Cassava post-harvest physiological deterioration genes

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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 in 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
  - Exclusion of oxygen
- Breeding - problems
  - High heterozygosity
  - Correlation between high dry matter & PPD
  - Genotype X Environment interactions
- Biotechnology
  - Increase understanding
  - Marker assisted selection (MAS)
  - Genetic modification

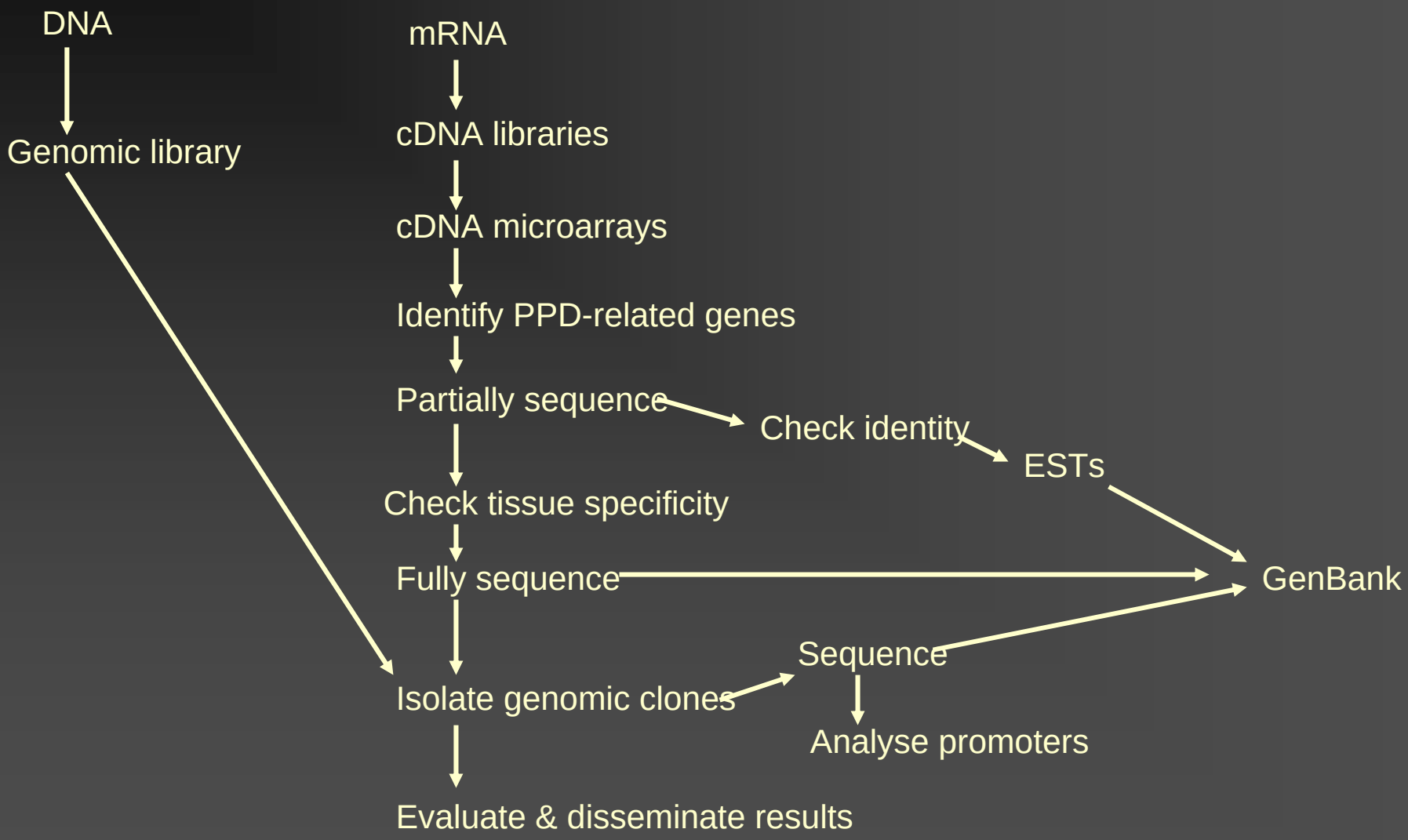
Hypothesis:

