Metabolic profiling of the response in susceptible and resistant Solanaceae to plant parasitic nematodes

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Rationale

Root knot *(Meloidogyne* spp.) and cyst (e.g. *Globodera* and *Heterodera* spp.) plant parasitic nematodes are responsible for global crop damage estimated at ~$100 billion annually.

Interactions with their host leads to either susceptible or resistant responses and involves localized changes in the root cell histology and in gene expression. These interactions have been the subject of some genetic and molecular analyses but almost nothing is known about metabolic changes in the host.

We are applying metabolite profiling, LC-MS and GC-MS, to monitor changes in tomato and potato hosts (leaves and roots) following nematode infection to determine if these profiles (indicative of both localized and systemic effects) can be used to differentiate uninfected, susceptible and resistant responses.

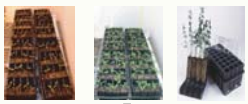
Meloidogyne spp. second-stage juvenile

Galled root of tomato infected with *Meloidogyne* spp. compared with non-infected root system.

Aim

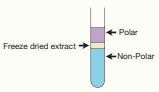
Monitor changes in metabolites that characterise compatible and incompatible plant responses to identify metabolic markers.

Method



Plant Material:

- 2 week old seedling are planted in root trainers
- 2 weeks later roots are inoculated with ~500 juvenile nematodes
- 14 days after infection these are harvested, frozen in liquid nitrogen, freeze dried then milled



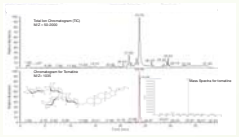
Extraction Method:

1. 100 mg freeze-dried material
2. 3 ml methanol containing internal standards
3. 0.75 ml dist. H_2O
4. 6 ml chloroform
5. 1.5 ml dist. H_2O

- polar and non-polar fractions separate out

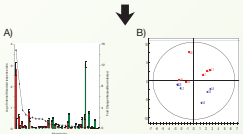
Instrumentation:

- Samples are run on HPLC-PDA-MS and GC-MS to allow better coverage of the whole metabolome.



Data analysis:

- Compounds are initially identified by retention times and mass. These are then processed using Xcalibur onboard software.



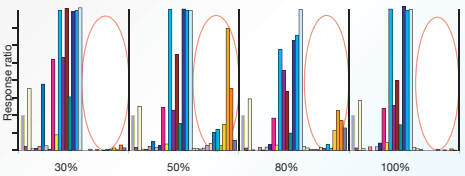
Statistics:

- Processed data is then statistically analysed to identify metabolites of interest.
- Analysis of variance allows the most significantly changed metabolites to be identified (A).
- Principal component analysis (PCA) reduces the data set for easier analysis and can show interactions between metabolites (B).

Results

Method Development

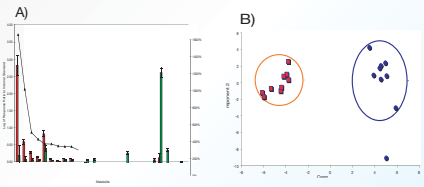
Percentage methanol to water for extraction



- Yield and number of metabolites relative to the internal standard was optimal in the extraction solution containing 50% methanol/water .

Galls

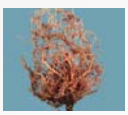
Infected galls Vs uninfected roots

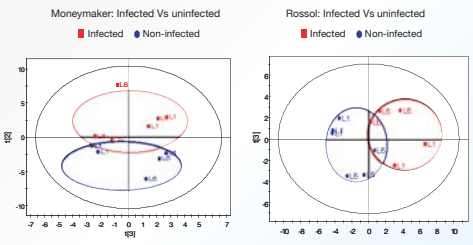


- 36 metabolites found to significantly change after infection (A).
- Principal component analysis shows clear separation between infected galls Vs uninfected roots (B).

Whole Root Systems

Susceptible (Moneymaker) and Resistant (Rossol) plants

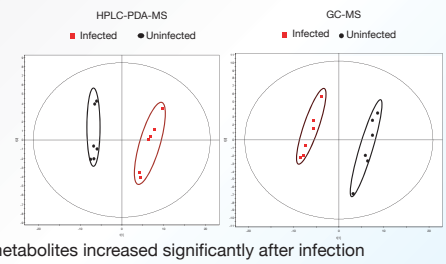




- Principal component analysis shows clear separation between resistant and susceptible cultivars before and after infection

Leaves

Motelle (resistant) infected Vs uninfected



- HPLC-PDA-MS: 34 metabolites increased significantly after infection
- GC-MS: 39 metabolites increased significantly after infection
- Principal component analysis shows clear separation before and after infection for both HPLC-PDA-MS and GC-MS

Conclusions

- A method has been developed for metabolic profiling nematode-plant interactions.
- Results from galls, whole root systems and leaves show that this method can be used to detect changes in localised and systemic responses due to nematode invasion.

Future Work

- Examine effects of plant to plant variation.
- Carry out time point experiment for *Meloidogyne* spp.
- Metabolite profile potato with various levels of resistance to *Globodera pallida*.
- Carry out correlation analysis to combine HPLC-PDA-MS and GC-MS data to model molecular pathways possibly involved in resistance.
- Identify molecular structures of metabolites of interest.

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