

Metabolomic approaches to understand host reprogramming by plant pathogens



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Research
funded by the

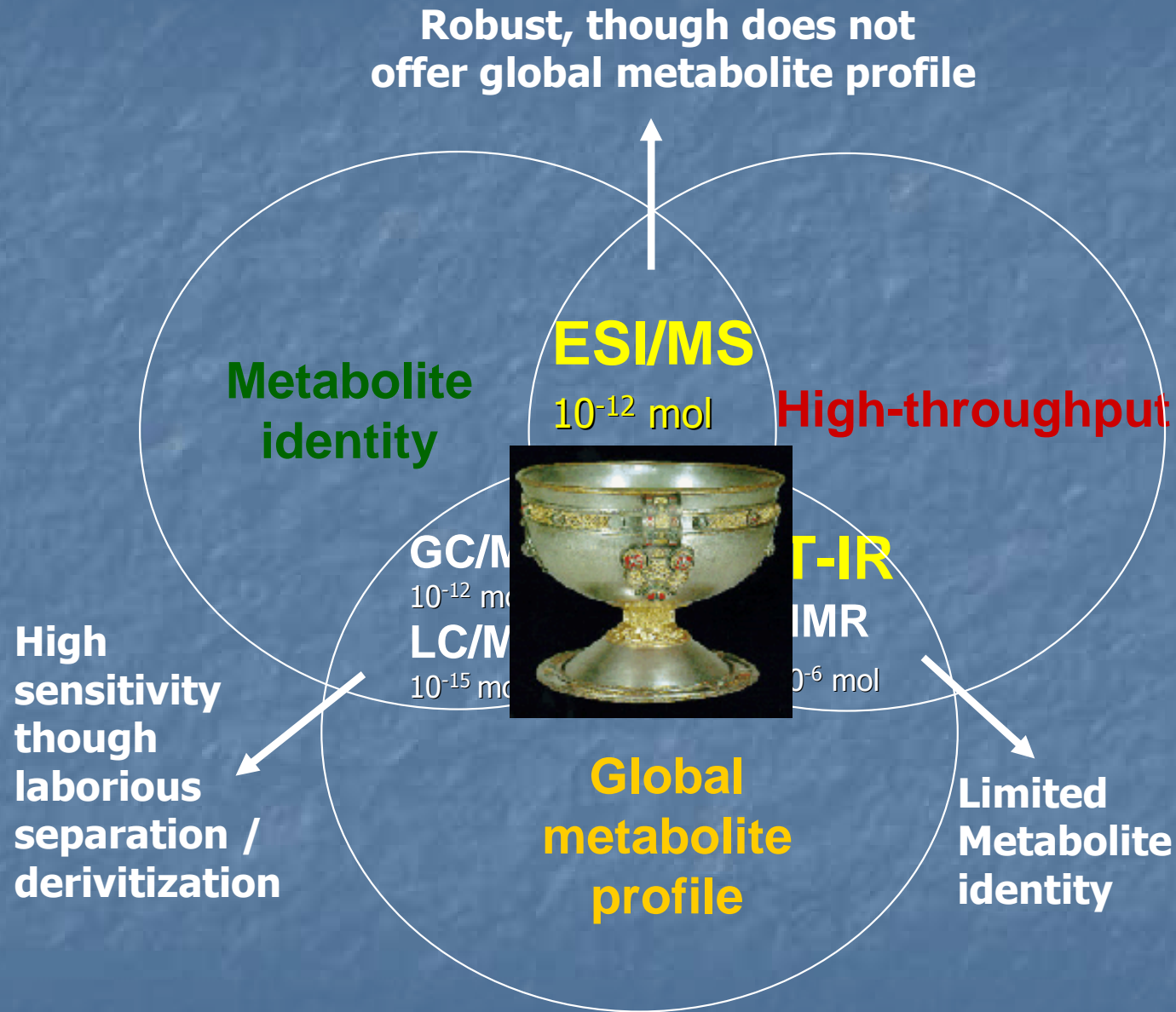


Plant reprogramming by pathogens

- Mobilisation of nutrients to pathogen
- Deployment of ineffective defences
- Plant breeding – resistance lines
- Fungicide/Pesticide targets

