Systematic functional characterization of small secreted proteins from the Fungal Wheat Pathogen *Mycosphaerella graminicola*

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Abstract

Mycosphaerella graminicola (Fuckel) J. Schröt., the causal agent of the wheat septoria tritici blotch (STB) disease, is one of the most economically destructive wheat pathogens in temperate regions with a high relative humidity during the growing season particularly in Western Europe. As a hemibiotrophic fungus, its life cycle consist of two distinct phase, biotrophy as well as necrotrophy phase where this fungus secretes a repertoire of proteins into the apoplast or mesophyll cell to promote disease or evade plant responses. Identification and characterization of such effector proteins provide a better understanding of the *M. graminicola* biology and molecular mechanisms which are involved in its infection. We mined the genome of *M. graminicola* IPO323 strain and identified more than 260 small secreted proteins (SSP) that share characteristic feature of well-known effector proteins (<300 aa residues and containing ≥4 cysteine residues). A set of 70 top candidates was selected based on the large EST databases and their expression profiles were analysed by quantitative PCR (qPCR) *in planta*. The expression data showed that although some of these genes are constitutively expressed *in planta,* the majority of them were differentially regulated suggesting that these genes may play certain roles during different stages of plant infection. These SSPs can be categorized in three main groups. The first group are genes that are highly up-regulated during the early stage of infection (<4dpi) coinciding with initiation of plant infection. The second group includes genes that are up-regulated during the switch of biotrophy to necrotrophy (~10 dpi) possibly triggering necrotrophy. The third group of genes are those that only up-regulated during necrotrophy stage starting after 12 dpi. These SSPs may have toxicity activity against plant cells. We are currently cloning some of these selected SSPs into expression vector to produce their proteins in *Pichia pastoris* GS115 strain using fermenter. The purified proteins, then, will be infiltrated into a set of wheat genotypes possessing *Stb* resistance genes to determine their accurate ETI or ETS activities.