Post-harvest Physiological Deterioration in Cassava

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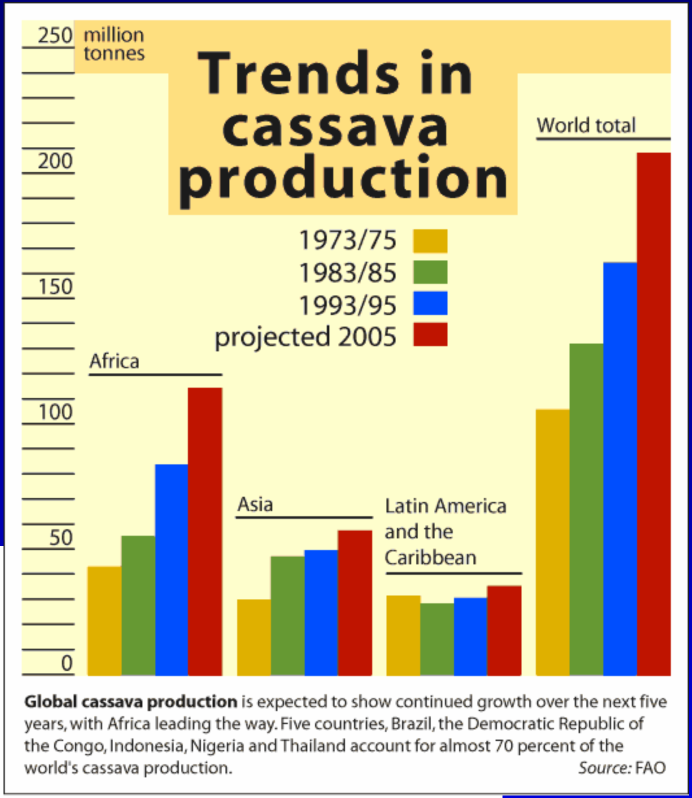
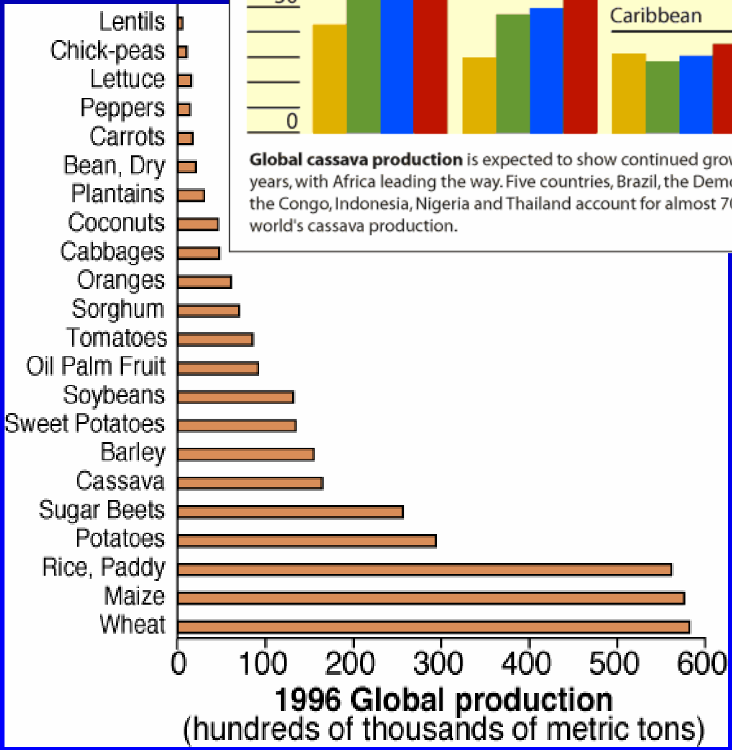
Overview of Presentation

- 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





Mann (1997) *Science*, 277: 1038-1043.

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

Negative feedback

Damage

Signals

Local: e.g. cell wall fragments, jasmonates, H_2O_2

Systemic: e.g. systemin

Hormones: e.g. ethylene

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

Defensive responses

Enzymes: e.g. chitinase, ß-1,3-glucanase, proteinase inhibitors

Low MWt compounds: phenolics, terpenoids, etc (anti-microbials & anti-oxidants)

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

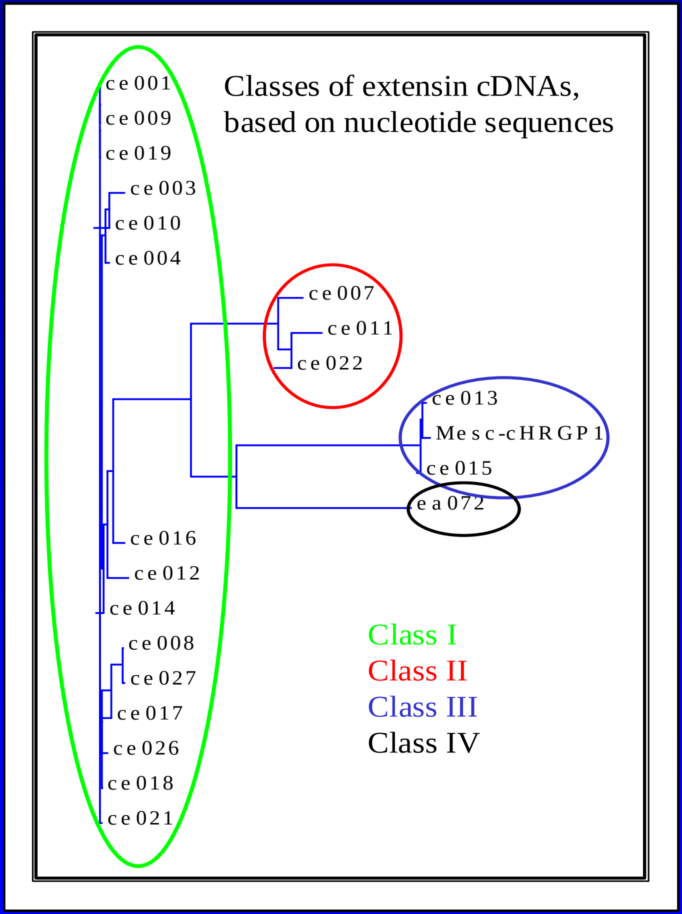
Scopoletin, H_2O_2 & Peroxidase Produce Blue-black Precipitate

Reactive Oxygen Species & PPD

- Excluding O_2 prevents PPD
- H_2O_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

