Identifying the full set of genes involved in cassava post-harvest physiological deterioration

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Overview of presentation

- Post-harvest physiological deterioration (PPD)
  - What is it?
  - Why is it important?
  - Can it be controlled?
- Towards identifying all genes
  - Strategy
  - cDNA microarrays
  - Results
  - Interpretation
- Where do we go from here?

What is post-harvest physiological deterioration (PPD)?

- Physiological / biochemical changes in the root (not due to micro-organisms)
- Becomes unpalatable and unmarketable within 24 - 72 hours of harvest
- Therefore, prompt consumption or processing is necessary
- PPD is a major constraint to cassava production, processing and consumption
- Impacts on sustainable livelihoods of resource-poor farmers

Economic & social effects of PPD

- Significant wastage
  - e.g. 5-25 %, which ends up as animal feed (FAO)
  - e.g. 10-60% losses depending on climate & distance (Colombia)
- Price reduction on deteriorated cassava:
  - e.g. 70-90% discounting on 3 day old cassava (Tanzania)
- High mark-up on fresh roots, especially in urban markets
  - up to 60 % of final price
  - urban consumers choose other starchy foods
- Non-uniform input to processing & industry
  - reduces quality & competitiveness of cassava products

Changes during PPD

- Increases in:
  - respiration
  - ethylene biosynthesis
  - phenolic biosynthesis
  - diterpene biosynthesis
  - enzyme activity e.g. PAL, CAT, PPO, invertase, peroxidase
- Changes in membrane lipids and sterols
- Active process involving changes in gene expression & protein synthesis
- Resembles wound responses in other plants, but lacks adequate wound repair

Approaches to controlling PPD

- Mechanical
  - Processing – OK on small scale
  - Exclusion of oxygen – for high price markets only
- Breeding - problems
  - High heterozygosity
  - Correlation between high dry matter & PPD
  - Genotype X Environment interactions
- Biotechnology
  - Increase understanding
  - Marker assisted selection (MAS)
  - Genetic modification

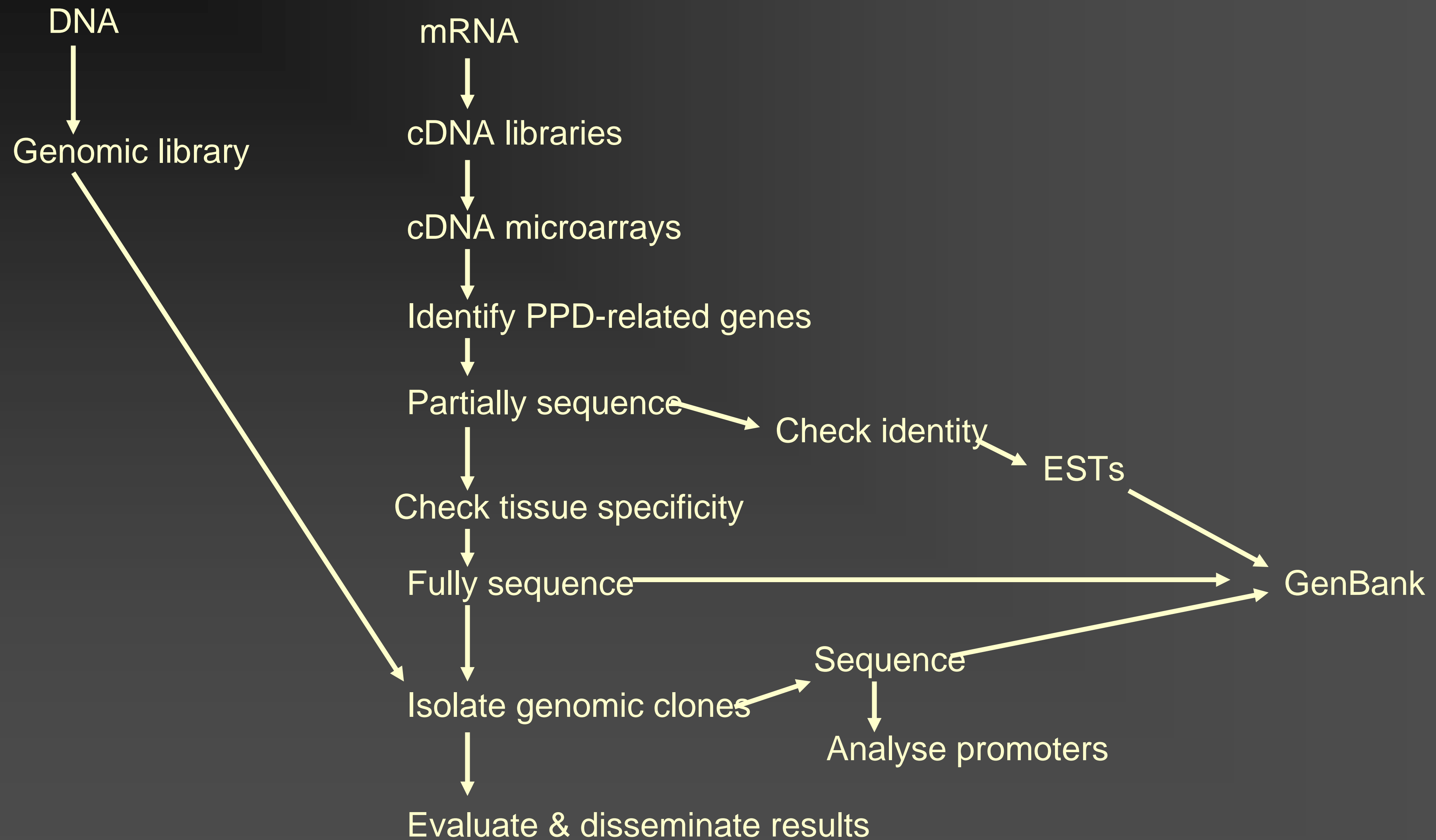
Hypothesis:

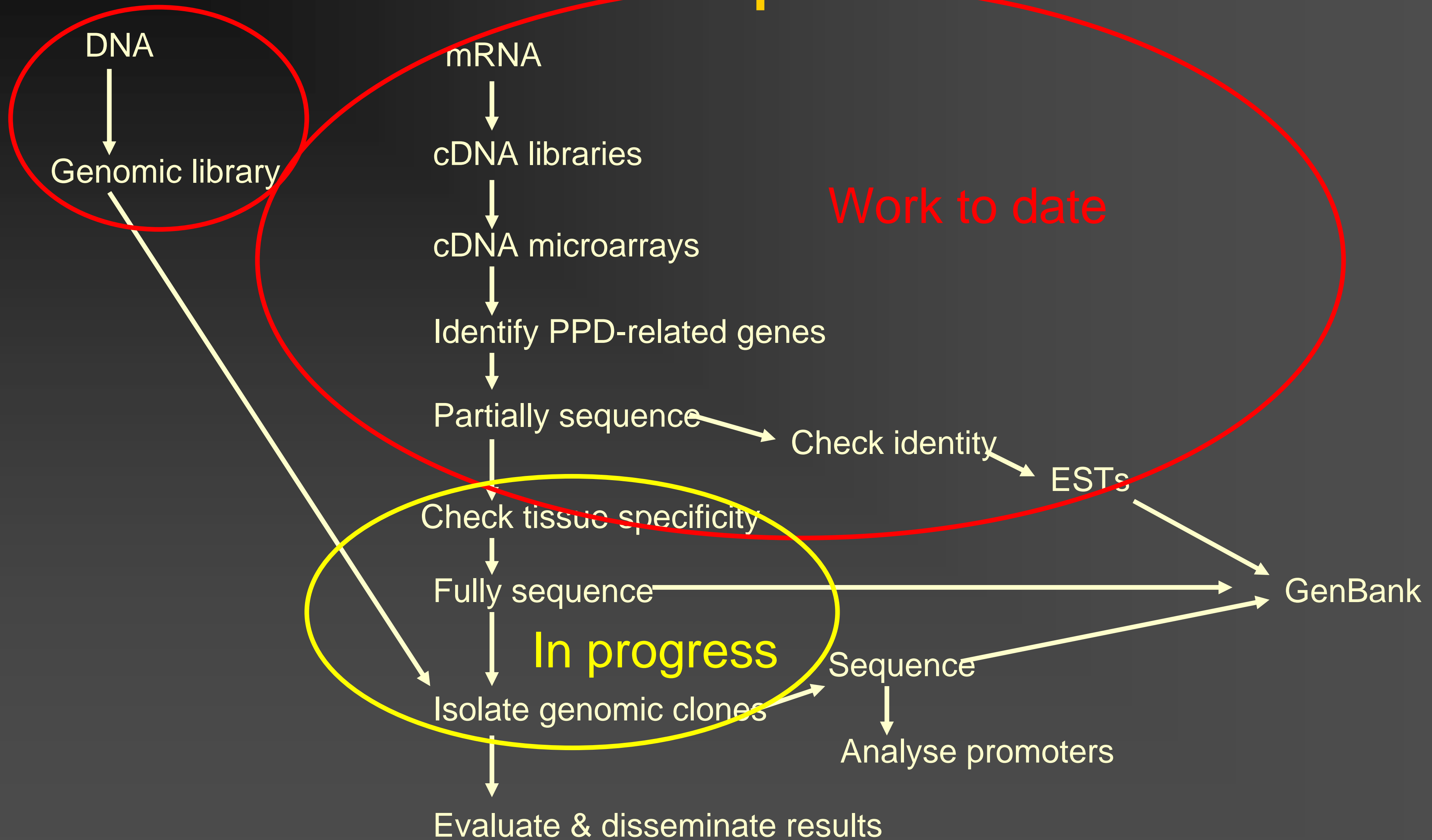
- Amongst the set of genes whose expression is altered during PPD there exists a sub-set, components of which can provide useful tools for the assembly of gene constructs that can be used to understand, modulate & ultimately control PPD

