

Role of *Phytophthora infestans* Protease Inhibitors and Their Target Tomato Proteases in Disease

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ABSTRACT

The oomycete *Phytophthora infestans* causes late blight, a reemerging and ravaging disease of potato and tomato. *P. infestans* has evolved gene families of Kazal-like extracellular serine protease inhibitors and cystatin-like cysteine protease inhibitors. Some of these protease inhibitors show inhibition activity towards tomato proteases. The study of the interaction between *P. infestans* protease inhibitors and tomato proteases will unravel the role of these molecules in the disease progress and provide possible ways to engineer plants for resistance against this pathogen.

SUMMARY

Protein-protein interactions play an important role in the battle between plants and pathogens. Among these proteins, proteases and protease inhibitors are molecules that are frequently involved in such molecular process. Proteases are enzymes that cleave peptide bonds linking amino acids in protein molecules. In plants, proteases perform their biological functions by processing proteins to generate bioactive mature forms or by degrading endogenous or exogenous proteins. P69 family from tomato, for example, contains six members, two of which are pathogenesis-related (PR) proteins involved in defense against pathogens' attack (Jorda et al., 2000). Protease inhibitors (PIs) are molecules that inhibit the function of proteases. Many naturally occurring protease inhibitors are proteins. Protease inhibitors interact with their target proteases to perform their biological functions, such as metabolism regulation; thereby blocking the proteases enzymatic activity. Based on their active sites, PIs are classified as serine, cysteine, or other protease inhibitors.

Data mining of genomic and cDNA sequences revealed that *P. infestans* evolved 18 extracellular protease inhibitor genes belonging to two major structural classes: (i) Kazal-like serine protease inhibitors (EPI1-14) and (ii) cystatin-like cysteine protease inhibitors (EPIC1-4) (Tian et al., 2004). We hypothesize that *P. infestans* secretes proteins that inhibit host proteases and facilitate infection by protecting secreted *P. infestans* proteins from proteolytic degradation and/or by perturbing host defense signaling cascades that include proteolytic steps.

Among the 14 Kazal-like serine protease inhibitor genes, *epi1*, *epi10* and *epi12* are up-regulated during infection of tomato by *P. infestans*, which suggests that they may play a role during the interaction. Previous biochemical studies showed that both recombinant EPI1 and EPI10 specifically inhibit subtilisin A among major serine proteases, and inhibit and interact with the pathogenesis-related (PR) P69B subtilisin-like serine protease of tomato. We extended our biochemical analyses to EPIC1 and EPIC2, secreted *P. infestans* proteins with similarity to cystatin-like protease inhibitor domains.

The *epiC1* and *epiC2* genes are strongly up-regulated during infection of tomato by *P. infestans*. EPIC1 and EPIC2B were unstable in tomato apoplastic fluids and were degraded by tomato P69B but EPI1 protected both proteins from degradation. Affinity purified P69B was sufficient to degrade EPIC1 and EPIC2B but not EPI1a, suggesting selectivity in degradation by P69B. Coimmunoprecipitation experiments revealed that EPIC2 interacts with a novel papain-like extracellular cysteine protease, termed Phytophthora Interacting Protein 1 (PIP1). The interaction was further confirmed by coimmunoprecipitation using in planta expressed PIP1 protein. Characterization of PIP1 revealed that it is a PR protein closely related to Rcr3, a tomato apoplastic cysteine protease that functions in fungal resistance. Altogether, our findings suggest that a cascade of inhibition of host proteases initiated by EPI1 occurs in the tomato apoplast during infection by *P. infestans*. We will expand our study on molecular characterization of these *P. infestans* protease inhibitors and the interacting tomato proteases to further investigate their roles in disease progression. The study of this proteases and protease inhibitor interaction is providing insights into key molecular processes regulating susceptibility to an economically important pathogen as well as providing hints for engineering plants to be more resistant to pathogens.

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