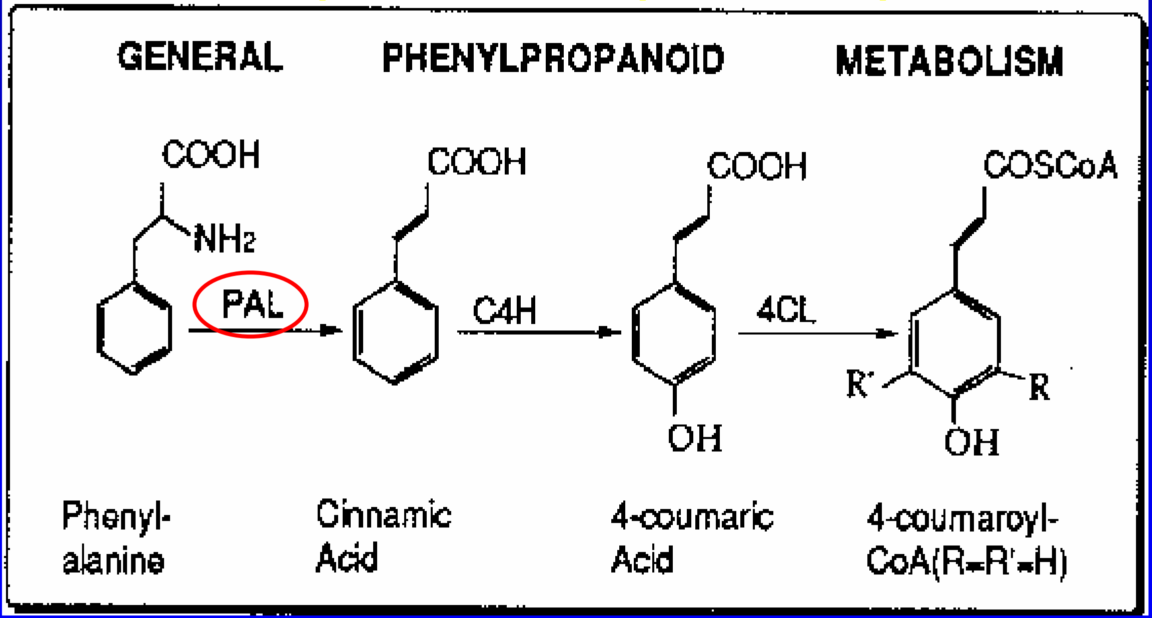
- All isolated genes located on genomic map
- Quantitative trait loci (QTLs) associated with PPD identified
- Correlation between carotenoids (provitamin A / anti-oxidants) & reduced PPD established
- Major influence of environment on PPD identified