- 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

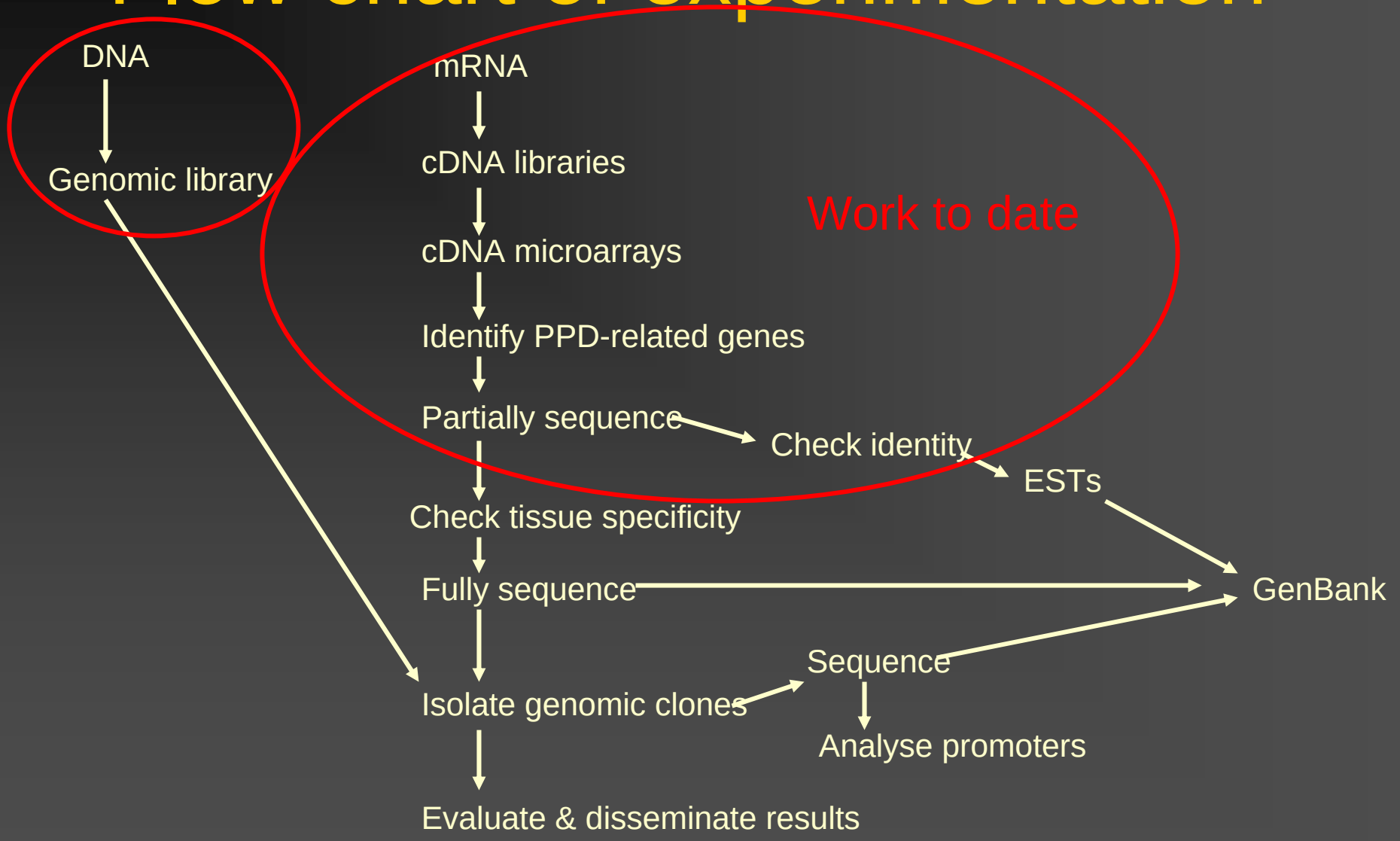
Strategy

- 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