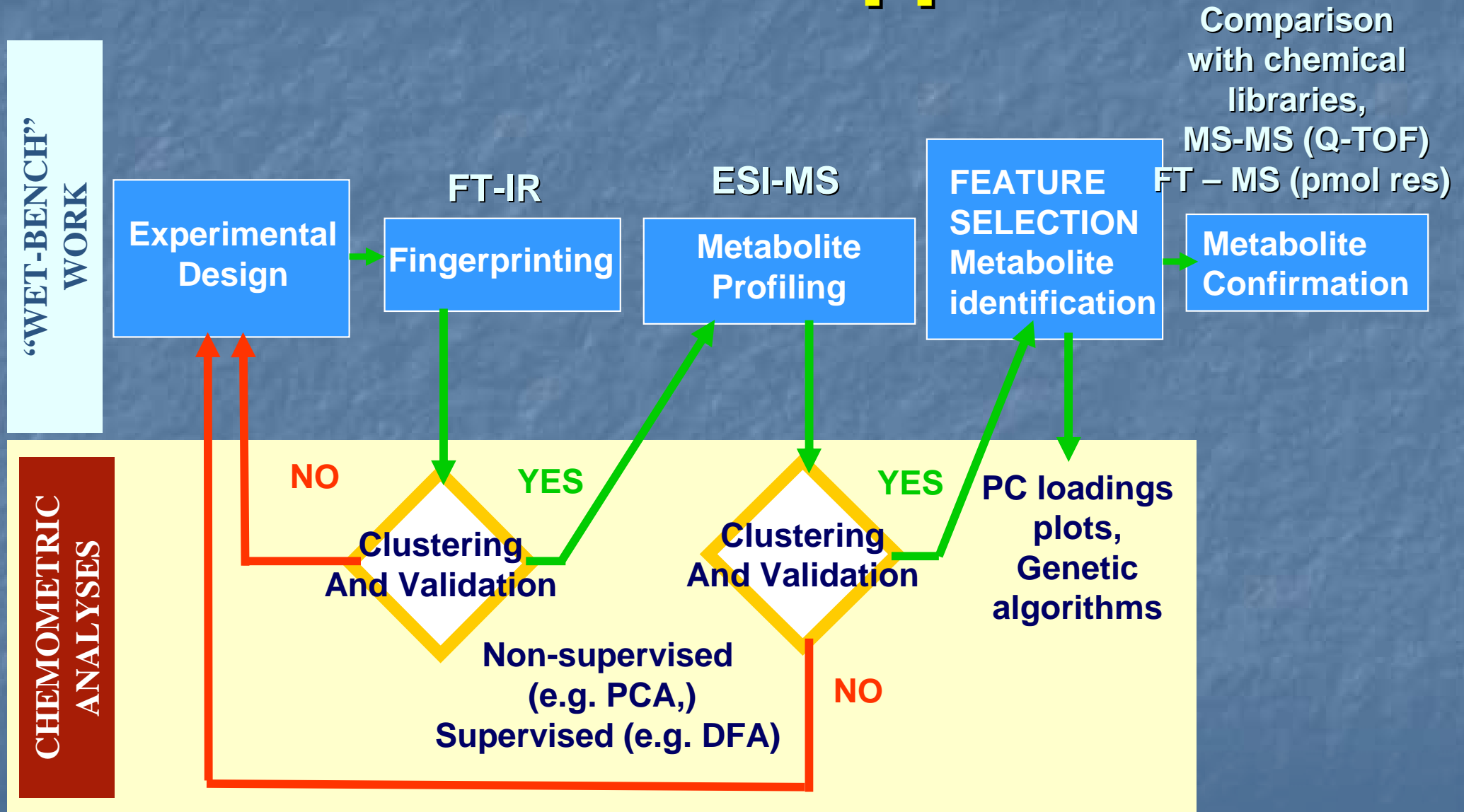
Powdery mildew (*Erysiphe cichoracearum*) on *Arabidopsis thaliana*

Metabolite profiling technologies



Relative sensitivities of various metabolomic tools (Sumner *et al.*, 2003)

Metabolomics: Our standardised approach



Experimental SOP

- **Biological Replication**

10 Replicates for each genotype / treatment at each time points

- **Machine Replication**

Many multiples for FT-IR fingerprinting

Typically, two for ESI-MS

- **Experimental Replication**

Typically, two experimental replicates.

- **Validation**

Comparisons of training and test sets in projection analysis. Targeted metabolite analysis

- **Extractions**

FT-IR = water,

ESI-MS Chloroform methanol and water based solvent mixture 6 (at 1:2.5:1 ratios)

Samples were reconstituted in propan-2-ol (70%) and water (30%) for analysis

- **Metabolite quenching** Quenching metabolism will be performed by

- (a) -58°C methanol, (b) 60% buffered methanol at -40°C, (c) Liquid N₂.

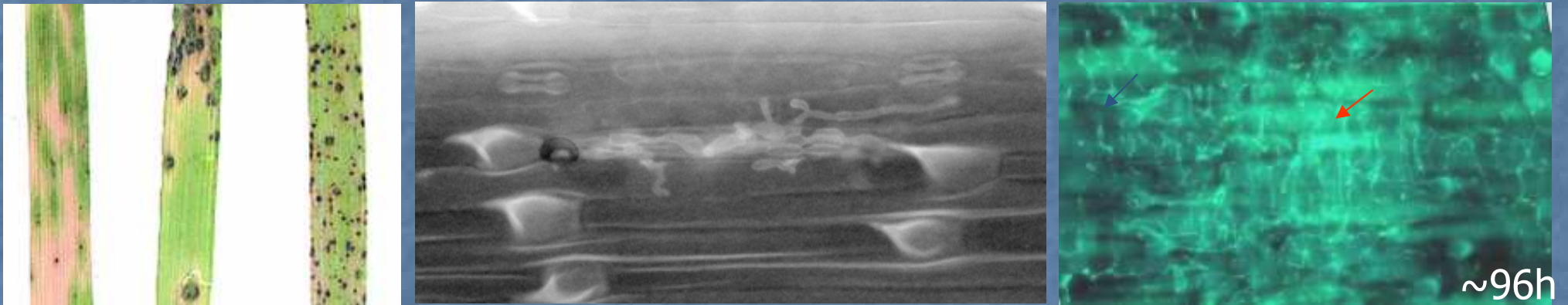
- **Use of internal standards (for MS)**

Pathosystems under investigation

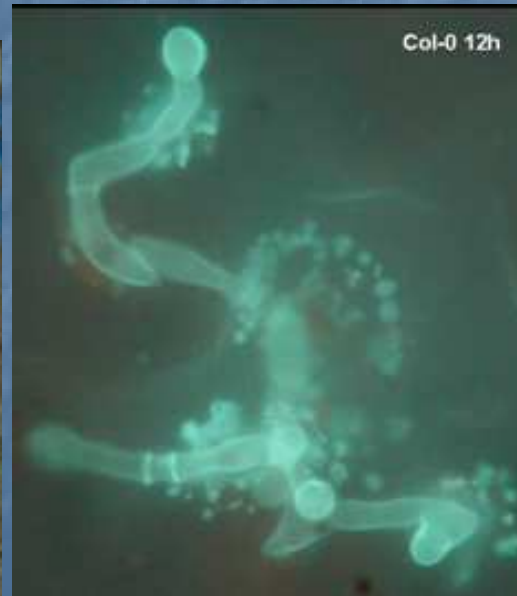


Fungal interactions

Rice Blast: (*Maganpothe grisea*). Allwood *et al.*, Plant Journal (*in press*)



