Plant-Pathogen Interactions; Implications for the Plant Metabolome

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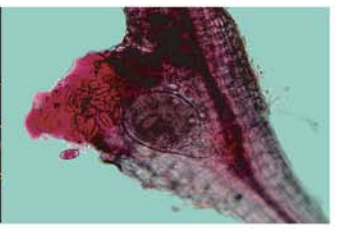
Introduction

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 Heteroderidae 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 know about the changes to cellular metabolism during host-pathogen 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* *Globodera* nematodes are being made to determine if these profiles can be used to differentiate between these interactions. To this end we are endeavoring to establish and exploit profiling procedures for tomato and potato roots derived from soil and pouch growth systems.

An extraction protocol has been established that allows the optimum extraction for freeze-dried tomato roots. From this fifty individual 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 group of compounds called glycoaklaloids. Theses are known to exhibit significant toxicity in mammals, particularly humans, and are thought to be involved in anti-parasitism. Data from these studies will complement corresponding transcriptome approaches and, *in toto*, should give valuable insights into the molecular basis of pathogenicity and resistance.

Tomato roots galled by the root-knot nematode, *Meloidogyne* sp.

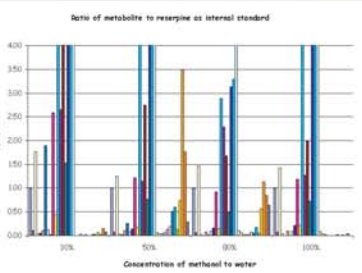


Tomato root gall with *M. incognita* female and egg sac (stained red)

Development of Extraction Protocol

Comparisons of frozen vs freeze dried root; ethanol vs methanol extraction solvent; 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 metabolite that could be extracted in one sample

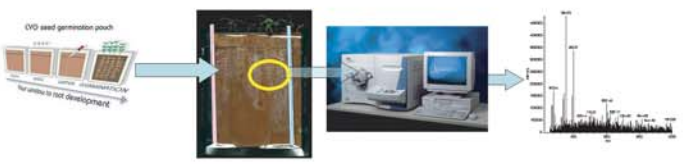
The graph opposite shows the results from varying the percentage of methanol/water. 50% methanol/Water was chosen because it was the best concentration for relative yield and number of metabolites. It also had the lowest overall standard deviation of 22%.



Methods

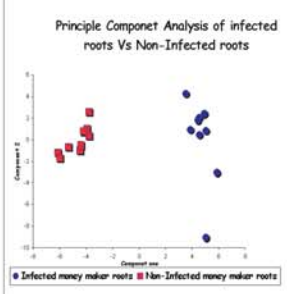
Two methods are being used for the preparation of root tomato (cv. Money maker) material; Cyg™ pouches and root trainers. Cyg™ pouches provide clean roots thereby facilitating nematode inoculation in an easily defined and controlled experimental system whereas root trainers are potted soil systems which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

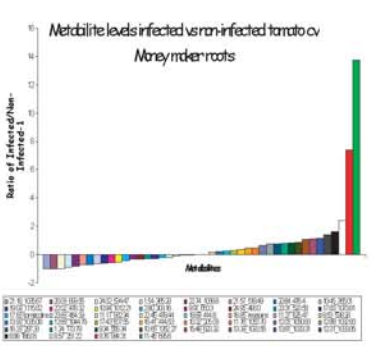
The roots are excised and frozen in liquid nitrogen to halt any metabolic activity. The frozen roots are then freeze-dried, milled and extracted with 50% methanol/water containing 0.5% acetic acid, with morin and reserpine as internal standards. The extract is then concentrated and resuspended in 0.5ml of buffer. LC-MS profiling is performed on a Thermo Finnigan LCQ-DECA 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 utilises Excalibur™ software.



Infected VS Non-Infected Money maker Roots

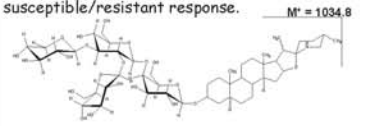
To assess if this metabolic profiling approach could be used to determine differences between infected and uninfected roots, 14-Day *M. javanica* infected tomato cv Money maker roots were compared to non-infected roots. Principle component analysis showed clear separation of infected Vs non-infected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



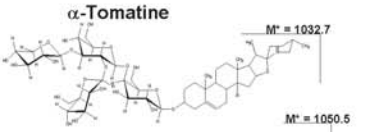


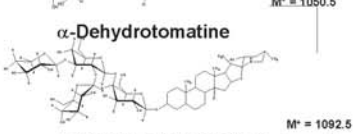
Compound Identification

Compounds are initially identified by retention times and mass. Further identification is carried out by combining PDA and MS^2 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 tomatine-related glycoalkaloids are, under these conditions, the predominant metabolites. The broad spectrum anti-pathogen activity of glycoalkaloids is well reported and may, in part, be at the "root" of the nematode susceptible/resistant response.

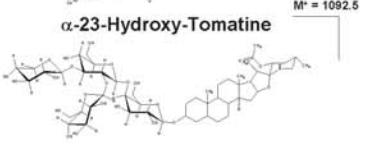


α-Tomatine

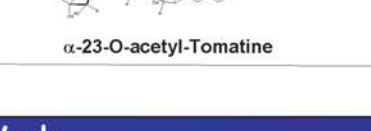




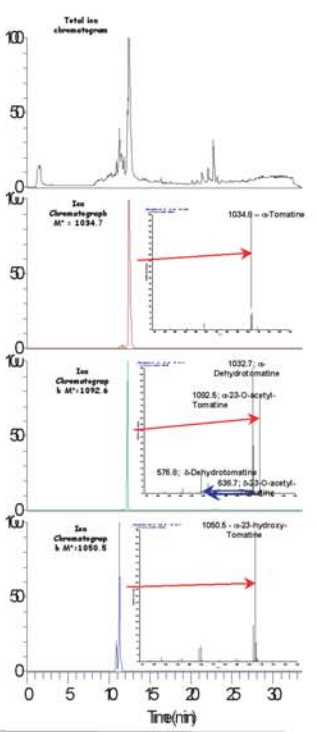
α-Dehydrotomatine



α-23-Hydroxy-Tomatine



α-23-O-acetyl-Tomatine



Future Work

Comparisons will be made for roots from tomato cultivars susceptible (Money maker) and resistant (Rossol) 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 those 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.

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