

University of São Paulo
"Luiz de Queiroz" College of Agriculture

Functional analysis of candidate effector proteins during *Sporisorium scitamineum*
x sugarcane interaction

Natália de Sousa Teixeira e Silva

Thesis presented to obtain the degree of Doctor in
Science. Area: Genetics and Plant Breeding

Piracicaba
2018

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To my amazing family

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RESUMO

Análise funcional de proteínas candidatas a efetores durante a interação *Sporisorium scitamineum* x cana

O carvão da cana-de-açúcar é uma doença cosmopolita de grande importância para o agronegócio, uma vez que pode afetar a produtividade da cultura. A doença é causada pelo basidiomiceto *Sporisorium scitamineum*, fungo biotrófico que coloniza exclusivamente a cana-de-açúcar. A interação cana-carvão vem sendo extensivamente estudada por este grupo de pesquisa nos últimos anos em seus vários aspectos, considerando as atividades de ataque e defesa do patógeno e da planta, respectivamente. Este trabalho teve como finalidade o estudo funcional de proteínas candidatas a efetores neste patossistema. Efetores são moléculas essenciais na manipulação do metabolismo e fisiologia do hospedeiro de forma a permitir sua colonização. A identificação de tais proteínas auxilia no reconhecimento de genes de resistência podendo gerar informações relevantes a programas de melhoramento genético na produção de variedades resistentes. A estratégia de seleção utilizada se baseia em características do secretoma predito e da expressão diferencial de genes do patógeno in planta. Os candidatos foram analisados quanto ao padrão de expressão gênica, à localização sub celular e sua influência sobre a defesa basal e imunidade em plantas. Os resultados demonstraram que a expressão dos genes que codificam para as proteínas efetoras de *S. scitamineum* e é influenciada pelo genótipo das plantas infectadas. Foram observadas variações no padrão de expressão entre o conjunto de efetores selecionados, bem como padrões diferenciais de localização sub celular e influência sobre a imunidade em plantas. Os resultados gerados por este trabalho servirão de subsídio para estudos futuros sobre os níveis de virulência dos diferentes isolados do patógeno bem como para auxiliar a tomada de decisão em programas de melhoramento genético de variedades resistentes ao carvão da cana.

Palavras-chave: Carvão da cana-de-açúcar; Biologia de efetores; Interação planta-patógeno; Perfil de expressão gênica; Localização sub celular; Genômica funcional

ABSTRACT

Functional analysis of candidate effector proteins during *Sporisorium scitamineum* x sugarcane interaction

Sugarcane smut is a worldwide distributed disease important to agribusiness, since it can affect sugarcane yield drastically. The disease is caused by the Basidiomycete *Sporisorium scitamineum*, a biotrophic fungus that colonizes mainly sugarcane. Sugarcane-smut interaction has been extensively studied by this research group for the past few years in their various aspects, considering both the pathogen attack and plant defenses. This work aimed to functionally address fungal candidate effector proteins associated with this pathosystem. Effectors are essential to modulate host metabolism to allow pathogen colonization. The identification of such proteins may assist in recognition of resistance genes relevant to genetic breeding programs. Based on the complete genome sequence of *S. scitamineum* and the dual transcriptomic data candidate genes were selected in silico. Selection strategies were based on the predicted secretome and differential expression levels of the genes in planta. Candidate effectors were analyzed regarding their expression pattern, subcellular location and influence over basal plant defenses and plant immunity. The results showed that the *S. scitamineum* candidate effector genes are expressed under the influence of the host genotype. It was observed various expression patterns in the set of selected genes and differential subcellular localization patterns. These results will enable future researches considering virulence level of different isolates and also help decision making in plant breeding programs.

Keywords: Sugarcane smut; Effector biology; Plant-pathogen interaction; Expression profile; Subcellular location; Functional genomics

1. GENERAL INTRODUCTION

Sugarcane is one of the most valuable crops worldwide for sugar and ethanol biofuel production (Arruda, 2011). The crop is cultivated in more than 100 tropical and subtropical countries, being Brazil its major producer (FAO 2016). Brazil accounts for 40% of the total sugarcane world's production, and São Paulo State is responsible for about 52% of the yield. Despite considered a robust and highly tolerant crop, sugarcane is affected by many pathogens, some of which drastically compromise productivity. Sugarcane smut is among the most severe diseases present in all sugarcane fields across the globe. The disease is responsible for significant losses due to reductions in sugar content and juice quality. Since sugarcane is a complex polyploid interspecific hybrid vegetatively propagated and breeding for resistance is demanding, the rapid emergence of new pathogen races is a significant concern among producers and breeders.