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

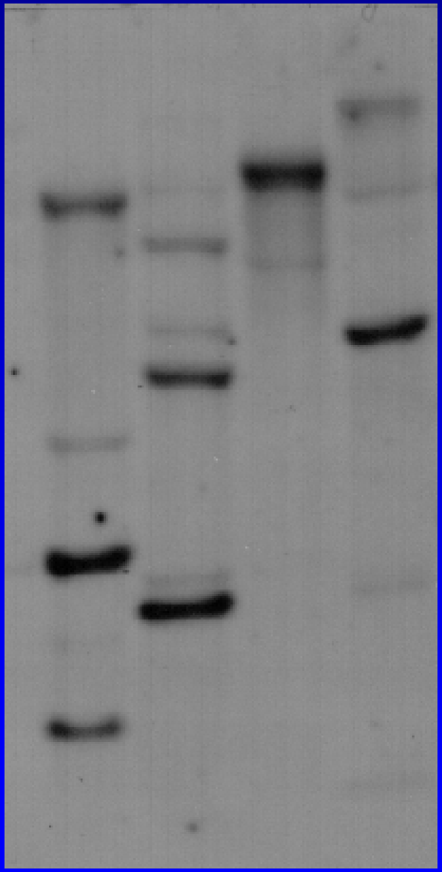


Signalling molecules: e.g.salicylic acid

Cell wall components: e.g. lignin, suberin

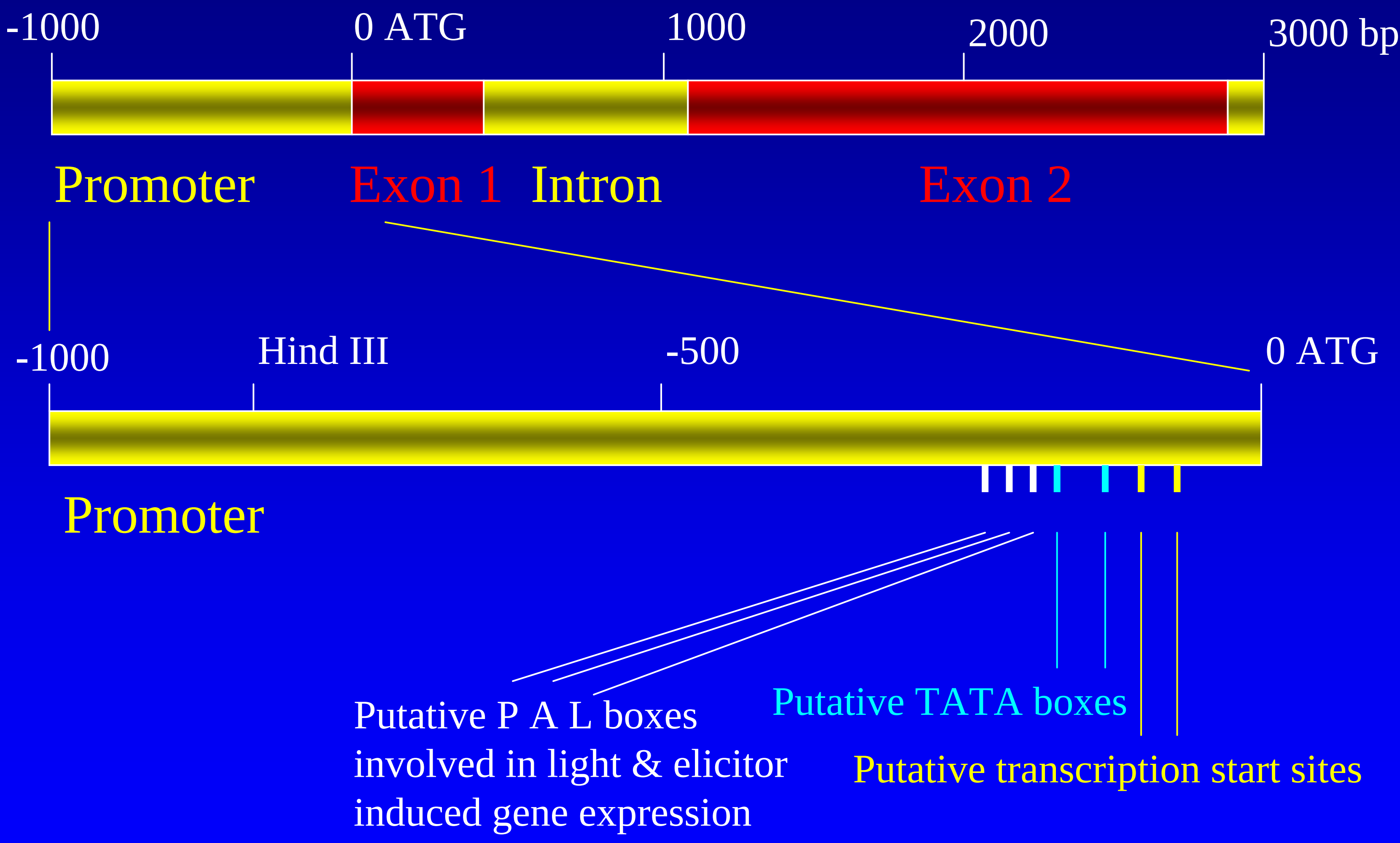
Cassava has 4 PAL genes

- 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

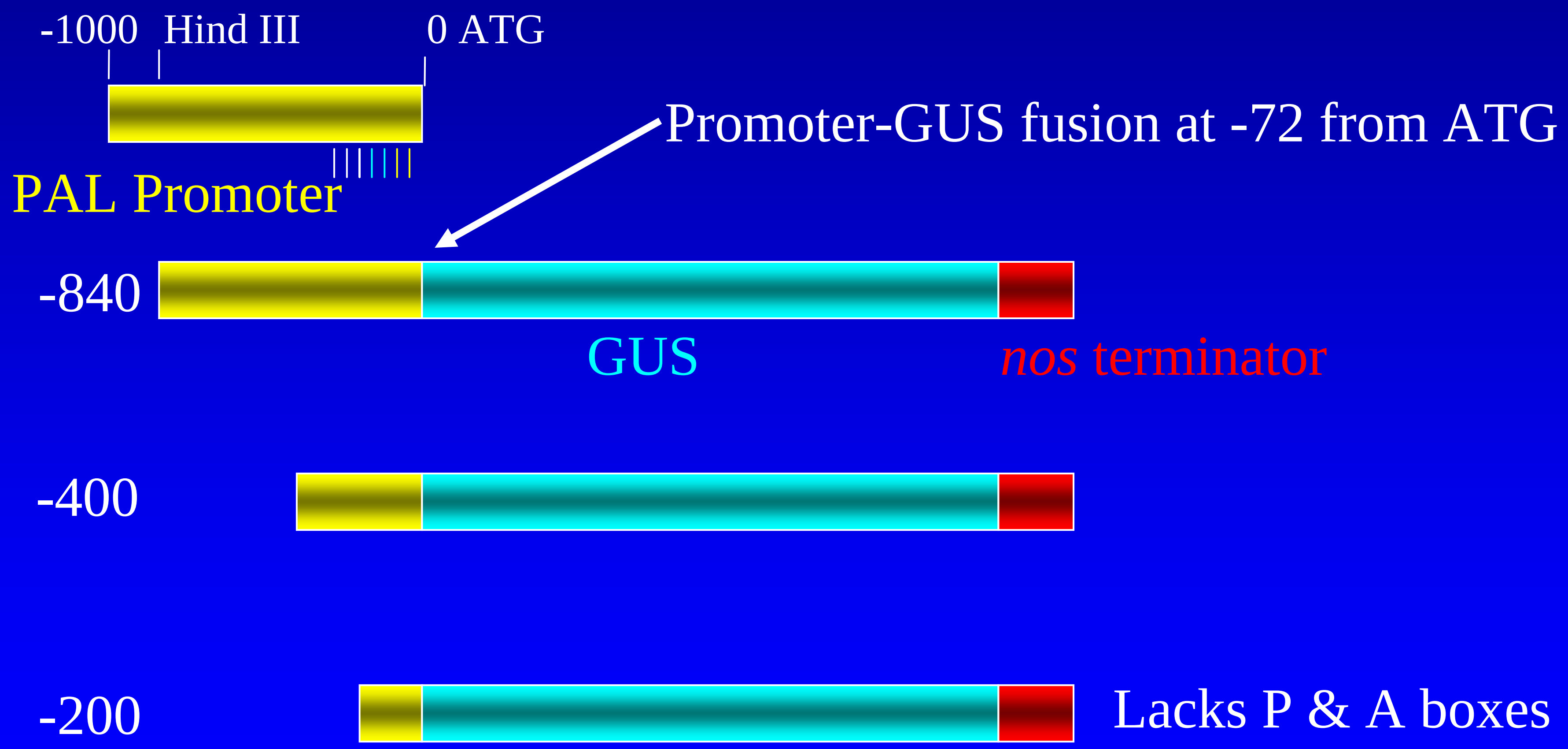


Cassava DNA probed with PAL cDNA clone

Cassava PAL2 Gene



PAL-Promoter GUS Constructs

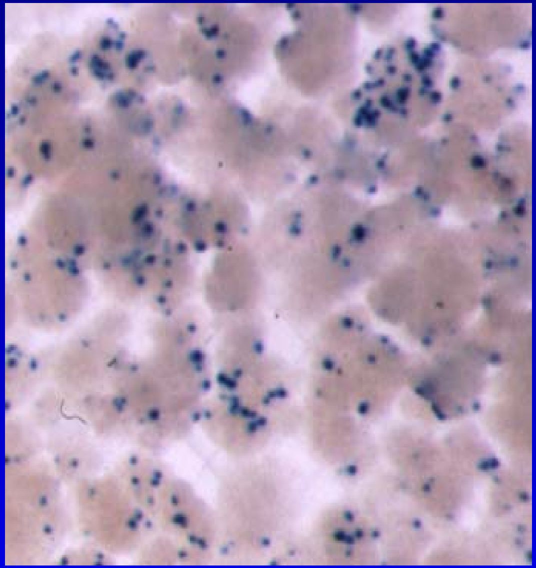


Transformation of Cassava

- -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:

-840PAL-GUS activity



Transient GUS expression in embryogenic suspension culture

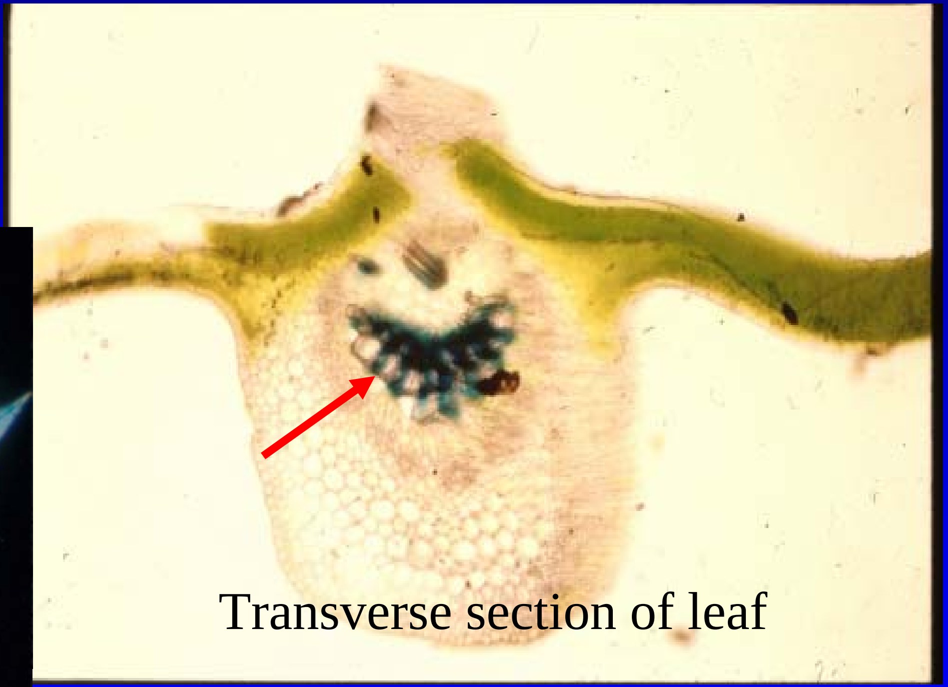
GUS expression in transformed callus

GUS expression in developing embryo

Transgenic Cassava Plants

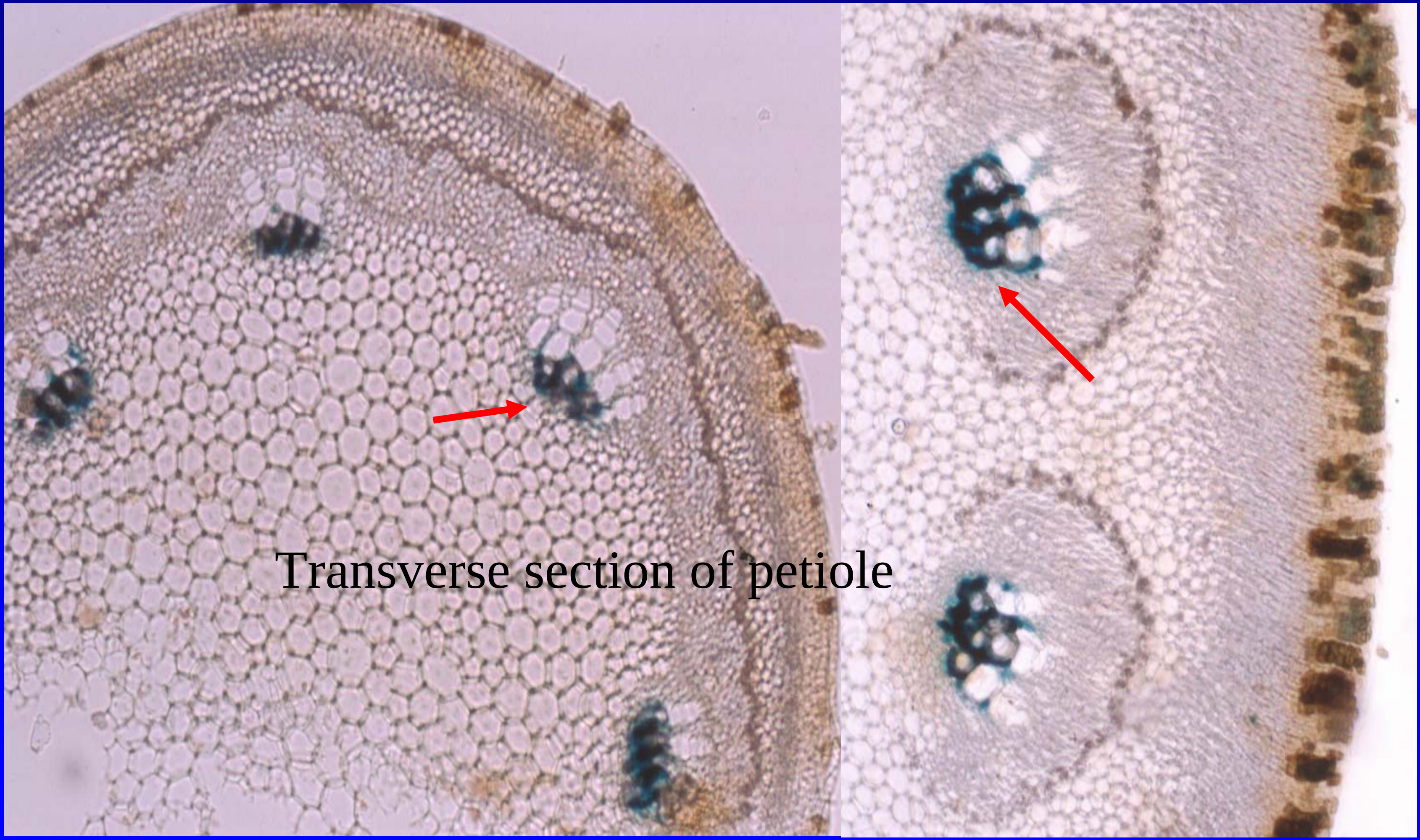
Cassava embryos transformed with -840PAL-GUS were grown on & produced storage roots

