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

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

DNA

Genomic library

mRNA

cDNA libraries

cDNA microarrays

Identify PPD-related genes

Partially sequence

Check identity

ESTs

Check tissue specificity

Fully sequence

Isolate genomic clones

Evaluate & disseminate results

In progress

Work to date

Sequence

Analyse promoters

GenBank
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

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hybridisation</th>
<th>Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time course</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyb 1</td>
<td></td>
<td>0 x 12 hours</td>
</tr>
<tr>
<td>Hyb 2</td>
<td></td>
<td>0 x 24 hours</td>
</tr>
<tr>
<td>Hyb 3</td>
<td></td>
<td>0 x 48 hours</td>
</tr>
<tr>
<td>Hyb 4</td>
<td></td>
<td>0 x 72 hours</td>
</tr>
<tr>
<td>Hyb 5</td>
<td></td>
<td>0 x 96 hours</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyb 6</td>
<td></td>
<td>12 x 24 hours</td>
</tr>
<tr>
<td>Hyb 7</td>
<td></td>
<td>24 x 48 hours</td>
</tr>
<tr>
<td>Hyb 8</td>
<td></td>
<td>48 x 72 hours</td>
</tr>
<tr>
<td>Hyb 9</td>
<td></td>
<td>72 x 96 hours</td>
</tr>
</tbody>
</table>
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 database
- Enable tentative identification of clones
- As expected, some are duplicates
## Classes of up-regulated genes

<table>
<thead>
<tr>
<th>Class</th>
<th>Nº of different genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall metabolism &amp; remodelling</td>
<td>4</td>
</tr>
<tr>
<td>Glucosinolate biosyntheses</td>
<td>2</td>
</tr>
<tr>
<td>Programmed cell death</td>
<td>3</td>
</tr>
<tr>
<td>Transcription / translation</td>
<td>6</td>
</tr>
<tr>
<td>Reactive oxygen spp. modulation</td>
<td>2</td>
</tr>
<tr>
<td>Signal transduction or perception</td>
<td>5</td>
</tr>
<tr>
<td>Ion, water or metabolite transport</td>
<td>6</td>
</tr>
<tr>
<td>Role unknown or uncharacterised</td>
<td>5</td>
</tr>
</tbody>
</table>
Expression of up-regulated genes

< 24 hrs

< 48 hrs

“Early”

72 hrs

“Late” 72-96 hrs

Ribosomal protein S3
H. hydroxytylaing Cytochrome P450 CYP79D2
Oligouridylate binding protein UBP1
PIP2 type aquaparin
Cytochrome P450 CYP79D1
Heat shock protein
Gamma adantin
Putative TIP type aquaporin

At2g33490
Phospholipase
Cytochrome P450 CYP71E
Vacuolar proton inorganic pyrophosphatase (H+ Ppase)
UDP glucosyltransferase
Class IV chitinase
M. esculenta allergenic related protein
PIP 1 type aquaporin

40S ribosomal protein S29
PIP2 type aquaparin
Peroxidase
ACC oxidase

Germin Like protein
Elongation factor EF1a
Catalase
Cytochrome P450
Ascorbate peroxidase
Cysteine protease
Ubiquitin
Ser/1 tRNA synthetase
Immunophilin
Auxin induced protein
MtN19 protein
Monoamine oxidase
Ethylene response factor
# Classes of down-regulated genes

<table>
<thead>
<tr>
<th>Class</th>
<th>No of different genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Programmed cell death</td>
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<td>3</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>3</td>
</tr>
<tr>
<td>Metabolism</td>
<td>1</td>
</tr>
<tr>
<td>Unknown or uncharacterised</td>
<td>5</td>
</tr>
</tbody>
</table>
Expression of down-regulated genes

< 12 hrs

60S ribosomal protein L7A
trRNA 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
Acknowledgements

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