The genetic and molecular changes involved in the interplay between host and pathogen for sugarcane pathosystems is still poorly understood. The basidiomycete fungus *Sporisorium scitamineum* (Syd.) [Piepenbring et al. (2002) (Syn: *Ustilago scitaminea* H. and P. Sydow)] causes sugarcane smut. *S. scitamineum* is a biotrophic pathogen specialized in defeat sugarcane defenses to complete its life cycle. The fungus is capable of interfering with sugarcane metabolism in such a way that the plant architecture is modified, leading to the production of a particular whip-like structure to allow its sporulation.

The fungus has a host-dependent life cycle starting with teliospore germination in bud surfaces at the basis of leaf insertions. Germination generates a promycelium where meiosis takes place to produce haploid sporidial cells. Haploid sporidia fuse to produce dikaryotic infective hyphae, the recognition of compatible mating types is a pheromone-receptor based system. After penetration using an appressorium formed at the end of a hyphal tip, fungal hyphae systemically colonize plant tissues, initiate sporogenesis by karyogamy and hyphal fragmentation to produce teliospores. The life cycle ends with the development of the main symptom of the disease: the emergence of a whip-like structure from the shoot apical meristem (SAM). Billions of teliospores produced in a single whip are easily dispersed in the field by the wind, rain and small animals. The disease establishment in young plants results in tillering and narrow leaves. Together these symptoms generate decreased biomass and poor juice quality.

One of the metabolic and physiologic orchestrators in plant-pathogen interaction are effector proteins. Effectors are secreted molecules produced by host-associated organisms contributing to host resistance or susceptibility, depending on both genetic backgrounds (Hogenhout et al., 2009). They have evolved to subvert host defenses and promote the Effector-

Triggered Susceptibility (ETS) (De Jonge et al., 2011). In recent years many effectors were functionally characterized, acting in remarkably diverse ways. When delivered to host apoplast they can act as degrading enzymes (De Jonge et al., 2011), host protease inhibitors, can interfere in chitin perception (Takahara et al., 2016), detoxify host environment (Ökmen and Doehlemann, 2014) and block oxidative burst responses (Hemetsberger et al., 2015). Whereas when translocated inside the host cell, effectors can target a variety of organelles and biomolecules to interfere in several pathways. These proteins can interact with resistance R proteins to avoid immune responses (Houterman et al., 2008), alter hormone signaling cascades (Caillaud et al., 2013), divert biosynthetic pathway of secondary metabolites (Tanaka et al., 2014) and redirect nutrient acquisition from the host to support their own feeding requirements (Wahl et al., 2010). All these mechanisms culminate in improved pathogen fitness against the host immune system. Effector biology has become a powerful tool to comprehend pathosystems and direct plant breeding programs that deal with crop yield losses (Hogenhout et al., 2009; Petre et al., 2015; Sharpee and Dean, 2016).

A robust and sustainable way to control fungal diseases involves the development of resistant genotypes, which requires a profound understanding of the host-pathogen interaction and years of breeding strategies. Comparing to other pathosystems, studies regarding sugarcane smut resistance fell behind in fungal determinants of virulence. Although effector biology is a recent theme of investigation in fungal-plant interactions, effectors are known for some time as critical molecules to defeat plant defense mechanisms. This thesis will discuss the topic of *S. scitamineum* effector biology by functionally addressing selected candidate genes.

1.1. Objectives

- a. Select effector candidates for functional studies based on the predicted secretome and dual transcriptomic data available;
- b. Detect and quantify the pathogen along disease establishment by quantitative PCR;
- c. Validate selected candidate effector gene expression profile by RT-qPCR during disease progression in contrasting sugarcane varieties regarding smut resistance;
- d. Characterize effector candidates by accessing their subcellular location;
- e. Assess the effects of single effector expression over disease symptoms and general immune responses in planta toward Hypersensitive Response (HR) and virulence assays.

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3.6. Conclusions and final considerations

In this study we used the dual transcriptomic data during interaction and the sequenced genome to select the most promising candidate effector proteins to start addressing functional aspects in the sugarcane smut pathosystem. The candidate selection was mainly based on a flexible pipeline aiming to group candidates potentially involved in colonization, response to plant defenses and also in the development of the whip. This strategy can provide information about why the genotypes considered to be resistant to smut are fully colonized by the pathogen, but the fungus does not induce whip emergence (Carvalho et al., 2016), *S. scitamineum* effectors are likely to be regulated under the influence of host genotype and the inoculation method in experimental analysis influence pathogen behavior. These two variables need to be taken into account in design the best experimental approach to define effector functions.

Although the work presented here is exploratory in nature we are able to describe variation in gene expression, subcellular localization, and speculate about function in the various phases of the fungal establishment in the plant tissues leading to resistance or susceptibility. Various gaps in knowledge of the molecular mechanisms involved in this pathosystem still need to be investigate. For the best of our knowledge this is the first initiative to uncover effector candidates of previous unknown function for sugarcane smut disease and the establishment of a set of molecular tools to further investigate effectors of *S. scitamineum*.

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