Grey Mould : *Botrytis cinera*



Arabidopsis– Bacterial Pathogen : Dual Metabolomics



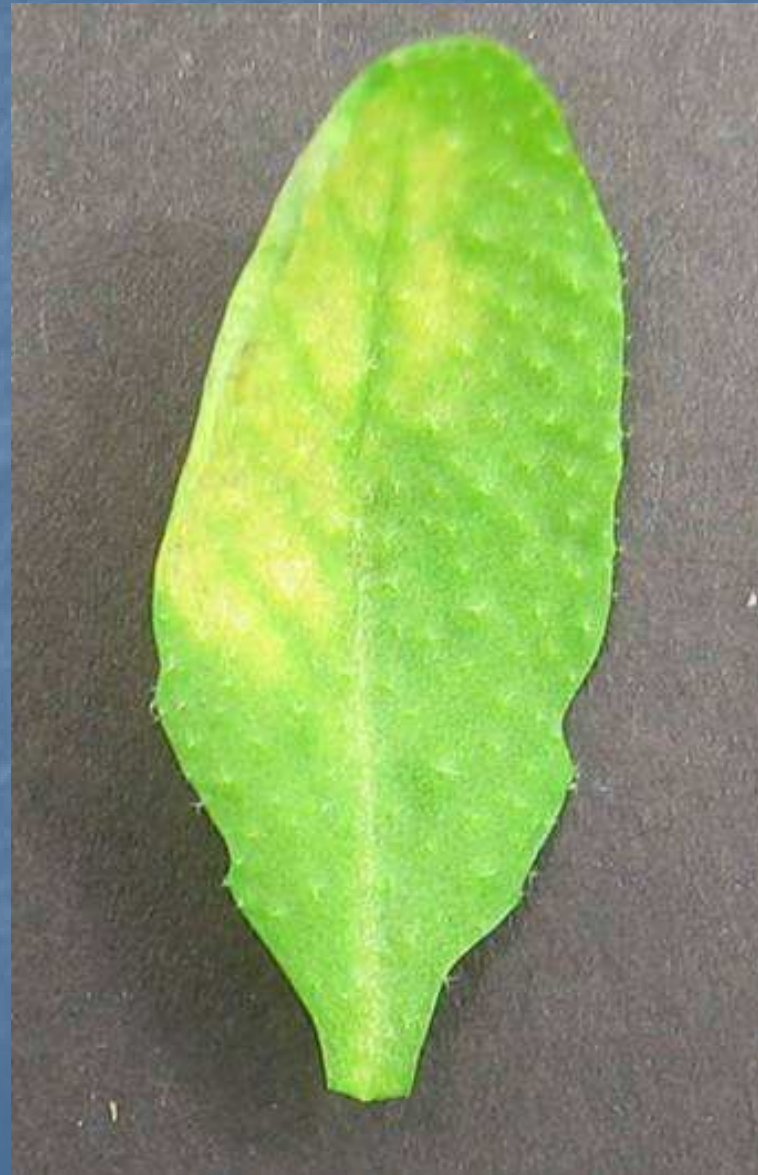
The aim is to develop a pathosystem where host and pathogen responses can be modelled.

We employed *Arabidopsis thaliana* suspension cultures inoculated with *Pseudomonas syringae*