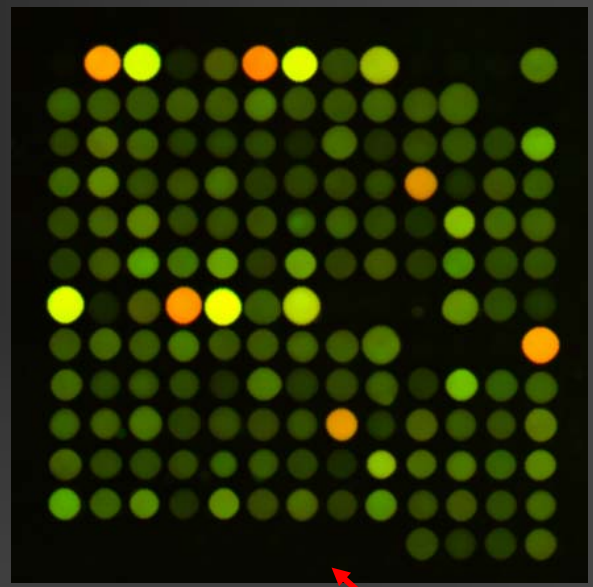
Flow chart of experimentation



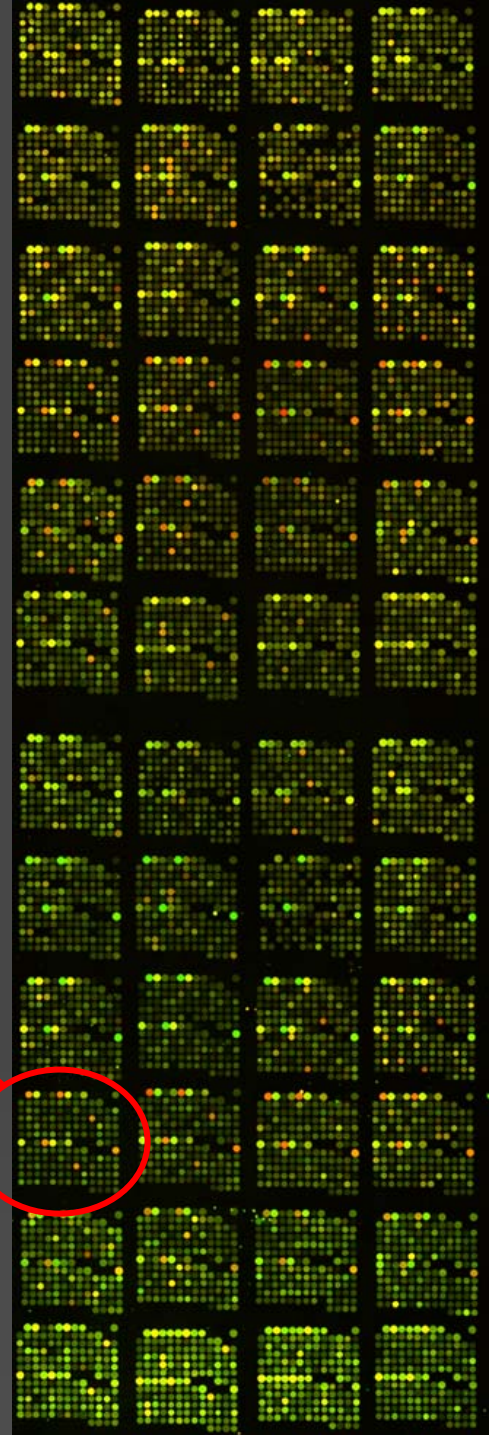
Construction of cDNA libraries

- 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