Strategy

- Employ massively parallel methods of gene discovery (cDNA microarrays) to identify those genes whose expression changes during PPD
- Evaluate these iteratively so as to fully characterise those genes whose components (promoters &/or cDNAs) could provide useful tools for modulating PPD in transgenic plants
- Identified genes can also be used for genomic mapping and marker assisted selection

Flow chart of experimentation



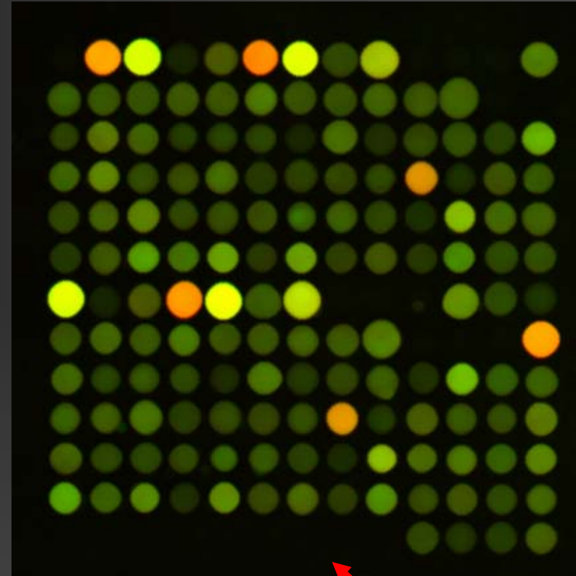


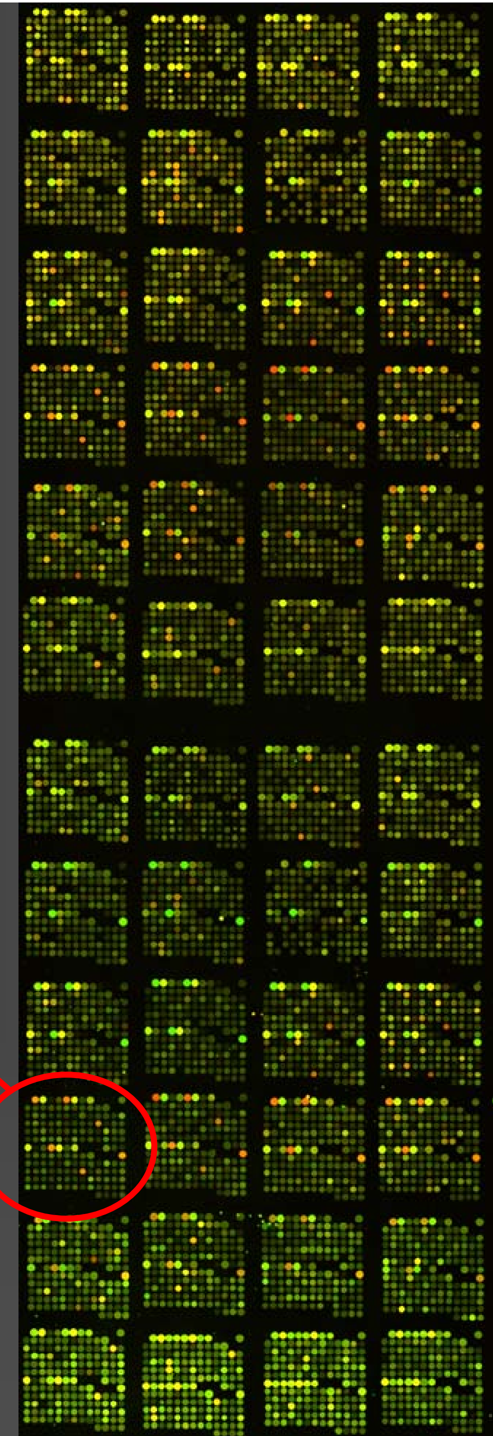
Flow chart of experimentation
DNA
Genomic library
mRNA
cDNA libraries
Work to date
cDNA microarrays
Identify PPD-related genes
Partially sequence
Check identity
ESTs
Check tissue specificity
Fully sequence
GenBank
In progress
Sequence
Isolate genomic clones
Analyse promoters
Evaluate & disseminate results

