Post-harvest Physiological Deterioration in Cassava

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Overview of Presentation

- Post-harvest physiological deterioration (PPD) & its economic importance
- Possible solutions
- Summarise current understanding of the problem
- Role of a key gene in PPD
- Conclusions
Why is cassava important?

- World’s 6th most important crop in terms of production
- Staple crop to over 500 M
- Especially important to the poor in sub-Saharan Africa
- Staple food & processed into many products
- Famine reserve crop
- Production predicted to increase

What is Post-harvest Physiological Deterioration?

- Physiological / biochemical changes in the root (not due to micro-organisms)
- Cassava roots become unpalatable and unmarketable within 1 – 4 days
- Therefore, prompt consumption and processing necessary
- PPD is a major constraint to cassava production, processing and consumption
Economic Impacts of PPD

• Wastage → animal feed → price reduction
• Price mark-up on fresh roots
• Add-on costs to limit deterioration
• In Colombia producers & processors estimate 10 – 50% wastage
• Affects farmers, processors, vendors and consumers
Possible Solutions to PPD

• Mechanical?
• Conventional plant breeding?
• Biotechnology?
Mechanical Solutions?

- Rapid processing: e.g. farinha, “chips”, starch
- Exclusion of oxygen: e.g. plastic bags, waxing, controlled atmospheres
- Freezing
- Only appropriate for specific uses and high price markets
Conventional Plant Breeding?

- Tried & tested method in other crops
- But PPD is complex multigene trait
- But close correlation between PPD and high dry matter content. Difficult to separate?
- But PPD is strongly influenced by the environment making it difficult to score progeny
Biotechnology?

• Powerful tool to manipulate important traits
• Largely untried in cassava, but successful in other crops
• Use to:
  – understand PPD
  – identify key control points
  – generate tools to assist with breeding
  – generate tools to manipulate PPD (GMOs)
Visible Changes During PPD

- Blue-black discoloration of vascular tissue
- Strong fluorescence under UV light
- Microscopy shows coloured occlusions & tyloses blocking xylem
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
• Changes in protein synthesis
• Changes in gene expression
• Resembles wound responses in other plants, but lacks adequate wound repair
Plant Wound Responses

Damage

Signals

Local: e.g. cell wall fragments, jasmonates, H$_2$O$_2$
Systemic: e.g. systemin
Hormones: e.g. ethylene

Defensive responses

Enzymes: e.g. chitinase, $\beta$-1,3-glucanase, proteinase inhibitors
Low MWt compounds: phenolics, terpenoids, etc (anti-microbials & anti-oxidants)

Boundary sealing (healing): e.g. callose, suberin, lignin, HRGPs

Negative feedback
Goal: To Understand & Control PPD

- Plant wound responses provide model & tools to understand PPD
- Approaches:
  - biochemical – roles of chemical & enzymes
  - molecular – roles of key genes
  - genetic / breeding – mapping of genes involved in PPD, evaluate & improve germplasm
Biochemical Summary

• Low molecular weight compounds peak during PPD. These include anti-oxidants & anti-microbials

• Reactive oxygen species (ROS), & enzymes & compounds that modulate ROS play important roles in PPD
Scopoletin, $\text{H}_2\text{O}_2$ & Peroxidase Produce Blue-black Precipitate
Reactive Oxygen Species & PPD

- Excluding O$_2$ prevents PPD
- H$_2$O$_2$ peak early in PPD
- Expression of catalase & peroxidase correlated with PPD response
- Anti-oxidant compounds & PPD
- β-carotene correlated with resistance to PPD
Classes of Clones Isolated & Characterised

- Cell wall strengthening 20
- Transcription 6
- Translation 7
- Signal transduction 6
- Stress 5
- Oxidative stress 4
- Senescence, PCD 5
- Defence 3
- Metabolism 7
- Secondary metabolism 7
- Cross-membrane transport 2
- Other 6
- Unidentified 5
Genetics/Breeding Summary