Plant reprogramming by bacterial pathogens

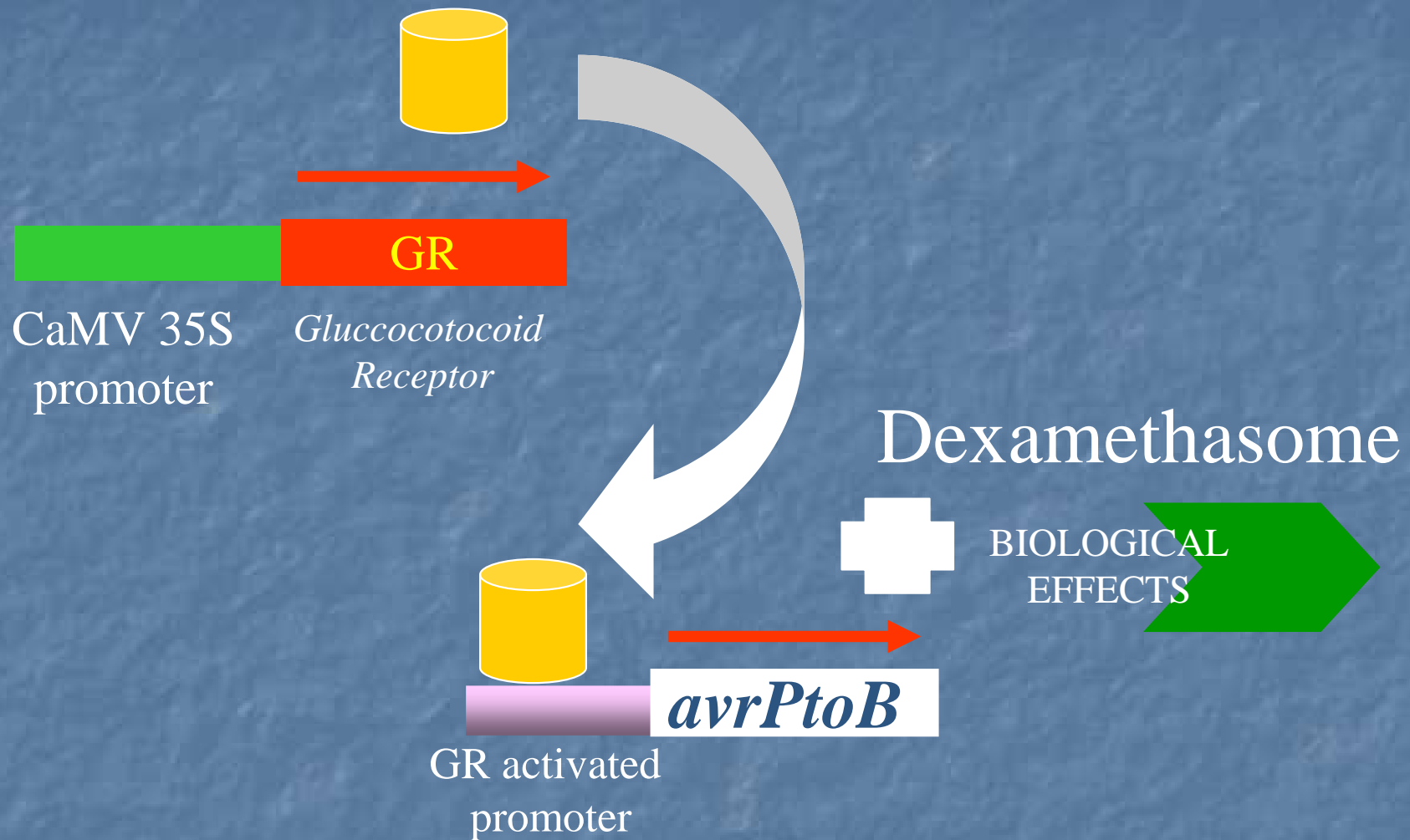


Arabidopsis thaliana



tomato

Glucocorticoid – inducible switch



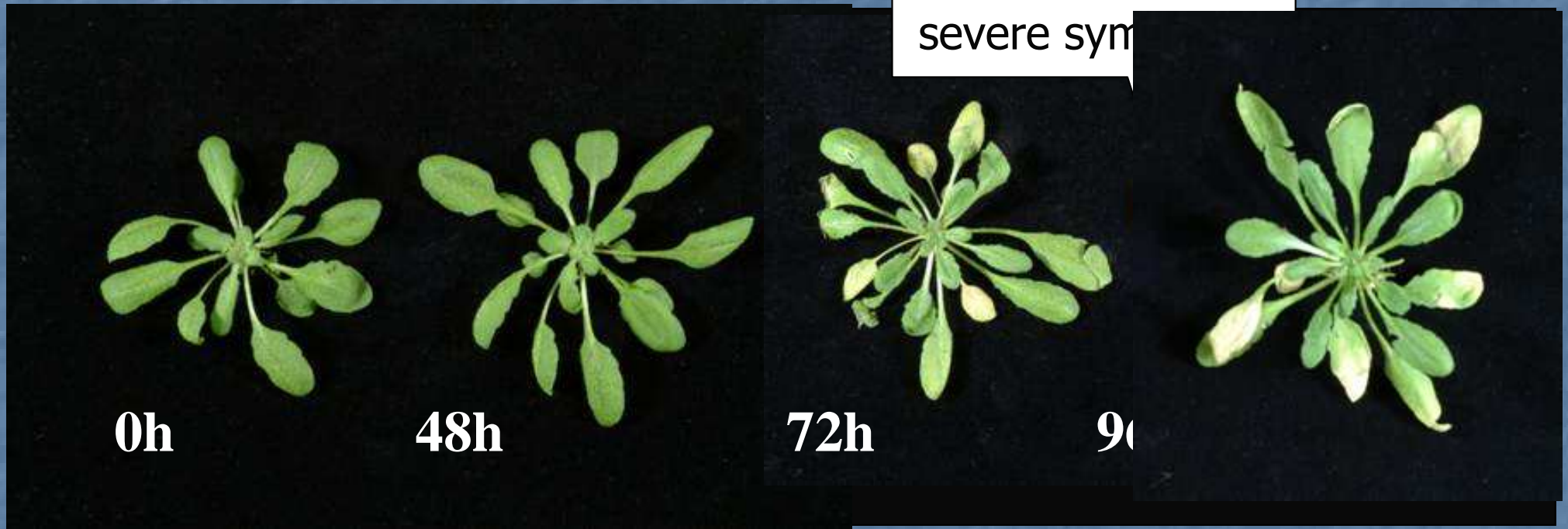
AvrPtoB is a cysteine protease – transgenic line provided by John Mansfield (Imperial College, UK) and Greg Martin (Purdue, USA)

Dexamethasone induced *avrPtoB* effects

234(*avrPtoB*)
236 (empty vector)

30 μ M dexamethasone

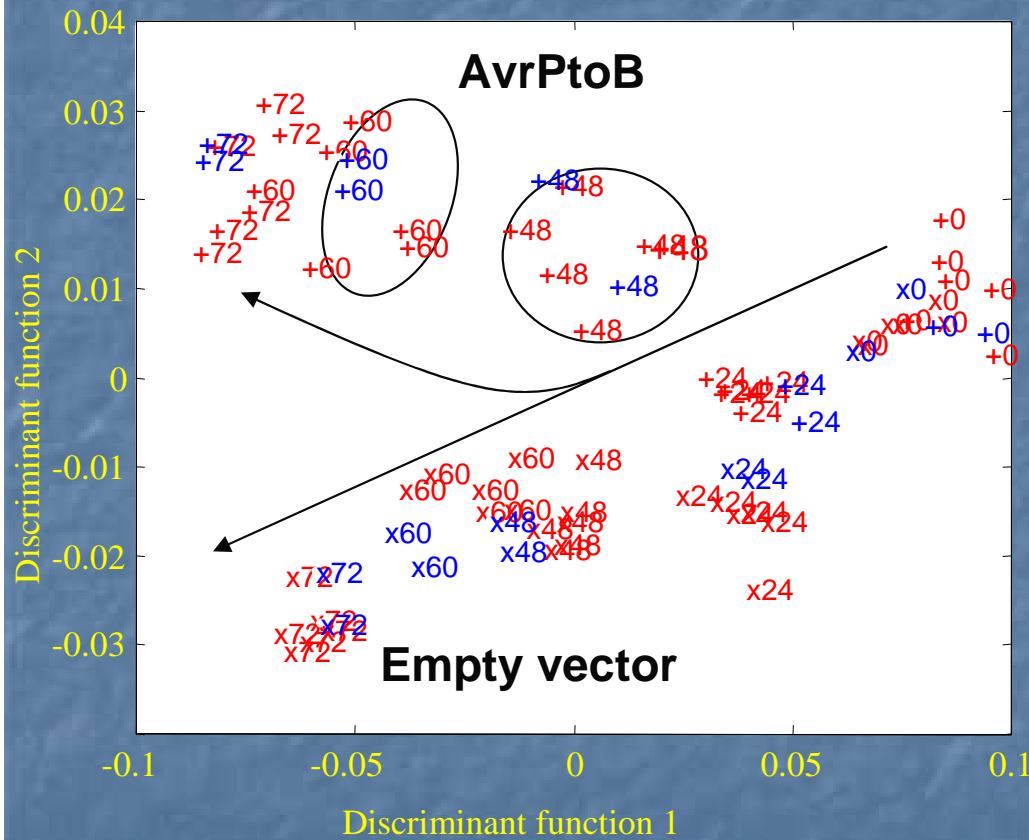
Older leaves
exhibited most
severe symptoms