-840PAL-GUS Activity in the Leaf

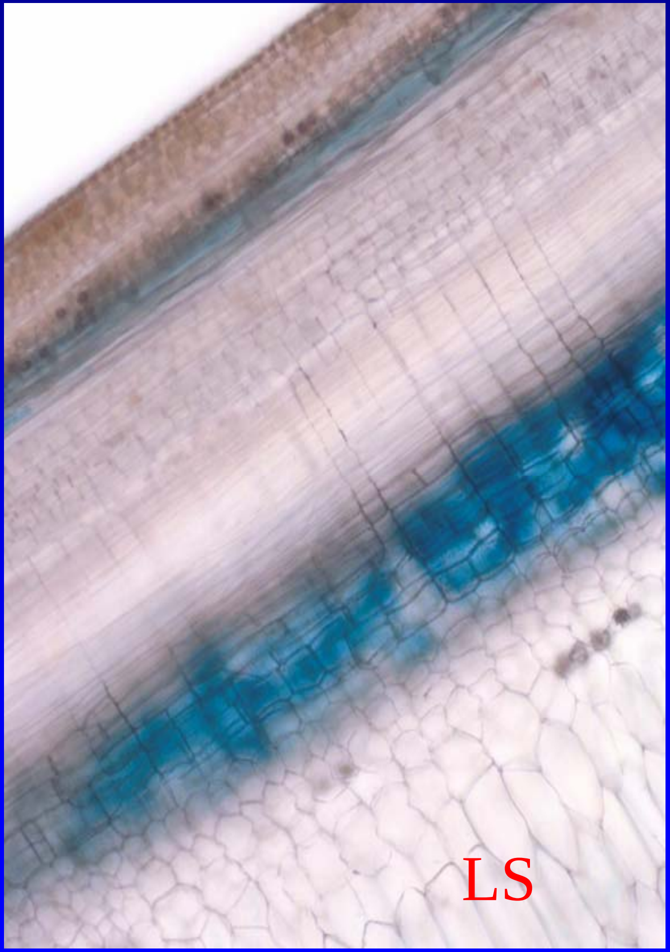


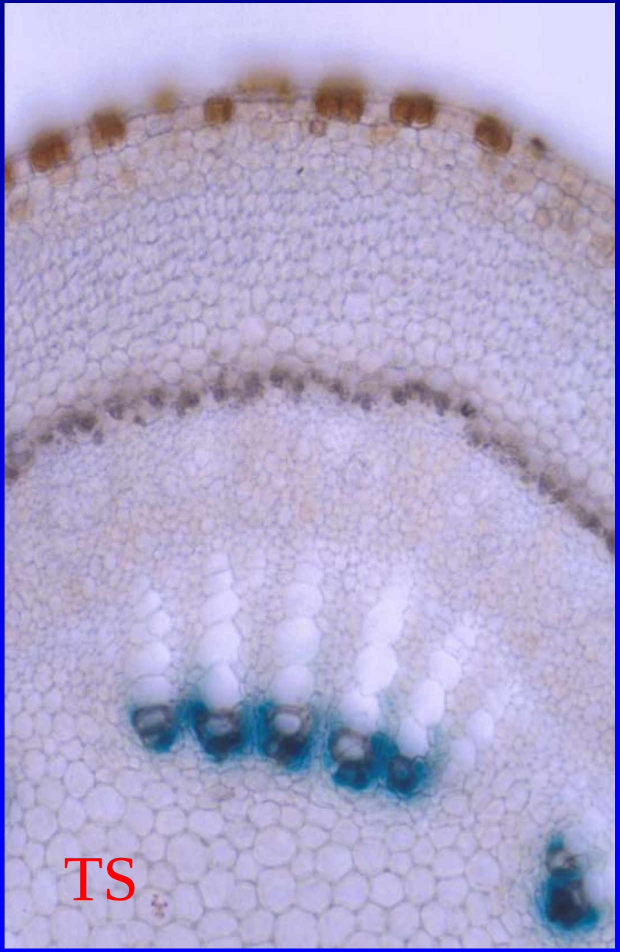
PAL promoter activity is confined to the vascular tissues during development