- All isolated genes located on genomic map
- Quantitative trait loci (QTLs) associated with PPD identified
- Correlation between carotenoids (provitamin A / anti-oxidants) & reduced PPD established
- Major influence of environment on PPD identified
Phenylalanine Ammonia-lyase: a Key Enzyme in the PPD Response

- Entry enzyme to phenylpropanoid metabolism
- Switch between primary metabolism (protein synthesis) & secondary metabolism
- Phenylpropanoid products important in:
  - signalling
  - protection, defence - (e.g. scopoletin)
  - wound healing
- Increases in activity during PPD
- Therefore, an important target for research
General Phenylpropanoid Metabolism & PAL

Pigments, UV protectants, anti-microbials, anti-oxidants: e.g. scopoletin

Signalling molecules: e.g. salicylic acid

Cell wall components: e.g. lignin, suberin
Cassava has 4 PAL genes

- Southern blots of genomic DNA & sequencing of isolated genes show that cassava has 4 PAL genes
- Most plants have ~ 4 genes
- Loblolly pine - 1 gene
- Potato - ~ 40 genes

Cassava DNA probed with PAL cDNA clone
Cassava PAL2 Gene

Promoter Exon 1 Intron Exon 2

Putative P A L boxes involved in light & elicitor induced gene expression

Putative TATA boxes

Putative transcription start sites
PAL-Promoter GUS Constructs

-1000 Hind III 0 ATG

PAL Promoter

-840

Promoter-GUS fusion at -72 from ATG

GUS

nos terminator

-400

Lacks P & A boxes

-200
Transformation of Cassava

-840, 400 & 200 PAL-promoter GUS constructs were transformed into cassava by particle bombardment (biolistics).

Transient expression showed that longer promoters had higher activity than shorter ones.

-840 & -400 constructs expressed in transgenics, -200 did not.
Transgenic Cassava Material:

- Transient GUS expression in embryogenic suspension culture
- 840PAL-GUS activity
- GUS expression in transformed callus
- GUS expression in developing embryo
Transgenic Cassava Plants

Cassava embryos transformed with -840PAL-GUS were grown on & produced storage roots.
-840PAL-GUS Activity in the Leaf

PAL promoter activity is confined to the vascular tissues during development.
-840PAL-GUS Activity in Petiole

Transverse section of petiole
-840PAL-GUS Activity in Stems
-840PAL-GUS Activity in Stem
Xylem Tyloses

Transverse section
-840PAL-GUS Activity in Storage Roots

GUS activity confined to vascular tissue & below cortex
-840PAL-GUS Activity in Storage Roots During PPD:

Transverse section

PPD symptoms

GUS expression
-840PAL-GUS Activity in Storage Roots During PPD:
Longitudinal section

PPD symptoms
GUS expression
-840PAL-GUS Activity in Transgenic Roots During PPD

![Bar chart showing GUS activity (pmol MU/min/mg protein) over time after harvesting (hours). The x-axis represents time in hours (0, 24, 48, 72, 96) and the y-axis represents GUS activity in pmol MU/min/mg protein.]
PAL-GUS Transgenics Show:

- First cassava transgene using cassava promoter
- 840 bp of the PAL2 promoter confers developmental specificity to a reporter gene
- PAL2 promoter activity confined to vascular tissue
- PAL2 promoter activity increases in roots during PPD
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Defensive responses

Enzymes: e.g. chitinase, β-1,3-glucanase, proteinase inhibitors
Low MWt compounds: coumarins, catechins (anti-microbials & anti-oxidants)

Boundary sealing (healing): e.g. callose, suberin, lignin, HRGPs, extensins

S/T kinase
ACC oxidase
PAL
H₂O₂
Catalase
Peroxidase
PCD enzymes

Negative feedback
Conclusions

• Biotechnology is providing:
  – the knowledge necessary to understand PPD
  – the tools to assist & accelerate breeding
  – the genes with which to manipulate PPD via genetic modification
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