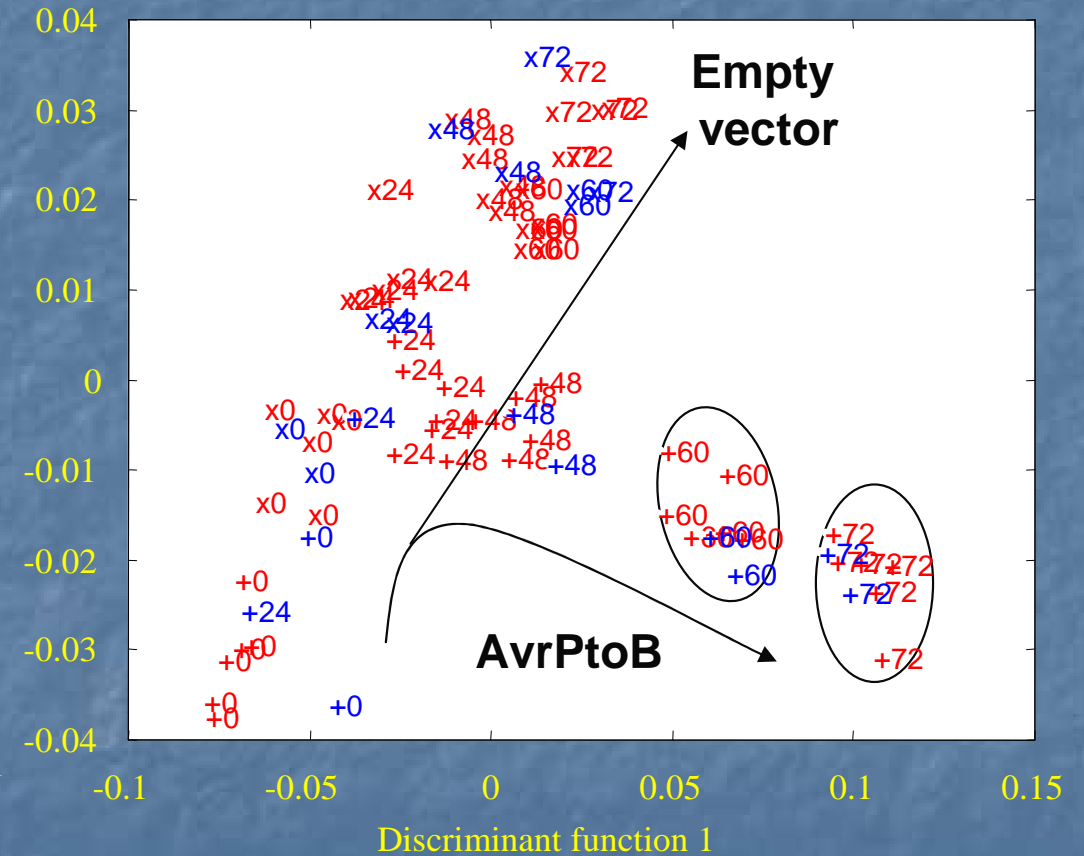
Discriminant function analysis (DFA)