-840PAL-GUS Activity in Petiole

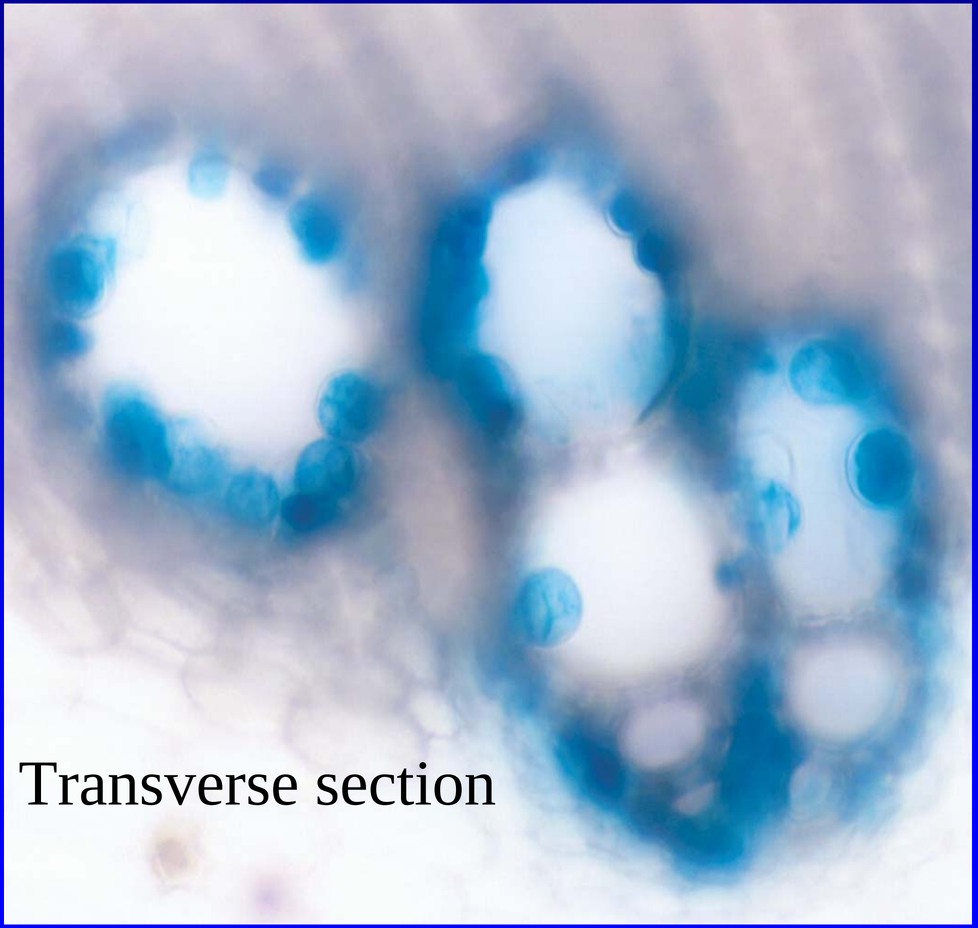


-840PAL-GUS Activity in Stems

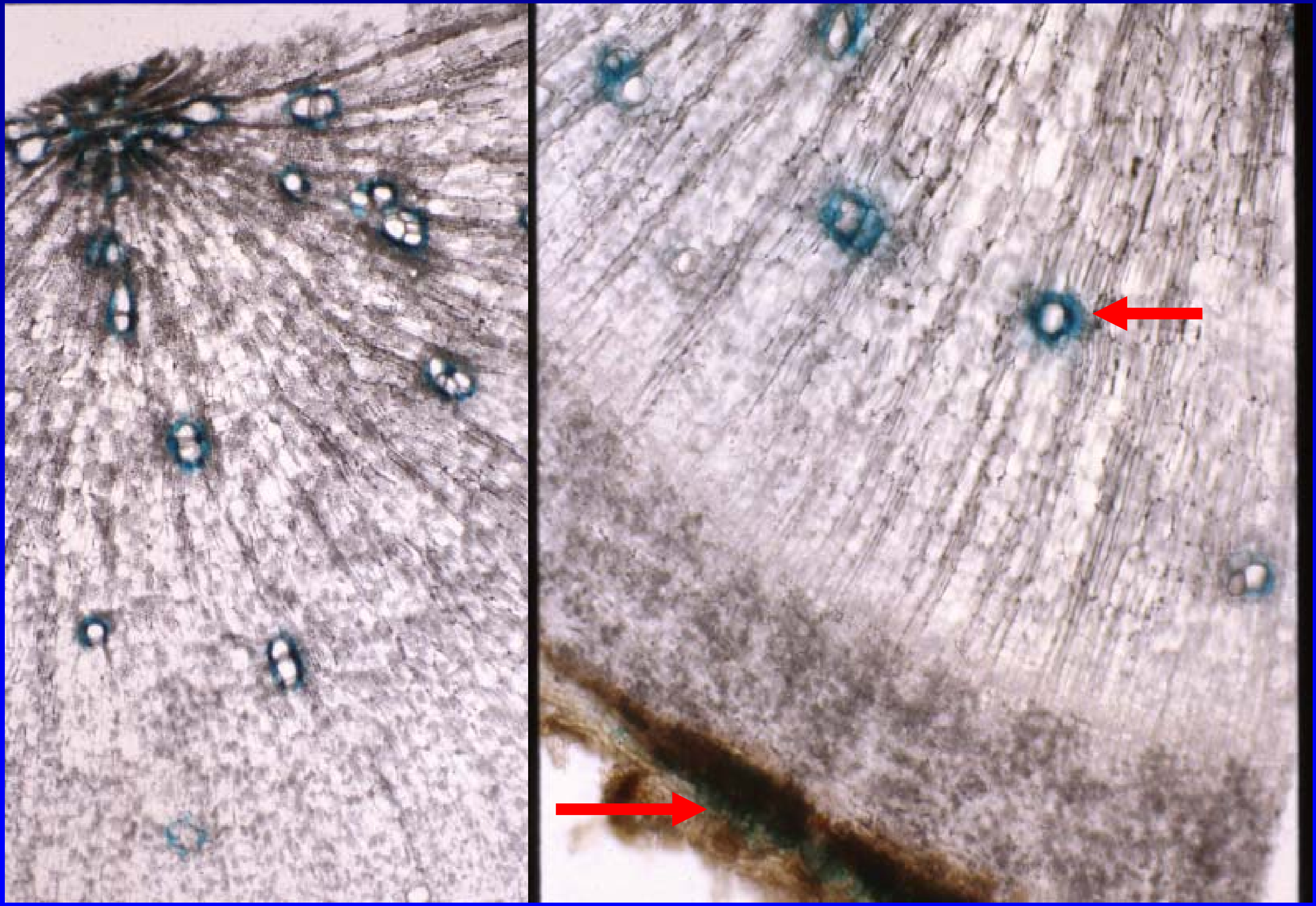




-840PAL-GUS Activity in Stem Xylem Tyloses



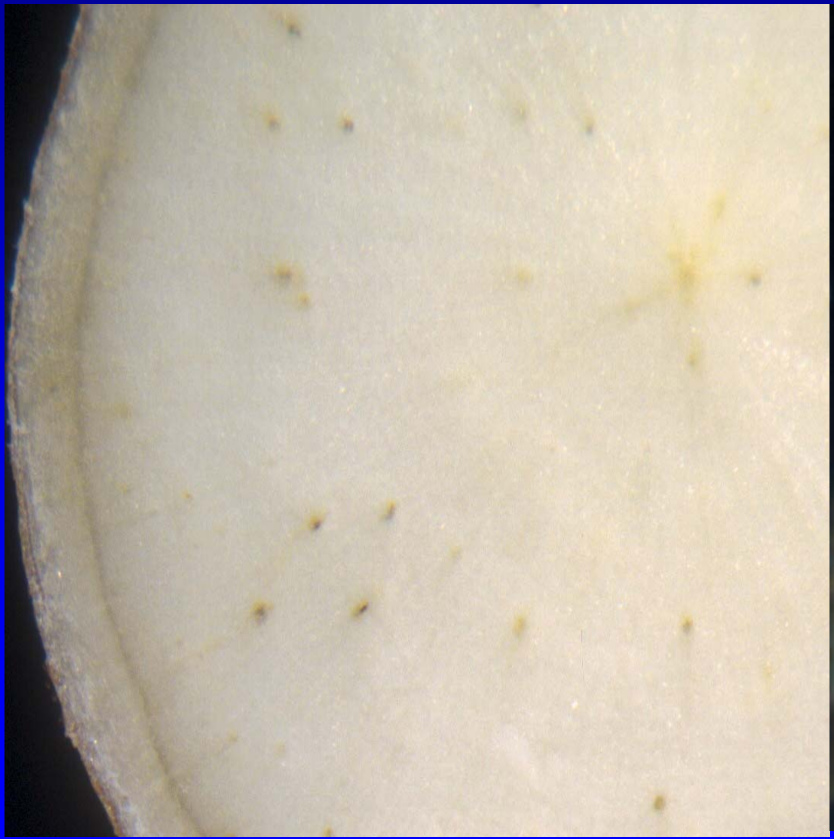
-840PAL-GUS Activity in Storage Roots