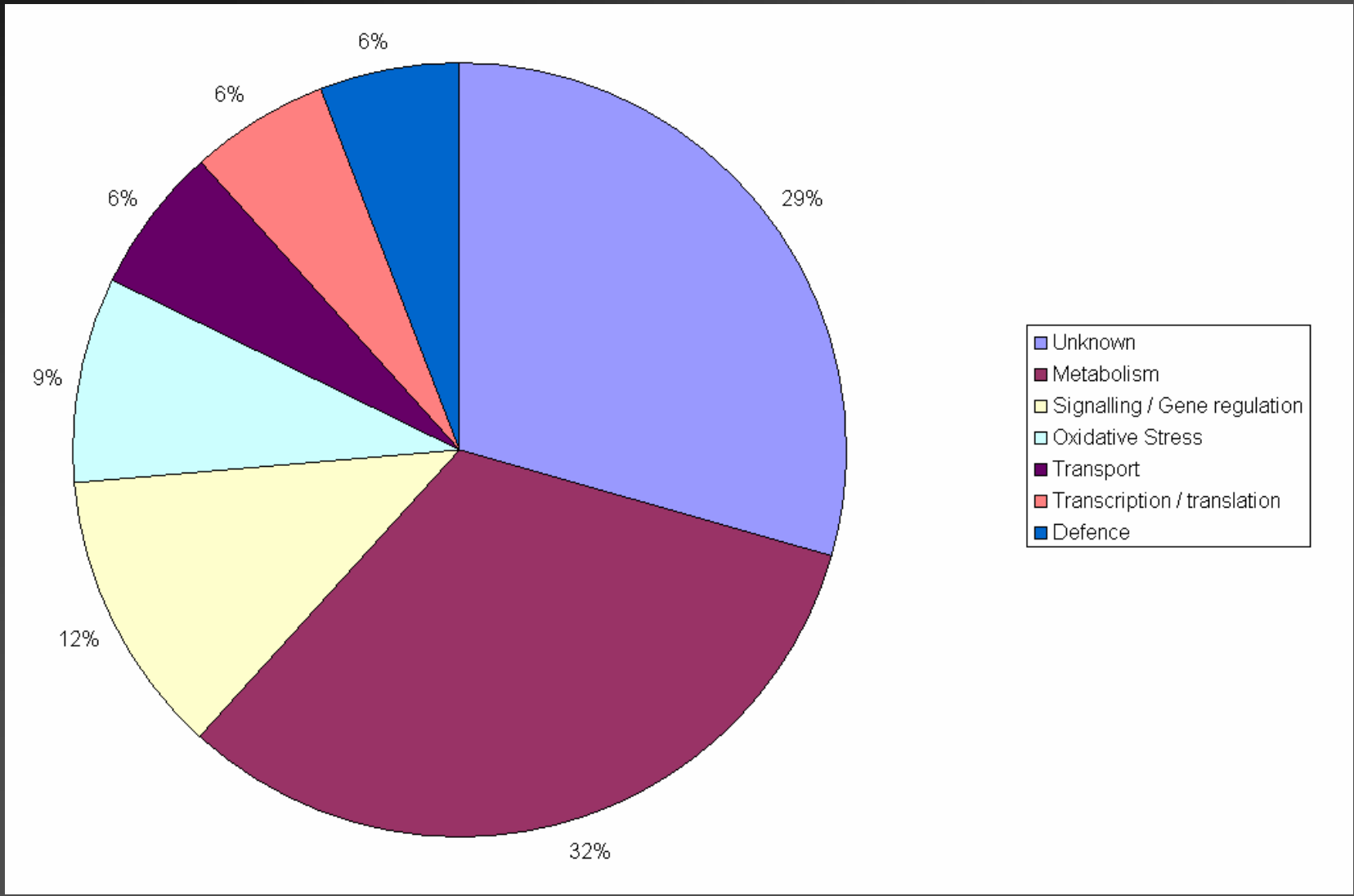
Microarray data analysis

- 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, 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