12 PC= 99.99% DFA - DF1 vs. DF2 red = train, blue = test

Polar metabolites



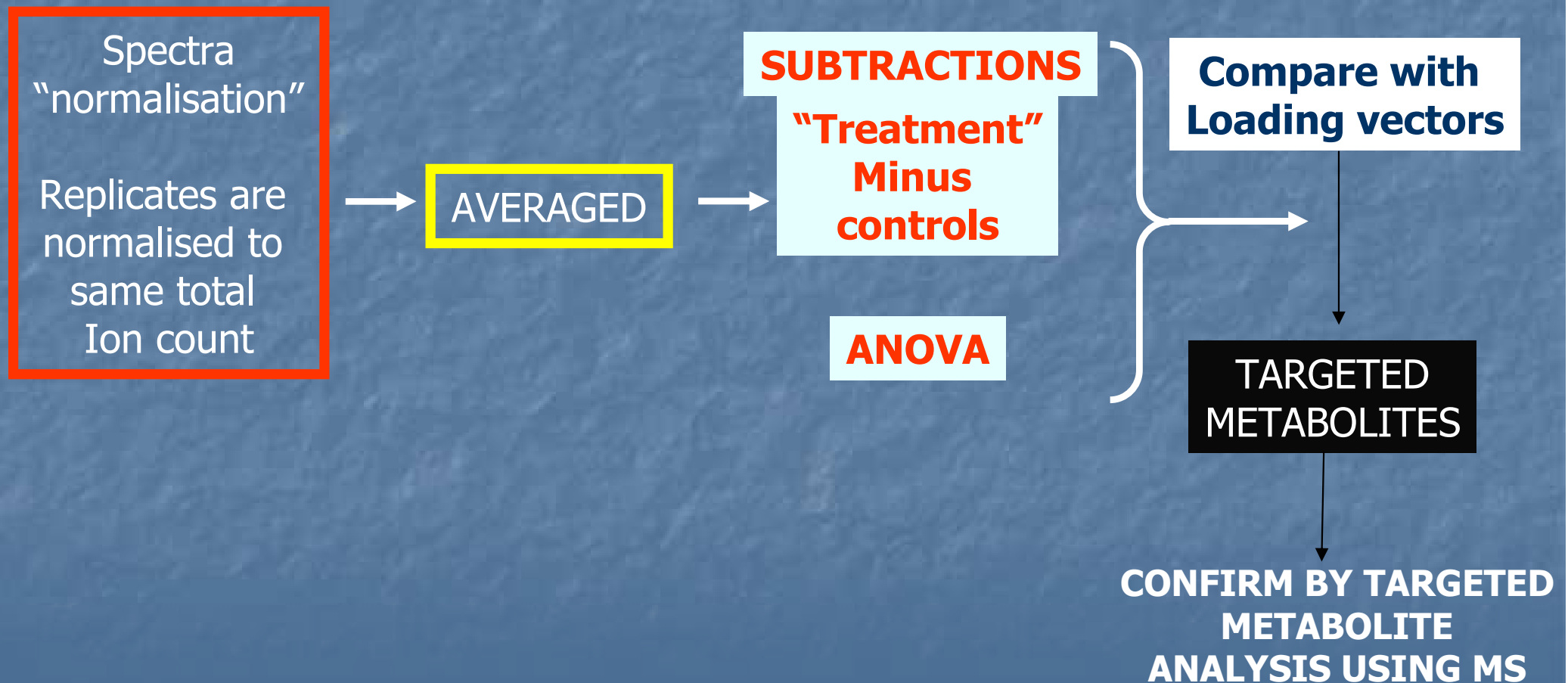
Non-polar metabolites

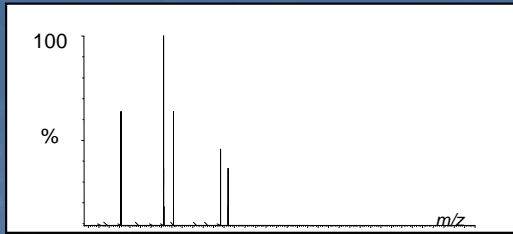


Feature Selection

Approach One : Loading Vectors

Loading (Eigen) vectors making up the PC on which DFA discriminatory models are based.





(1) AvrPtoB effect at (e.g.) 72h

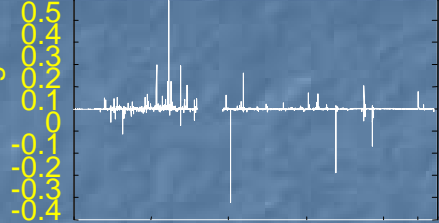
Two component chemometric models for 72 h

Normalised MS Spectra

Subtract 72h Spectra

PC-DF loading

LOADING VECTORS



REJECT

Do the subtracted m/z appear in the discriminatory loading vectors?

(2) Remove inter-genotype (non-avrPtoB) variation

Normalised MS Spectra

Subtract 0 from 72h treatment

Do the subtracted m/z appear as the most discriminatory loading vectors?

REJECT

Chemometric models For all time point

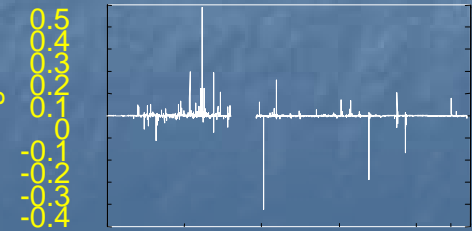
LOADING VECTORS

(3) Identify m/z which explain most avrPtoB-associated variation over time

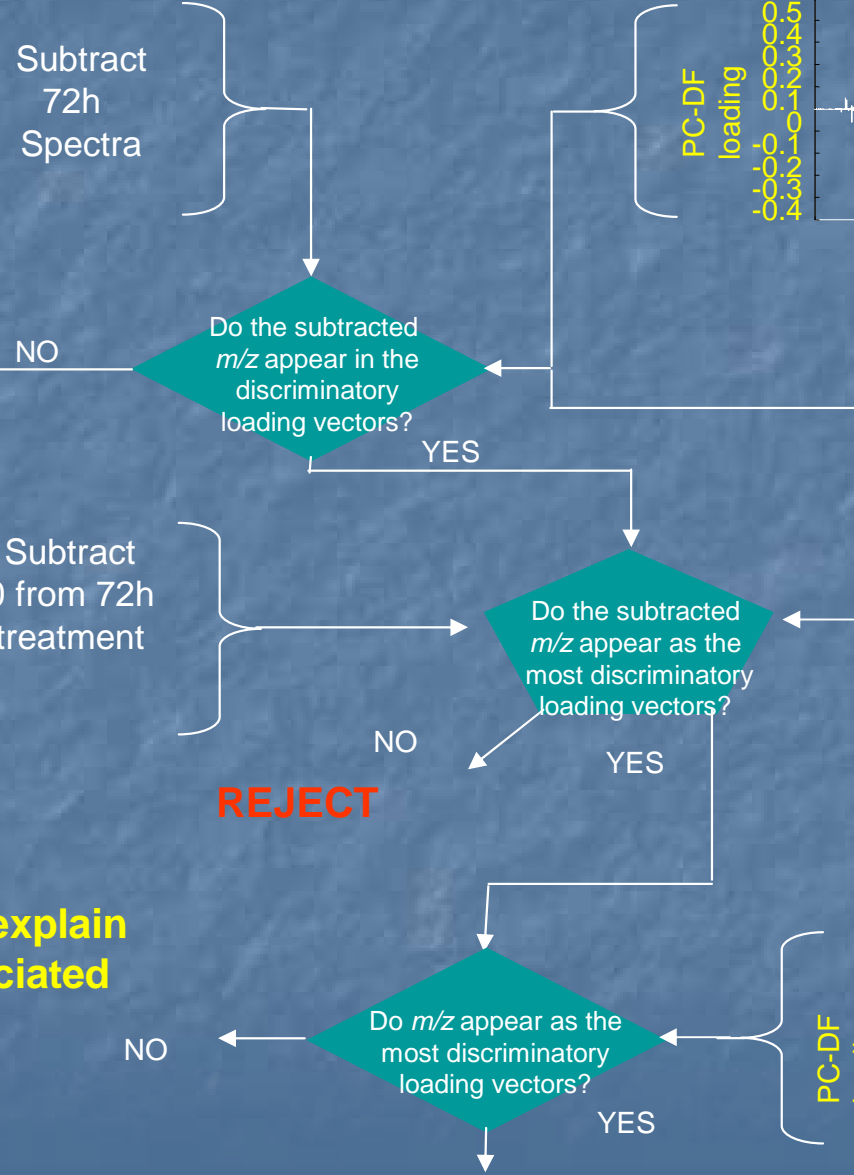
NO

Do m/z appear as the most discriminatory loading vectors?

