ABSTRACT

IDENTIFICATION AND CHARACTERIZATION OF TOMATO (SOLANUM LYCOPERSICUM) PROTEINS INVOLVED IN RESISTANCE TO INSECT HERBIVORES

By

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In response to wounding or herbivore attack, plants synthesize proteins that negatively affect the growth and development of arthropod herbivores. Many of these proteins are induced in plant tissue in response to herbivory and, following ingestion by the herbivore, target processes involved in insect digestive physiology. The objective of this thesis research is to identify and characterize plant proteins that impair the ability of insect herbivores to obtain nutrients from host tissue. To address this objective, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to identify proteins in tomato {Solarium lycopersicum L.) that are excreted in the insect feces (frass). This approach is based on the premise that plant anti-insect proteins are stable during passage of food through the insect digestive system, and therefore enriched in the frass. The results establish the utility of insect feces as a source of material for proteomic-based discovery of defensive proteins that target insect digestive processes. Comparative proteomic analysis of frass from three tomato-reared insect species, including lepidopteran (Manduca sexta and Trichoplusia ni) and coleopteran (Leptinotarsa decemlineata) herbivores, provided evidence that the lepidopteran insects digest bulk tomato leaf protein more efficiently than the coleopteran insect. This study also identified a subset of tomato leaf proteins that are highly stable in the digestive tract of all three

insect species. Including in this subset were proteins previously shown to have a role in defense against insect attack. These findings are consistent with the hypothesis that plant anti-insect proteins are inherently stable in the insect digestive track.

One of the most abundant tomato proteins excreted in the frass from all three insects was a jasmonate-inducible isoform of threonine deaminase (TD2) that converts threonine (Thr) to a-ketobutyrate and ammonium. TD2 and other plant TDs contain a Cterminal regulatory domain that, upon binding isoleucine (He), feedback inhibits the Nterminal catalytic domain. Following ingestion of tomato foliage by lepidopteran insects, the regulatory domain of TD2 is removed by a chymotrypsin-like protease of insect origin. This processed form of TD2 efficiently degrades Thr in the presence of He, thereby starving the insect of an essential nutrient. The increased growth rate of Spodoptera exigua larvae on transgenic tomato lines silenced for TD2 expression showed that this enzyme serves a role in anti-insect defense. Tomato contains a second TD isoform (TD1) that catalyzes the committed step in the biosynthesis of He. Based on the comparison of the expression pattern and biochemical properties of TD1 and TD2, it is concluded that the two TD isoforms have evolved specialized functions in plant primary metabolism and anti-insect defense, respectively.