Construction of cDNA libraries

- Cassava cultivar CM 2177-2
- mRNA isolated over time course of PPD
- 0, 6 & 12 hours → "Early PPD library"
- 24, 48 & 96 hours → "Late PPD library"
- 7,680 "Early" clones spotted onto slide
- 3,456 "Late" clones spotted onto slide
- + control DNAs

cDNA microarrays





- •cDNAs spotted by robot onto slide
- •4 technical replicates
- •Control DNAs
- •Early time point cDNA probe (e.g. time 0) labelled with Cy3 (green)
- •Late time point cDNA probe (e.g. 24 hours) labelled with Cy5 (red)
- •Probes hybridised to cDNAs on slide
- •Up-regulated clones are red
- •Down-regulated clones are green
- •Identify clones of interest based on various criteria

Microarray hybridisations

Experiment	**Hybridisation**	**Probes**
Time course	Hyb 1	0 x 12 hours
	Hyb 2	0 x 24 hours
	Hyb 3	0 x 48 hours
	Hyb 4	0 x 72 hours
	Hyb 5	0 x 96 hours
Range	Hyb 6	12 x 24 hours
	Hyb 7	24 x 48 hours
	Hyb 8	48 x 72 hours
	Hyb 9	72 x 96 hours

Microarray data analysis

- Microarrays analysed using two methods:
  - Clones flagged in each hybridisation
  - Analysis of data normalised across arrays
- Clones selected if they show at least a 2-fold increase, or 2.8-fold decrease, in expression in at least two hybridisations using both methods
- These are strict conservative criteria to reduce false positives; however, they may also miss important genes showing transient expression

Results - preliminary analyses

- 114 clones show at least 2x up-regulation
- 70 clones show at least 2.8x down-regulation
- Single-pass sequencing in 5’ to 3’ direction
- BLASTn comparison to DNA sequences in GenBank database
- BLASTx comparison to protein sequences in GenBank data base
- Enable tentative identification of clones
- As expected, some are duplicates

Classes of up-regulated genes

Class	Nº of different genes
Cell wall metabolism & remodelling	4
Glucosinolate biosyntheses	2
Programmed cell death	3
Transcription / translation	6
Reactive oxygen spp. modulation	2
Signal transduction or perception	5
Ion, water or metabolite transport	6
Role unknown or uncharacterised	5