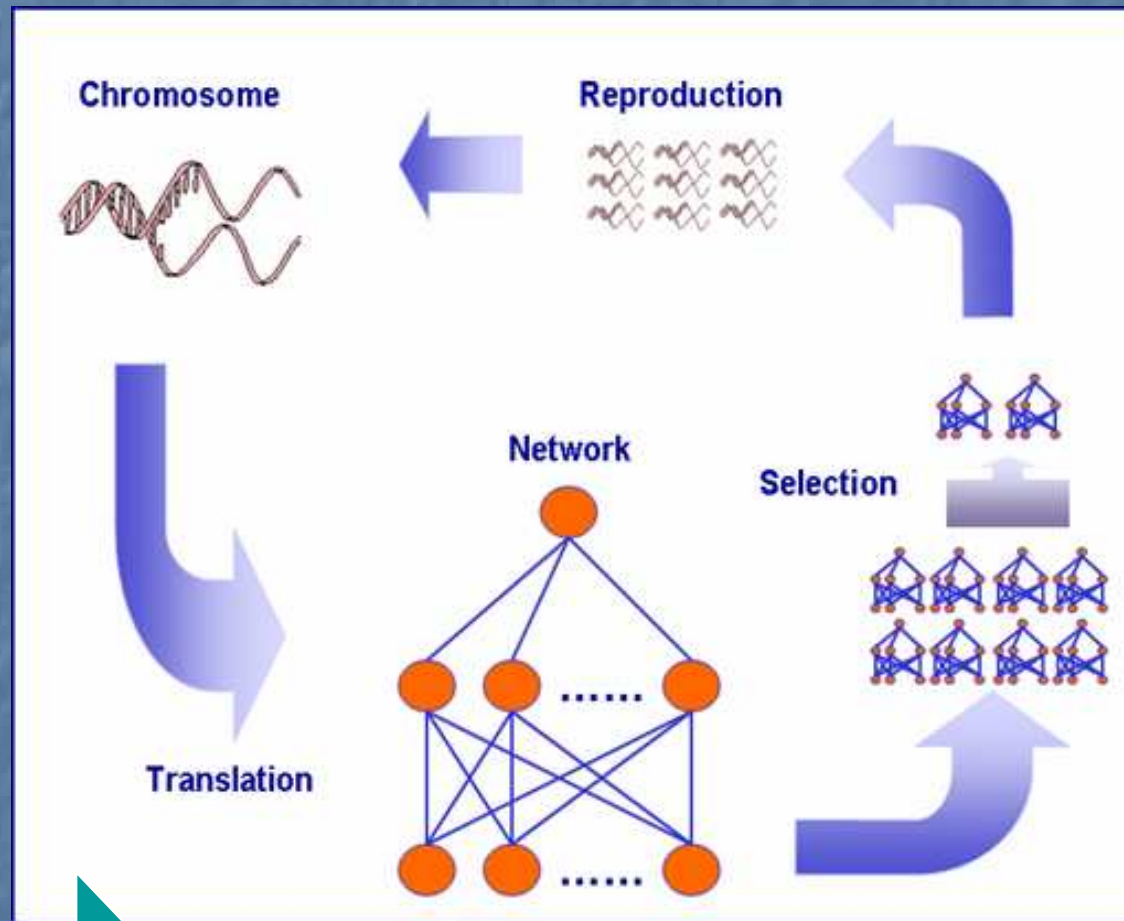
PC-DF loading



MS-MS/FT-MS



Approach Two : Genetic Algorithms – Mimicking evolution



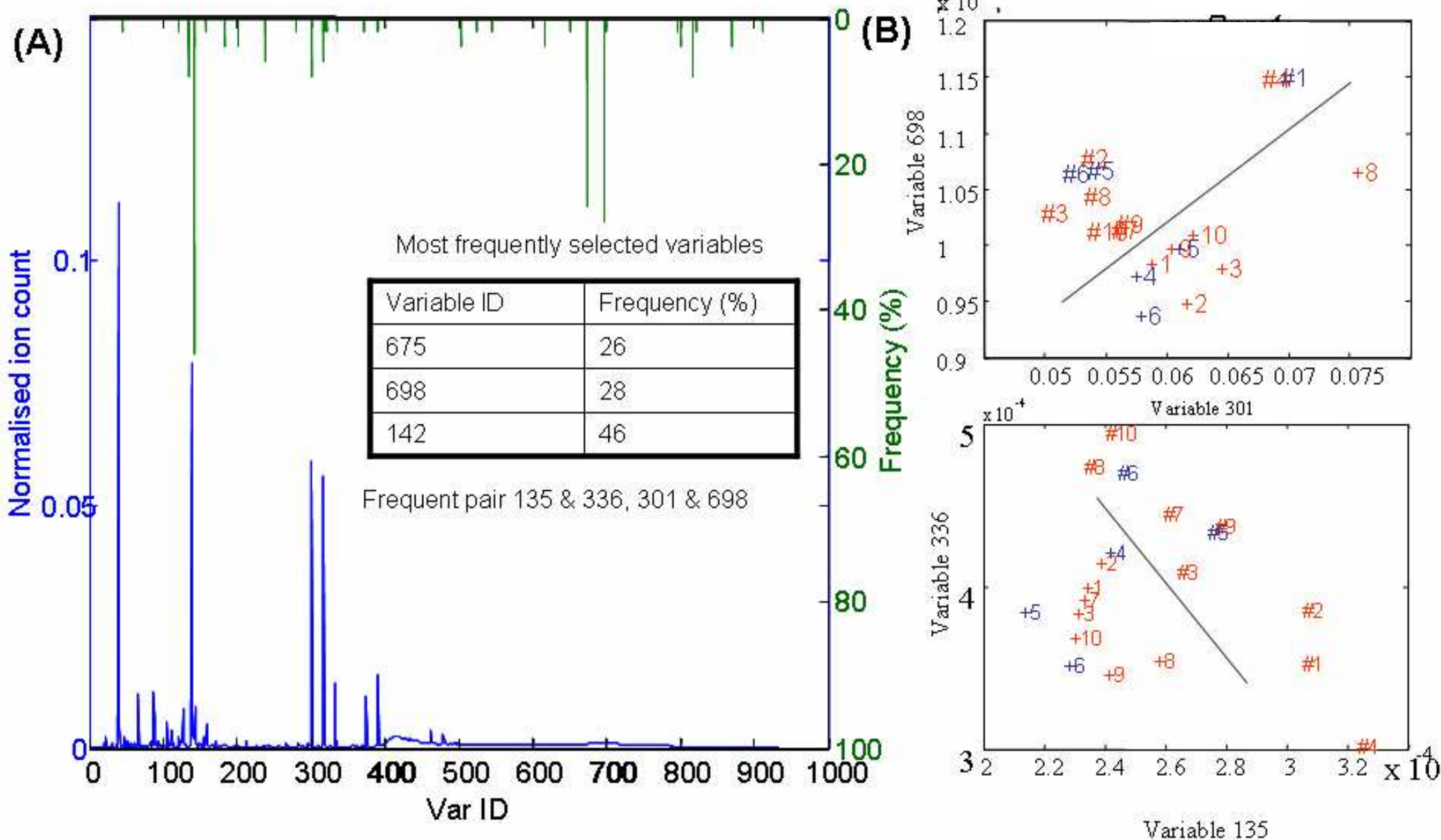
**Create New
Population
By Mating &
Mutation**