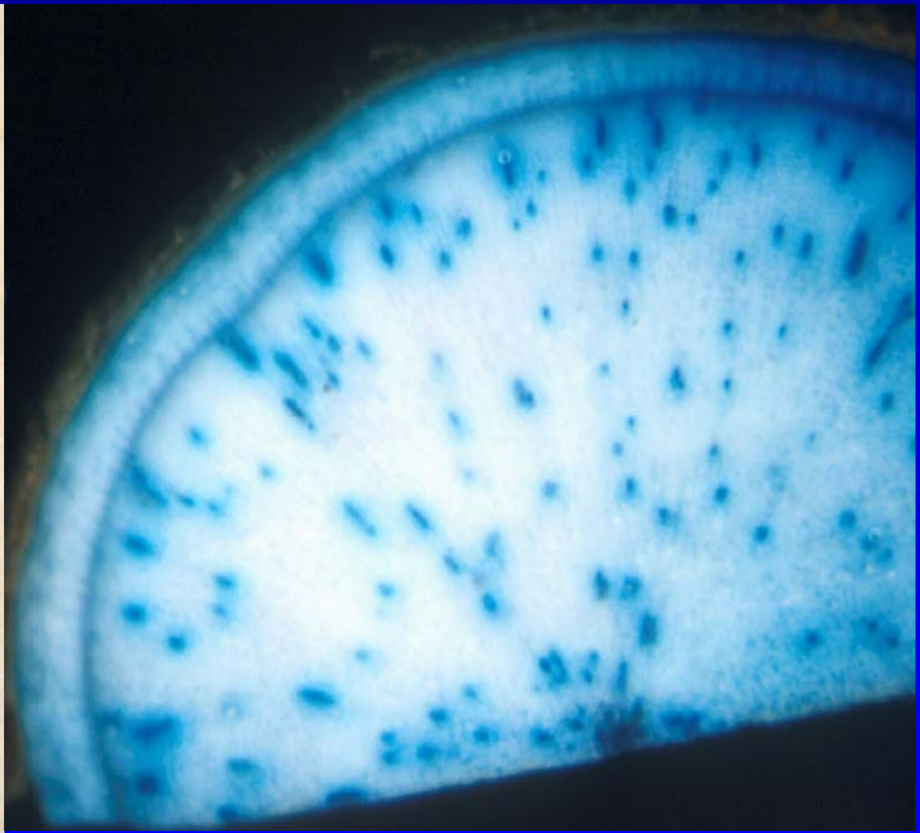
GUS activity confined to vascular tissue & below cortex

-840PAL-GUS Activity in Storage Roots During PPD:

Transverse section



PPD symptoms

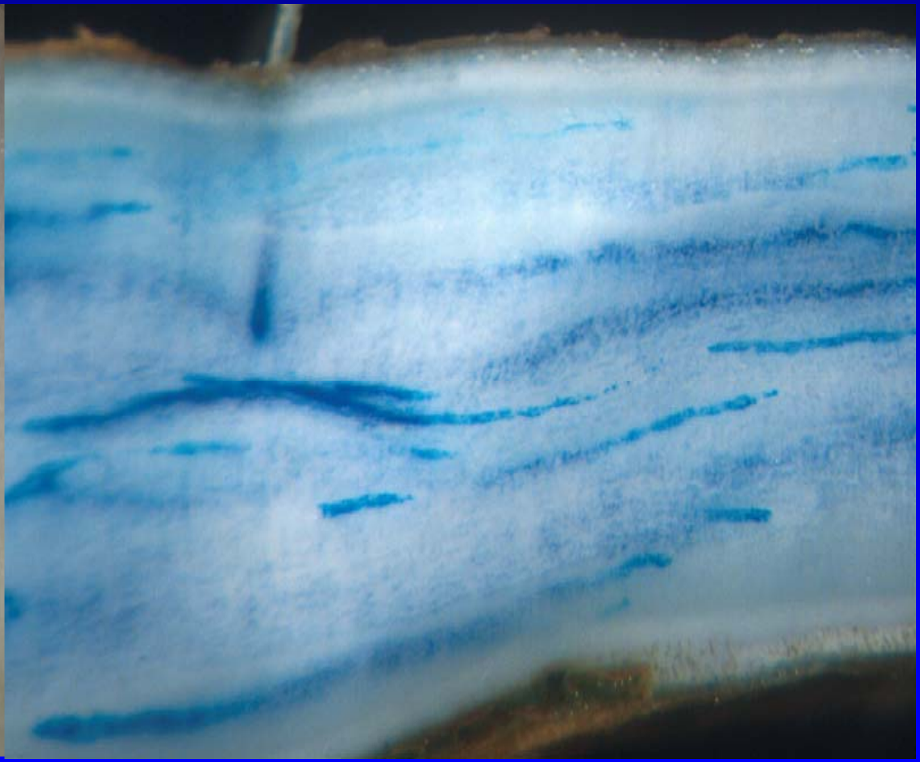


GUS expression

-840PAL-GUS Activity in Storage Roots During PPD:

