Plant Pathogen Interactions: Implications for the Plant Metabolome

Tim Miller¹, Derek Stewart² and Vivian Blok¹

¹Plant Pathogen Interaction and ²Quality Health & Nutrition Programs
Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK

Introduction

Plant parasitic nematodes are responsible for global crop damage estimated at ~$100 billion annually. Of these, root-knot (Meloidogyne) and cyst (Globodera and Heterodera spp) nematodes in the family Heteroderaeae are commonly associated with crop losses. During the interaction between plant endoparasitic nematodes and their hosts, changes in both localized and systemic gene expression occur leading to either compatible or incompatible interactions. Little is known about the changes to cellular metabolism during host-nematode interactions, how these profiles are affected by the interaction and how these relate to changes in gene expression. Comparisons of metabolite profiles from uninfected and infected tomato and potato roots from compatible and incompatible interactions with Meloidogyne and Globodera nematodes are being made to determine if these profiles can be used to differentiate between these interactions. To this end we are undertaking work to establish and exploit profiling procedures for tomato and potato roots derived from soil and pot growth systems.

An extraction protocol has been established that allows the optimum extraction for freeze-dried tomato roots. From this study 37 compounds were identified from the chromatograms by their retention times and mass. Of these the most intensive peaks have been characterized; they belong to a family of compounds called glycolaloids. This study shows that these peaks have significant toxicity in mammals, particularly nematodes, and are thought to be involved in anti-parasitism. Data from these studies will complement corresponding transcriptome approaches and, in turn, should give valuable insights into the molecular basis of pathogenicity and resistance.

Development of Extraction Protocol

Comparisons of frozen vs freeze-dried root, ethanol vs methanol solvent extraction, percentage methanol/water in extraction solvent and the concentration (mg of root powder to volume of extraction buffer) of the final sample were examined to determine the conditions that gave the optimum number of metabolites that could be extracted in one sample.

The graph above shows the results from varying the percentage of methanol/water. 50% methanol/water was chosen because it was the best concentration for relative yield and number of metabolites. It also had the lowest overall standard deviation of 22%.

Infected VS Non-Infected Money maker Roots

To assess if this metabolic profiling approach could be used to determine differences between infected and uninfected roots, 14-day M. javanica infected tomato cv Money maker roots were compared to non-infected roots. Principle component analysis showed clear separation of infected vs non-infected roots. Although not yet statistically proven the chart below shows the first indication of metabolites with increased (above the X-axis) and decreased (below the X-axis) levels at 14-days after infection.

Methods

Two methods are being used for the preparation of root tomao (cv. Money maker) material. CysG™ pouches and root trainers. CysG™ pouches provide clean roots by facilitating nematode inoculation in an easily defined and controlled experimental system whereas root trainers are used to train soil systems which simulate a more natural plant-pathogen interaction and encourage optimum root formation. Both being assessed to determine if metabolic profiles differ between soil and CysG™ pouch-trained plants.

The roots are excised and frozen in liquid nitrogen to halt any metabolic activity. The frozen roots are then freeze-dried, killed and extracted with 50% methanol/water containing 0.5% acetic acid, with Moran and Nenaros as internal standards. The extract is then concentrated and reconstituted in 0.05ml of buffer. LC-MS profiling is performed on a Thermo Finnigan LCQDECAQ with an 150 x 2 mm Synergy 4 μm Hydro RP column. Metabolites are detected by both PDA and ESI-MS and post acquisition processing utilizes Excalibur™ software.

Compound Identification

Compounds are initially identified by retention times and mass. Further identification is carried out by combining PDA and MS data with literature research. Future identification will also be carried out by running standards of common plant metabolites under the same chromatographic conditions.

Clearly the tomato-related glycolaloids are, under these conditions, the predominant metabolites. The broad spectrum anti-pathogen activity of glycolaloids is well reported and may, in part, be at the root of the nematode susceptible/resistant response.

Future Work

Comparisons will be made for root from tomato cultivars susceptible (Money maker) and resistant (Rosella) to the root knot nematodes. In addition, this will be extended to potato genotypes which differ in susceptibility to the potato cyst nematodes Globodera rostochiensis and G. pallida. The changes in all known, and unknown, metabolites (including these identified by GC-TOF-MS) should allow us to elucidate the basis of the susceptible and non-susceptible plant response and, ultimately, lead to the identification of specific targets for marker assisted breeding.

Funding from the BBSRC for an Industrial CASE Studentship with Advanced Technologies Cambridge, University of Dundee (TM) and the Scottish Environment Agriculture and Rural Affairs Department (VB & DS) is acknowledged.