**Select
Mating Pairs**

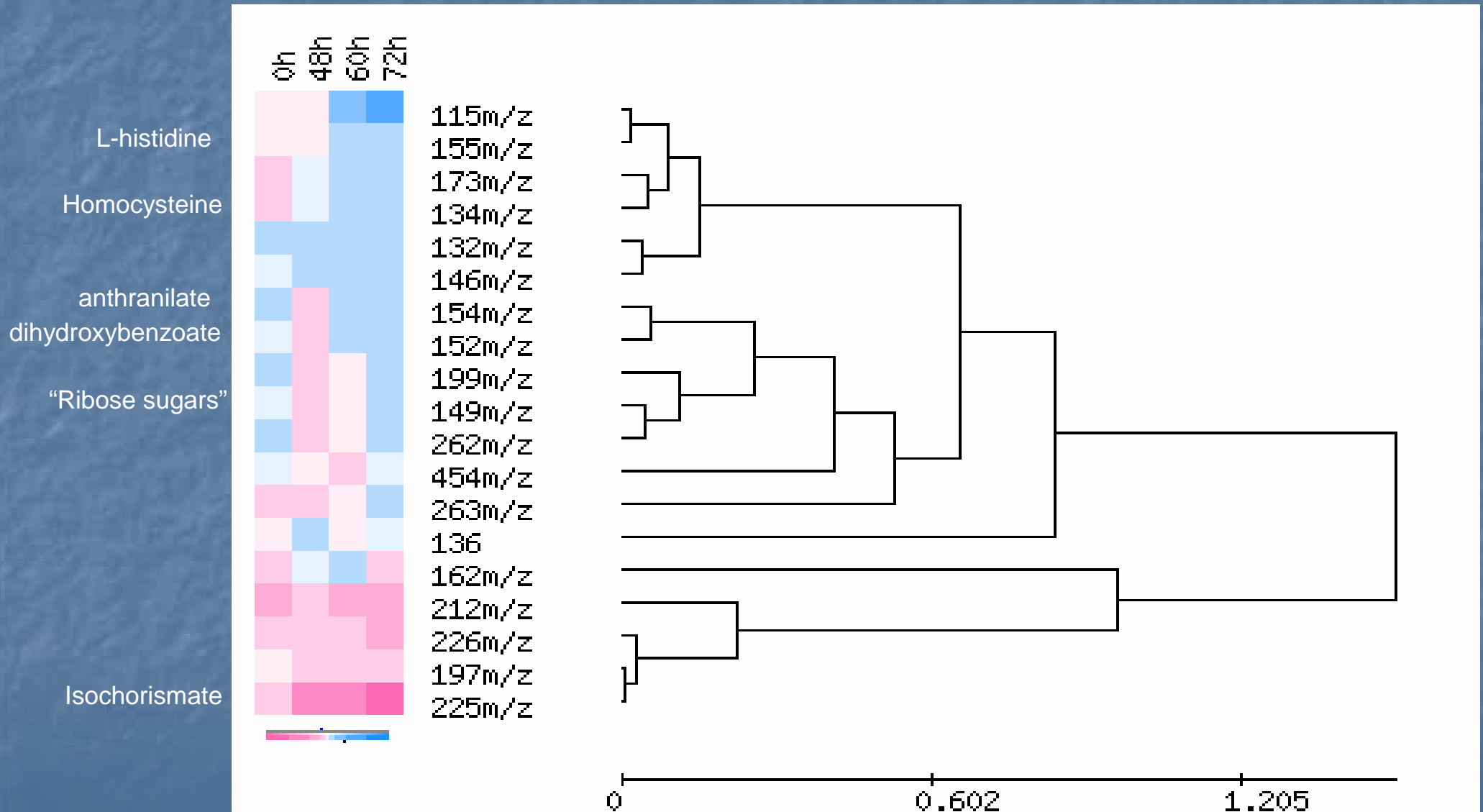
**Rank
Individuals**

**Evaluate Fitness of
Individuals**

Selection of key mass ions via GA-DFA



Hierarchical Cluster Analysis (HCA) of most discriminatory polar metabolites



Delivering the promise in plants?

- **Demonstrating added value**
- **Confirmation of discriminatory m/z**
- **Biological validation**
 - i) **Arabidopsis mutants .**
 - ii) **Addition of chemicals/inhibitors**

Acknowledgements

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