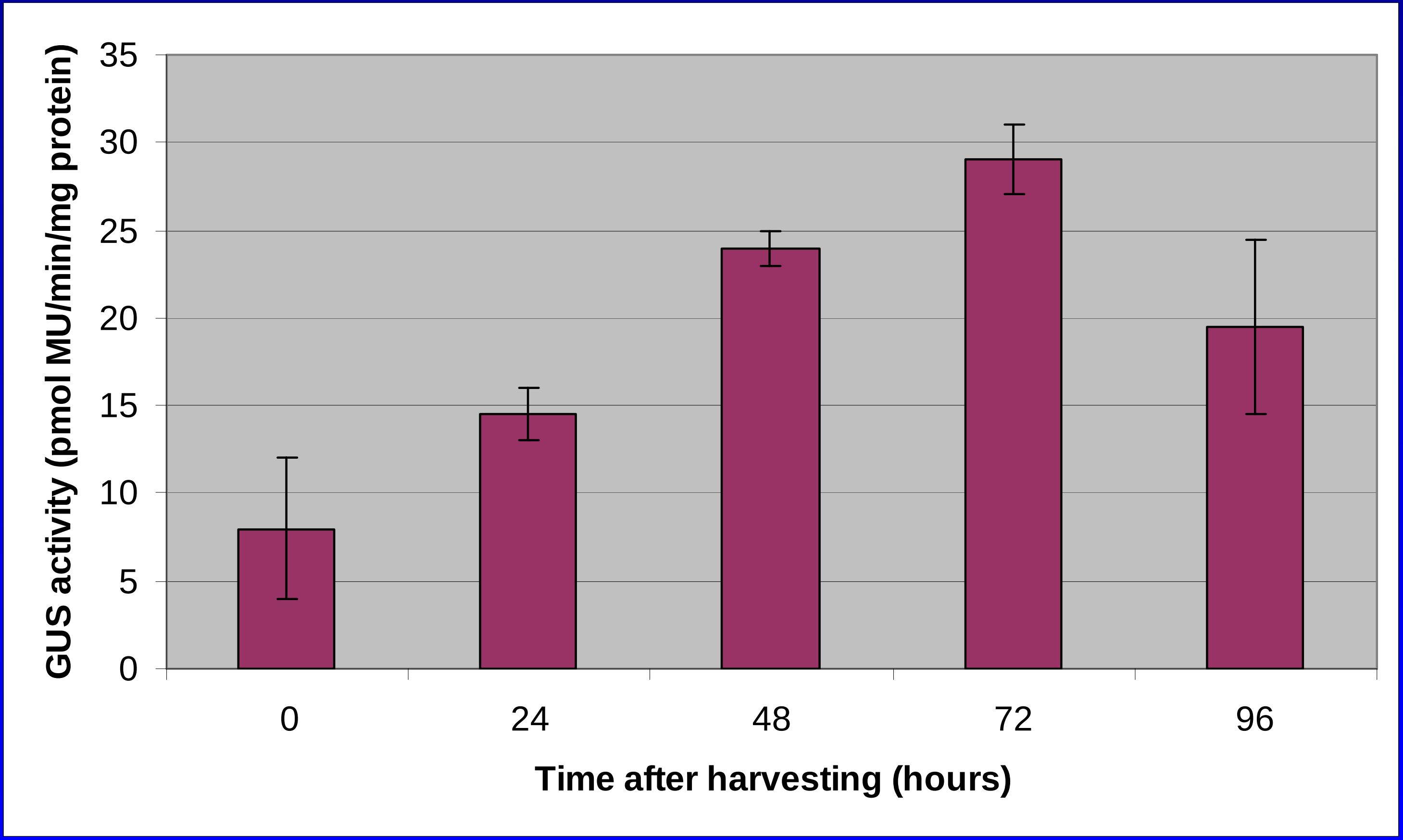
Longitudinal section

PPD symptoms



GUS expression

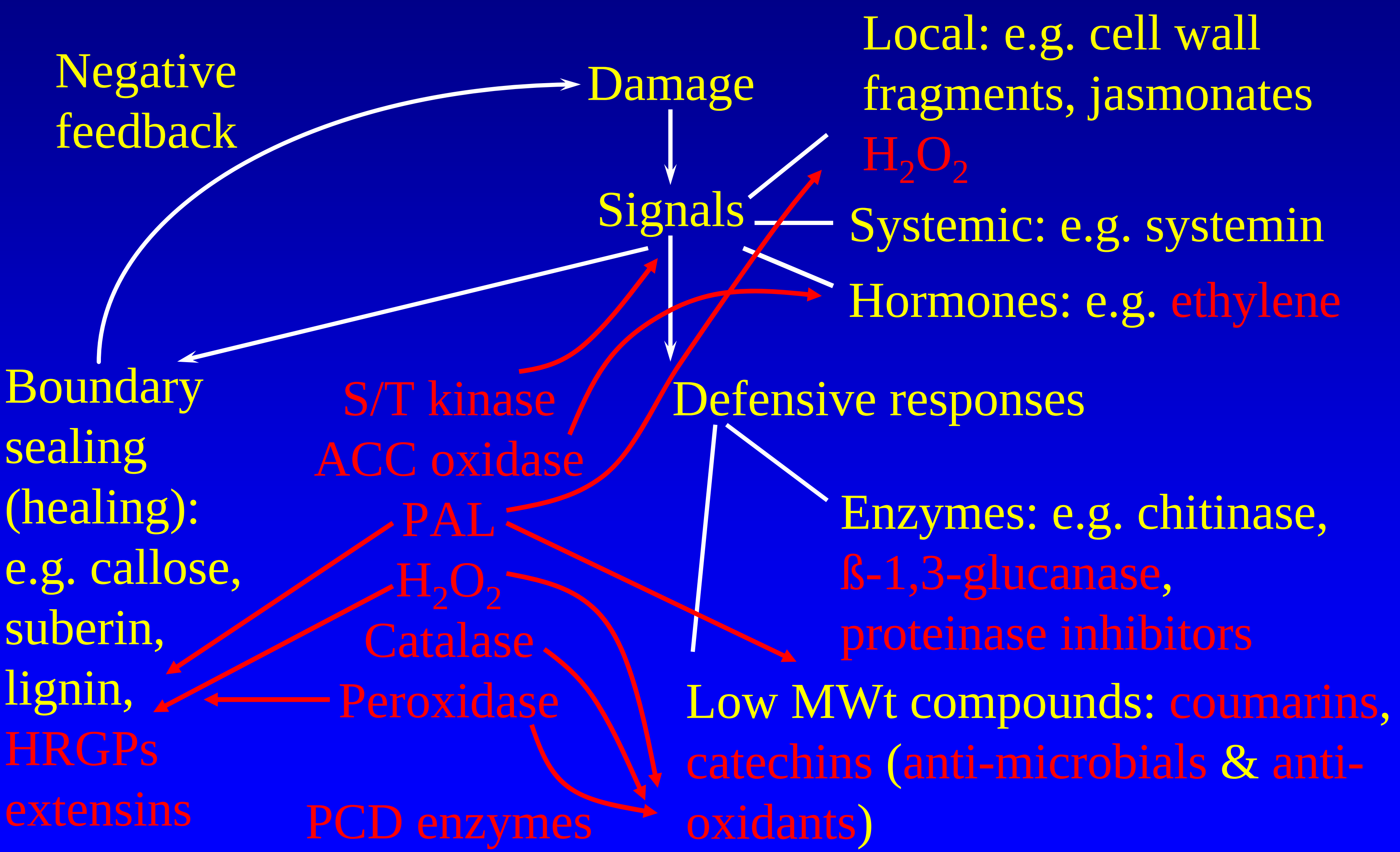
-840PAL-GUS Activity in Transgenic Roots During PPD



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

Cassava PPD Responses



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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