn data (see Fig. 3) In this network, molecules are connected when considered as structurally closed, *i.e.* when they share common fragments. This approach will be used to infer putative structures of unknown mass signals.

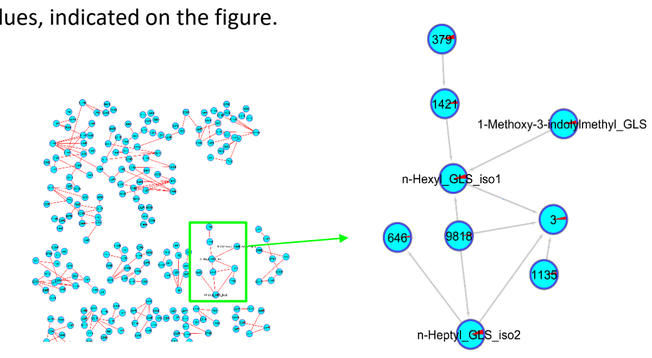


Fig. 3 | *B. napus* molecular Networking used for identification of unknowns metabolites. Here, we are an example : heptyl and hexyl GLS are members of Non-methionine derivated Aliphatic GLS (atypical GLS never characterized in *B. napus*).

<u>Identification of a series of atypical glucosinolates accumulated during stem infection by *Leptosphaeria.*</u>

A chromatographic procedure was improved for the profiling of about 50 glucosinolates. This list includes well known compounds with authentified biosynthesis pathways, and also poorly described compounds such as alkyl-GLS. Among those compounds, our results indicate that one isomer of hexyl-GLS and one isomer of heptyl-GLS are specifically induced by one strain of *Leptosphaeria* (see Fig. 4).

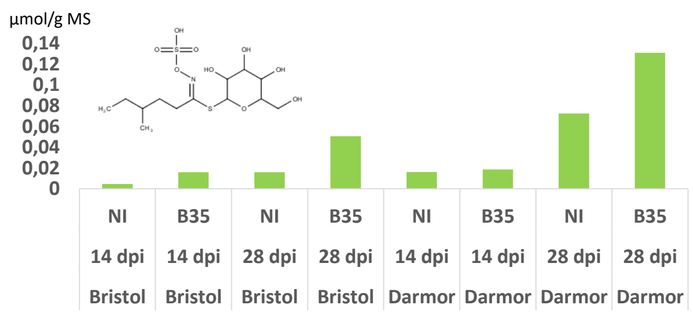
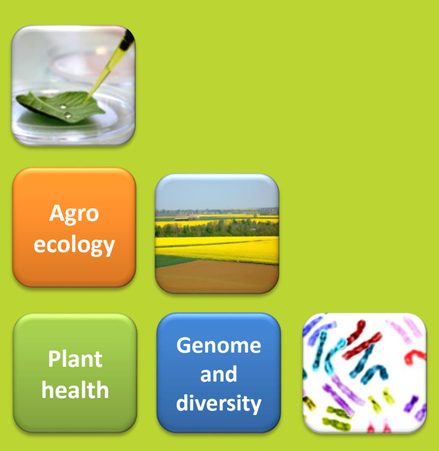


Fig. 4 | Induction of M402_T15.14_cdt_n-Hexyl_GLS_isomers2 in in both partially resistant (Darmor) and fully susceptible genotypes (Bristol) at 14 dpi and 28 dpi with and without petiole infection with *Leptosphaeria.*

Perspectives

We plan to apply those methodologies with other *B. napus* genotype and *P. brassicae* strains interaction.



References

(1) M. Soledade C. Pedras, Estifanos E. Yaya, 2010. Phytoalexins from Brassicaceae: News from the front.

(2) Andrew P. Klein and Elizabeth S. Sattelya, 2017. Biosynthesis of cabbage phytoalexins from indole glucosinolate.