

Metabolic Analysis of Soybean-Nematode Interactions: A Prelude to Metabolic Engineering for Nematode Resistance

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In this project we analyzed metabolic changes resulting from the expression of a plant parasite nematode *chorismate mutase* (CM) gene in a soybean hairy root model system. CM is a key regulatory enzyme in the shikimate pathway of plants and microorganisms, but does not exist in animals. The shikimate pathway produces the aromatic amino acids, so animals do not require this pathway since they can obtain their essential amino acids from their diet. The discovery of a CM in the phytoparasitic nematode *Meloidogyne javanica* was unusual in that this was the first CM found in an animal. This new CM was named *Mj*-CM-1 and is currently found only in some phytoparasitic nematodes and not in other animal or non-plant parasitic nematodes. The observation that *Mj*-CM-1 is secreted from the nematode into the soybean plant tissue at the onset of plant parasitism, suggests the nematodes directly alter this essential primary metabolic route in its host plant. It was assumed that *Mj*-CM-1 is used by the nematode to assist it in parasitizing plants; however, the exact metabolic consequence of *Mj*-CM-1 enzyme activity in the plant cell is unknown. In this project we sought to study the changes in shikimate-derived metabolites in soybean hairy roots expressing *Mj*-CM-1.

For these experiments we generated soybean hairy roots lines transformed with the *Mj*-CM-1 gene. These root lines have allowed us to measure the main products of the metabolic pools of the shikimate and phenylpropanoid pathways using HPLC-MS and GC-MS. This analysis allows for the identification of compounds that are different in *Mj*-CM-1 expressing hairy roots compared to the control roots. To date, we found that soybean hairy roots expressing *Mj*-CM-1 accumulate 3-fold high concentrations of phenylalanine (Phe) and tryptophan (Trp). In spite of markedly increased levels of Phe in roots expressing *Mj*-CM-1, the concentrations of isoflavones decreased up to 10 times in *Mj*-CM-1 expressing lines. We hypothesized that the increased Phe was causing the decrease in isoflavones. To test this idea we incubated hairy roots, which do not contain *Mj*-CM-1, with exogenous Phe (4mM) in culture medium. This experiment caused a three-fold increase of Trp and a two-fold decrease of isoflavone concentrations in the hairy roots, which is similar to those produced by *Mj*-CM-1 expression.

Our results suggest a potential model for how a nematode CM could manipulate plant metabolism during plant parasitism. Expression of an unregulated nematode CM in a plant's cytoplasm produces excess Phe in the cell. Apparently this extra Phe shifts this highly regulated pathway in favor of the cytosolic branch of the shikimate pathway, out competing the plastid pathway for chorismate and inhibiting synthesis of plastid chorismate-derived Phe by feedback inhibition. The Trp biosynthetic enzymes are known

to be stimulated by Phe (feed-back activation); therefore, this model also explains an increase in Trp. Our model suggests isoflavonoids are derived from plastid Phe, so as plastid Phe is reduced, isoflavonoids and other secondary metabolites are also lowered. Isoflavones are important for plant defense against nematodes and other plant pathogens, thus plant nematode CM seem to function as a suppressers of plant defense compounds. This suppression of plant defenses is probably essential for the nematodes ability to parasitize soybean and other crop plants.

If the primary role of the nematode CM is to lower toxic plant defense compounds or defense signaling compounds and if it is possible to prevent this from occurring via metabolic engineering of the host plant, then significant nematode resistance should be imparted. New sources of nematode resistant crop plants are desperately needed to control the increasing threat posed by phytoparasitic nematodes.

Leveraged funds:

The data from this project enabled the PI and Co-PI to obtain additional grant funds to continue the study (Soybean Disease Biotechnology Center, Soybean Cyst Nematode subproject. Metabolic analysis of SCN chorismate mutase, \$72,060).