

MOLECULAR STRATEGIES INVOLVED IN PLANT-INSECT INTERACTIONS

Marcio de Castro Silva Filho

Luiz de Queiroz Agriculture School / University of São Paulo (ESALQ/USP)

FAPESP Process 2008/52067-3 | Term: Mar 2009 to Feb 2013

Plant responses to insect damage have been investigated and these studies have resulted in new methods to enhance host resistance to insect pests, including the use of insecticidal proteins that can be expressed in selected crops by genetic engineering. Integration of the knowledge of how plants react to insect damage with the techniques of molecular biology should be able to increase even more the methods

available for the control of insect pests. Understanding how insects cope with plant defenses also has proved instrumental into designing new strategies for crop protection. The sugarcane transcriptome project (SUC-EST) has allowed the identification of several genes involved in the plant response to insect damage. There are numerous classes of naturally occurring phytochemicals that are thought to confer resistance to plants against herbivorous insects. These classes include lectins, waxes, phenolics, sugars, alpha-amylase inhibitors and proteinase inhibitors. Analysis of sugarcane-expressed genes involved in secondary metabolism suggests that most of the expressed compounds may be acting as defensive barriers to



Figure 1. Sugarcane plants exposed to *Diatraea saccharalis* attack and damage produced by the caterpillars after insect wounding and colonization by opportunistic fungi

insect attack. Our main goal is to understand the defense strategies adopted by sugarcane when challenged by its numerous pests (Figure 1). We are also interested in the counter-measures adopted by the insect pests in order to overcome plant defenses. To accomplish these, we are studying leaf proteins and gene responses involved in signal transduction and direct defense of sugarcane plants challenged by *Diatraea saccharalis*, *Spodoptera frugiperda* and *Telchin licus licus*. To understand the insect responses to plant defenses and to evaluate the potential use of genetic engineering to control these insects, we are investigating gene expression and protease activities extracted from intestines of insects exposed to proteinase inhibitors.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

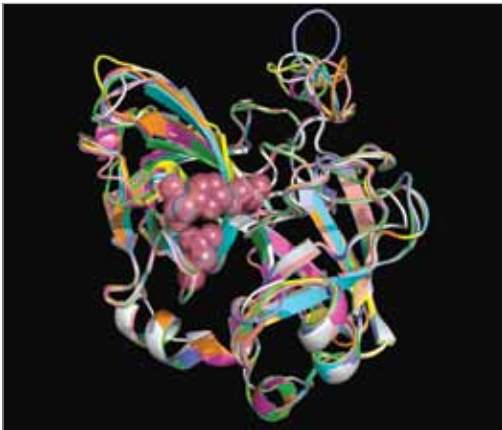


Figure 2. Structural alignment of eight chymotrypsins paralogues modeled by similarity. In detail, the position of the catalytic site

In sugarcane fields, colonization of the stalk by opportunistic fungi usually occurs after the caterpillar *Diatraea saccharalis* attacks sugarcane. Plants respond to insect attack by inducing and accumulating a large set of defense proteins. In a search for defense-related proteins in sugarcane, two homologues of a barley wound-inducible protein (barwin) were identified by in silico analysis, and were designated sugarwin1 and 2 (sugarcane wound-inducible proteins). Using quantitative real-time polymerase chain reaction for monitoring of transcripts, we showed that the induction of sugarwin transcripts is late induced, restricted to the site of damage and occurs in response to mechanical wounding, *D. saccharalis*, and methyl jasmonate treatment. Subcellular localization using GFP indicates that SUGARWINS are secreted proteins. Recombinant SUGARWIN1 protein incorporated into *D. saccharalis* diet, showed no effect on insect development. BARWIN proteins have been described as wound- and pathogen-inducible proteins that possess *in vitro* antipathogenic activities against fungi. We hypothesized that sugarwin gene induction by herbivory is part

of a concerted strategy against opportunistic pathogens that are commonly found in the site of caterpillars' attack.

We are also investigating the gene expression profile of proteinases involved in the adaptation when larvae of *Spodoptera frugiperda* are removed from the chronic ingestion of proteinase inhibitors. Larvae of the 6th instar moved to an inhibitor-free diet after the chronic ingestion of PIs showed a decreased in gene expression levels for all proteinases evaluated. Three proteinases showed a distinct pattern of expression when compared with controls: two of them returned to levels of expression below the initial level and one maintained its high level of expression induced by the inhibitors. Our data show that, although transient and dependent of the presence of the inhibitors, the "shotgun" approach changes the initial pattern of proteinases expressed in caterpillars challenged by the inhibitors even after its removal.

Insect proteinases that were induced by challenging the caterpillars with proteinase inhibitors had their tertiary structure modeled and refined in silico by sequence similarity modeling technique. The modeled structures were used to identify possible changes in the structural parameters that might impair the recognition of the catalytic site by inhibitors (Figure 2).

MAIN PUBLICATIONS

Brioschi D, Nadalini LD, Bengtson MH, Sogayar MC, Moura DS, Silva-Filho MC. 2007. General up regulation of *Spodoptera frugiperda* trypsins and chymotrypsins allows its adaptation to soybean proteinase inhibitor. *Insect Biochem Mol Biol.* **37**: 1283-1290.

Lam E, Shine JrJ, Silva J, Lawton M, Bonos S, Calvino M, Carrer H, Silva-Filho MC, Glynn N, Hessel Z, Ma J, Richard JrE, Souza GM, Ming R. 2009. Improving sugarcane for biofuel: engineering for an even better feedstock. *Global Change Biology Bioenergy.* **1**: 251-255.

Mingossi FB, Matos JL, Rizzato AP, Medeiros AH, Falco MC, Silva-Filho MC, Moura DS. 2010. SacRALF1, a peptide signal from the grass sugarcane (*Saccharum spp.*), is potentially involved in the regulation of tissue expansion. *Plant Molecular Biology.* DOI 10.1007/s11103-010-9613-8.

Marcio de Castro Silva Filho

Escola Superior de Agricultura Luiz de Queiroz
Universidade de São Paulo (USP)
Departamento de Genética
Av. Pádua Dias, 11
CEP 13400-970 – Piracicaba, SP – Brasil

+55-19-3429-4442
mdcsilva@esalq.usp.br