DECIPHERING THE MOLECULAR MECHANISMS INVOLVED IN THE LOCALIZATION OF ORGANELLAR PROTEINS AS WELL AS THE COMPLEX PLANT-INSECT-PATHOGEN INTERACTIONS

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*Figure 1. The Plant Journal front page with the "*FtsH2 and FtsH5: two homologous subunits use different integration mechanisms leading to the same thylakoid multimeric complex*" manuscript from Rodrigues et al. 2011. The Figure illustrates the two-step processing mechanism of the FtsH2 and FtsH5 subunits and their integration into thylakoid membrane by Tat and Sec pathways, respectively*

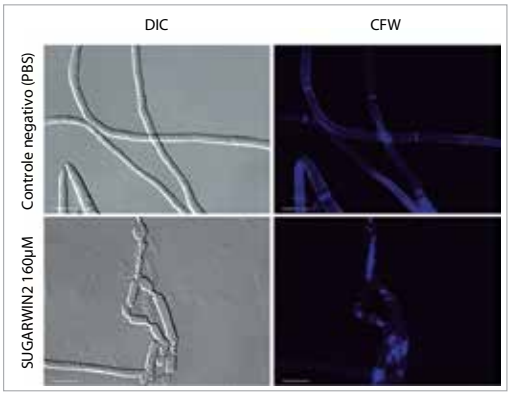

Figure 2. Effects of recombinant sugarcane wound-inducible protein 2 (HisSUGARWIN2) on the hyphal morphology of Colletotrichum falcatum. Calcofluor assay on C. falcatum. C. falcatum germlings were grown in the absence of HisSUGARWIN2 for 16 h of exposure to phosphatebuffered saline (PBS) at 25uC (control) or in the presence of 160 mM HisSUGARWIN2 for 16 h at 25uC. CFW = Calcofluor White. The bars represent 10 mm. (Franco et al., 2014)

Over the last two decades our laboratory has been involved with research and higher education training on the understanding of the molecular mechanisms related to protein subcellular localization, using the model plant *Arabidopsis thaliana*, as well as on the characterization of the complex plant-insect-pathogen interactions.

Regarding the intracellular protein trafficking, we have been working on the identification and characterization of the protein import specificity into their respective subcellular compartments, mainly mitochondria and chloroplasts. We have been able to identify and characterize dual-targeted (DT) proteins and also describe the mechanisms of membrane proteins insertion at organelles (*Figure 1*). Furthermore, we are also working on the identification of auxiliary protein factors acting as regulatory mechanism of organellar membrane proteins.

Our interest on plant-insect-pathogen interactions is related to the bioenergy crop, sugarcane, mainly on the identification and characterization of plant genes involved with the sugarcane borer (*Diatraea saccharalis*) response as well as with opportunistic fungi associated with this interaction. We are also particularly interested on the insect adaptation mechanisms, which allow them to overcome the plant defense barriers, as the peptidase inhibitors (PIs). PIs are essential proteins involved in plant resistance to herbivorous insects. In turn, many insect species are able to escape from the negative effects of these molecules by different mechanisms. One of our main questions is to understand the arms race between plants and herbivore insects in terms of evolutionary mechanisms leading to insect adaptation or successful barriers to prevent insect damage.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In the intracellular protein trafficking study, we have been able to identify dual-targeted (DT) proteins and also to describe for the first time a post transcriptional mechanisms in plants based on an alternative translation initiation, capable of determining the differential localization of THI1 protein in Arabidopsis. In addition, we also performed evolutionary studies with respect to DT proteins and its conservation among plants. Our results showed that the dual-targeted condition observed in the aminoacyl-transfer RNA (tRNA) synthetases (aaRS) is a gain-of-function derived from gene duplication events and that this condition is maintained among other plants. Regarding the mechanism of integration organellar of some proteins we showed that even high homologues proteins, as the FtsH type A and type B subunits, could use distinct integration mechanisms to lead to the same thylakoid multimeric complex (*Figure 1*).

In the plant-insect-pathogen study we characterized an antifungal protein, SUGARWIN, induced after the sugarcane borer (D. saccharalis) damage and pathogen infection. This protein affects the *Colletotrichum falcatum* (*Figure 2*) and *Fusarium verticilioides* morphology leading to the fungal cell death by apoptosis. This relationship between insect and fungal was further investigated, showing that the presence of both fungal species attract and positively influence *D. saccharalis* feeding, at the same time that the presence of this insect is favorable for the fungal proliferation in sugarcane.

In our studies with peptidase inhibitors, we observed that different Lepidoptera species have different susceptibility levels to these molecules, particularly, we observed that *D. sacharalis* (Crambidae) is more susceptibility to soybean PIs than *S. frugiperda* (Noctuidae). In order to understand this variable response, we developed several experimental and in silico studies, which showed that *S. frugiperda* larvae present a more variable set of trypsin enzymes and a general up regulation in their chymotrypsin and trypsin genes after the inhibitor challenge, while *D. sacharalis* larvae present less variable trypsin genes and absence of expression modulation in their chymotrypsin and trypsin genes in the same conditions. An evolutionary study showed that this could be result of an adaptation of Noctuidae species, originated from an expansion of the trypsin gene family.

MAIN PUBLICATIONS

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