Expression of up-regulated genes

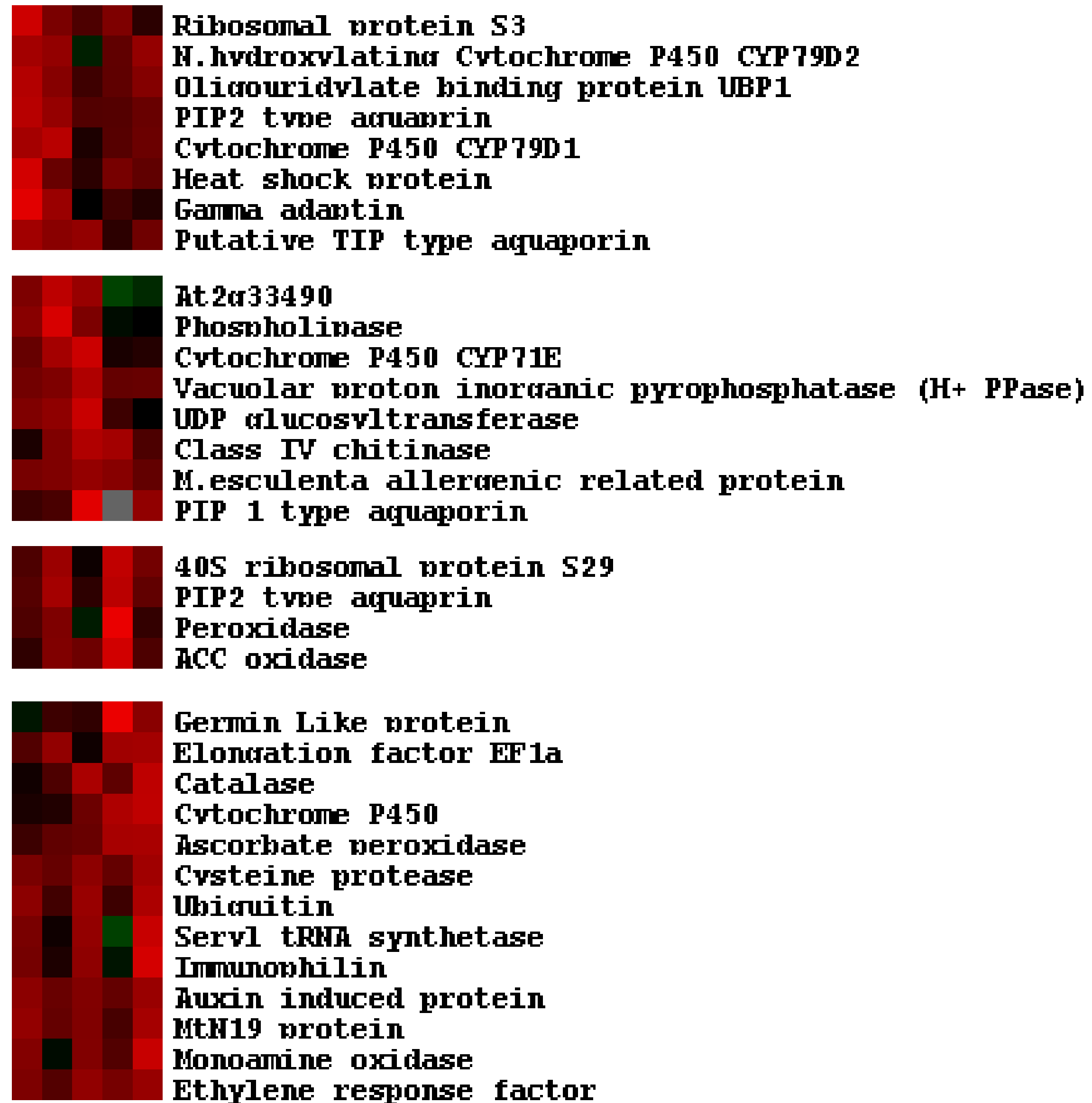
"Early"

< 24 hrs

< 48 hrs

72 hrs

"Late" 72-96 hrs



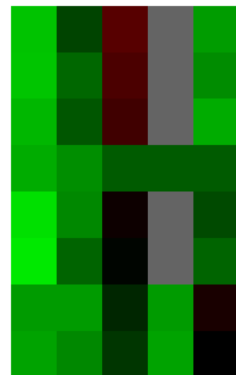
Classes of down-regulated genes

Class	Nº of different genes
Programmed cell death	2
Transcription / translation	3
Signal transduction	3
Metabolism	1
Unknown or uncharacterised	5

Expression of down-regulated genes

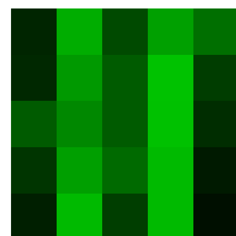
0 v 12 | 0 v 24 | 0 v 48 | 0 v 72 | 0 v 96

< 12 hrs



60S ribosomal protein L7A
tRNA transglycosidase
Unknown protein
Auxin repressed protein
Hypothetical protein
Putative senescence associated protein
Auxin repressed protein
PWWP domain protein

> 12 hrs



Expressed protein
Expressed protein
Expressed protein
ADP ribosylation factor
Translationally controlled tumour protein

Genes for further analysis

Up-regulated

- Ascorbate peroxidase
- Peroxidase
- Ethylene transcription factor
- Auxin-induced protein
- Cysteine proteinase
- Germin-like protein
- Glucosyltransferase
- Catalase
- FKBP-like immunophilin (peptide isomerase)

Down-regulated

- Translationally-controlled tumour protein
- PWWP domain protein
- Auxin-repressed protein
- ADP ribosylation factor

Analyses include:

- Full sequence of cDNA
- Tissue & temporal specificity
- Isolation & characterisation of corresponding genomic clone, including promoter

What remains to be done?

- Complete sequencing of selected cDNAs
- Northern blots & *in situ* hybridisations
- Isolate & sequence corresponding genomic clones & their promoters
- Identify gene components with possible potential to modulate PPD
- Publish results & their analyses

Where do we go from here, or, rather, there?

- At the end of the project we should have:
  - cDNAs of genes that play important roles in PPD
  - Promoters of some of these genes
  - Knowledge of gene components than could be used as constructs with the potential to modulate PPD in transgenic cassava
- Require collaborators & funding to test these constructs

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