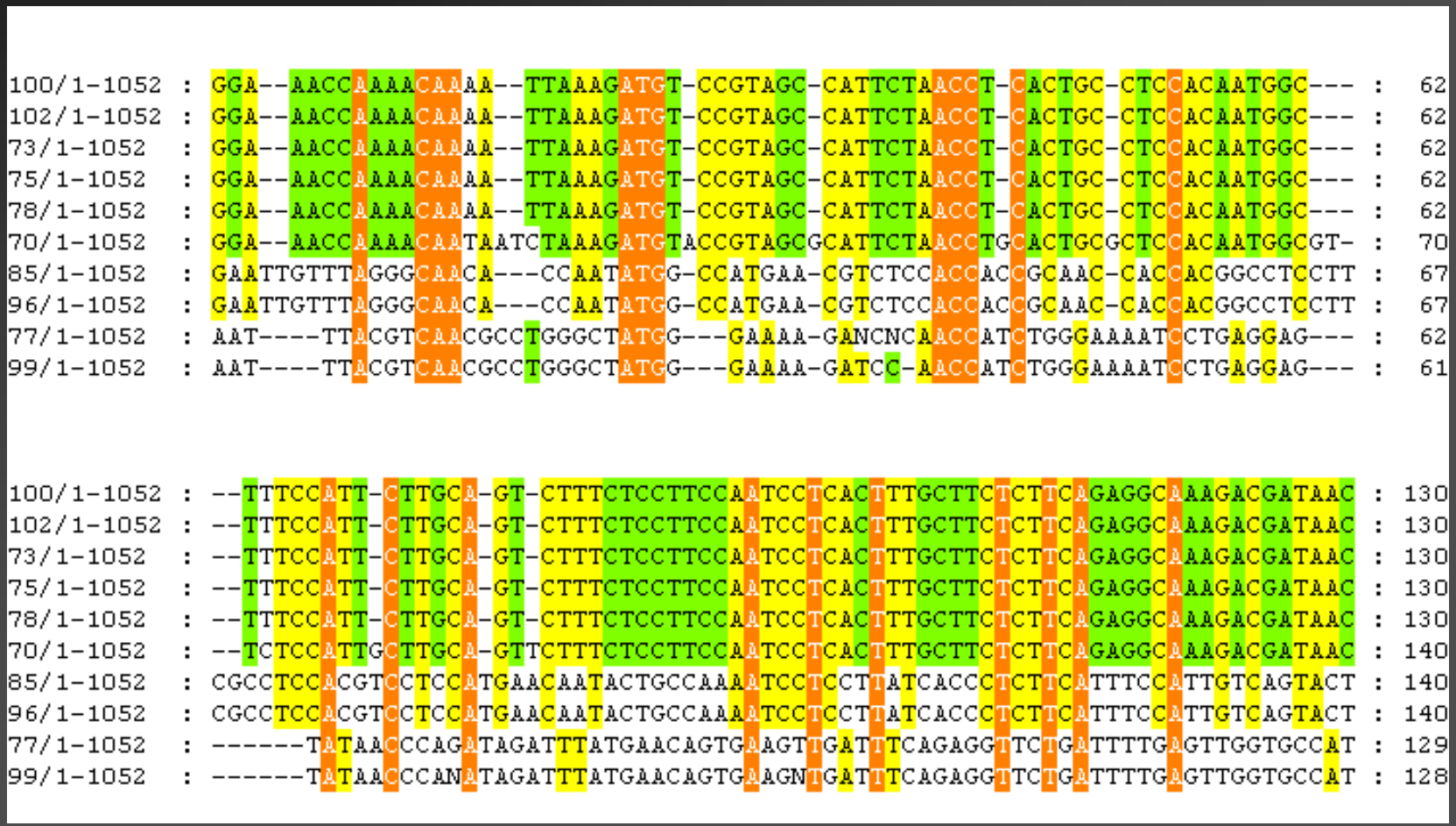
Up-regulated clones



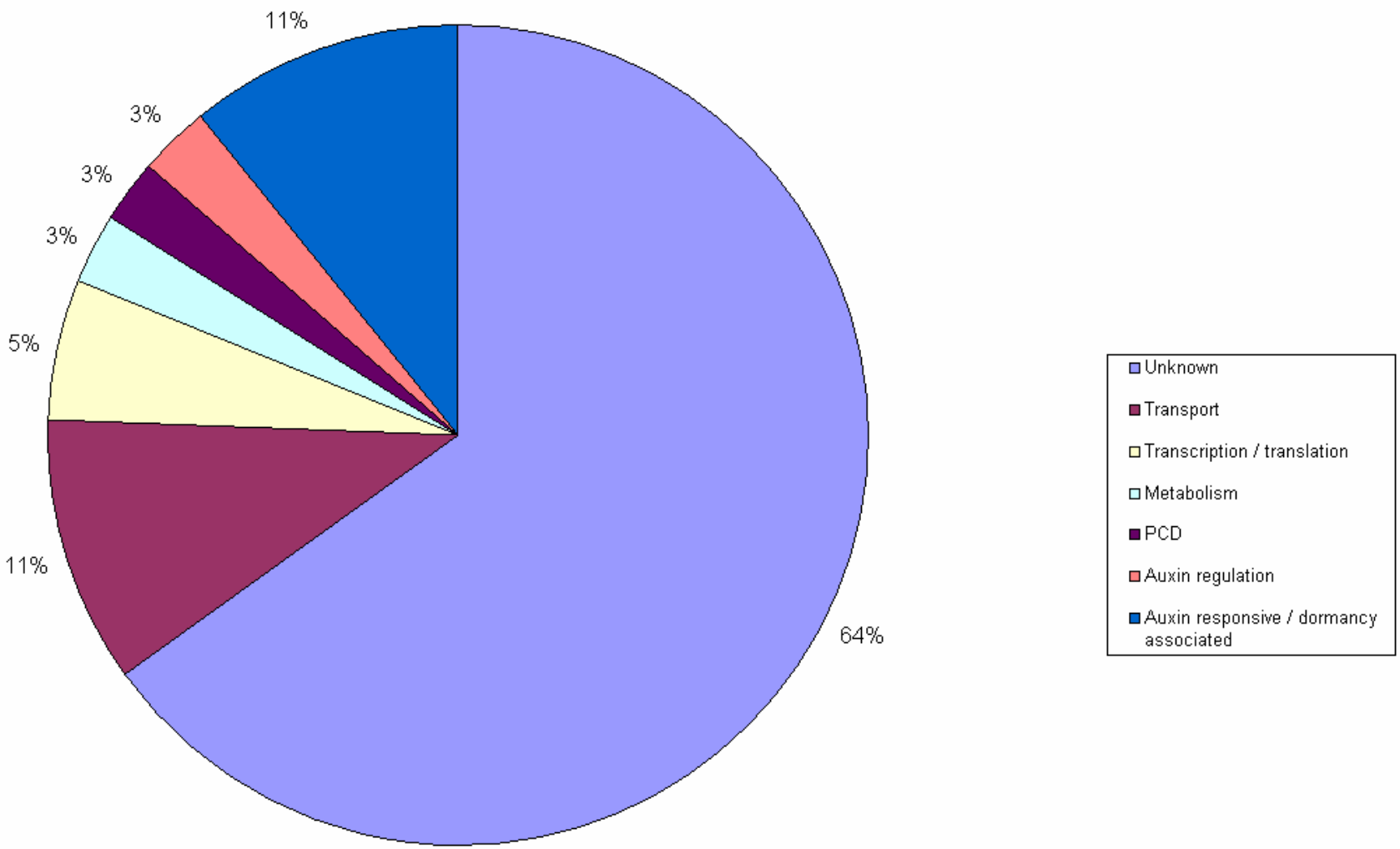
Up-regulated genes of interest

- 2 new peroxidases – oxidative stress
- Germin-like protein – stress & defence responses
- ACC oxidase – ethylene biosynthesis
- 2 glucosyl transferases – transfer sugars to acceptor molecules
- Phospholipase – role in signal transduction
- 10 cytochrome P450s –4 distinct types

Cytochrome P450s: alignment suggests 4 discrete types



Down-regulated clones



Down-regulated genes of interest

- Auxin-repressed protein – dormancy?
- IAA amidohydrolase – regulation of levels of free IAA
- ADP ribosylated factor – G-proteins involved in intracellular vesicle transport & signalling
- Senescence-associated protein

Where do we go from here?

- Complete sequencing & BLAST analyses
- Perform 2 biological replicates of whole experiment to improve reliability of data & sensitivity of clone selection
- Isolate & sequence corresponding genomic clones & their promoters
- Publish results & their analyses

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