Molecular approaches to overcoming post-harvest deterioration in cassava

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Overview of talk:

- Post-harvest physiological deterioration (PPD):
  - what is it?
  - socio-economic impacts
  - current approaches to control
- What happens during PPD:
  - physiological, biochemical & molecular insights
- Reactive oxygen species:
  - central to the PPD response?
- Senescence:
  - is PPD an apoptotic event?
- Conclusions
- Hypothesis

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 & consumption

Impact of PPD today:

- In traditional village societies PPD is a manageable problem as roots are left in the ground until required
- However, changing societies, increasing urbanisation & entry of rural societies into the cash economy, extend distances & times between farm & market
- Therefore, today PPD impacts on farmer, consumer & processor alike

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, often imported
- Non-uniform input to processing & industry
  - reduces quality & competitiveness of cassava products
- Controlling PPD would turn cassava into a modern crop, unlocking its full potential for Africa and the world

Approaches to controlling PPD:

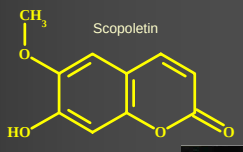
- Current
  - leaving roots in the ground – *of local use only*
  - processing – *OK on small scale or for low quality products*
  - waxing / freezing – *for high price markets only*
- Conventional breeding - *problems*
  - *high heterozygosity*
  - *correlation between high dry matter & PPD*
  - *genotype X environment interactions*
- Biotechnology
  - increase understanding of PPD
  - marker assisted selection (MAS) – *as yet untried*
  - genetic modification – *as yet untried*

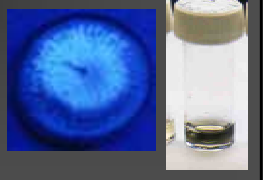
What is happening during PPD?

- Wounding due to harvesting causes:
  - water loss
  - ingress of air (oxygen)
- Visible symptoms:
  - blue/black vascular streaking
  - fluorescence under UV light
  - symptoms spread from wound sites throughout the root via the vascular system

Accumulation of secondary metabolites:

- Diterpenes
- Hydroxycoumarins
- Flavan-3-ols
- Function?
  - antimicrobials
  - antioxidants
  - other?
- E.g. scopoletin (hydroxycoumarin)
  - responsible for much of fluorescence
  - peroxidase-mediated reaction with H_2O_2 to give blue/black precipitate
  - addition of exogenous scopoletin accelerates PPD response

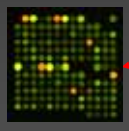




Phytochem. 27:3769 *J. Agric. Food Chem.* **48**:5522 *Ann. Bot.* **86**:1153 *J. Exp. Bot.* **36**:783

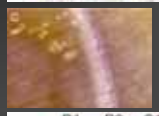
PPD is an active process:

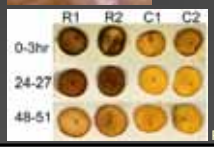
- *De novo* synthesis of proteins – *in vivo* labelling
- Changes in gene expression - Northern hybridisations
- E.g. 11,000+ cDNA microarray showed significant changes in gene expression of 72 genes during PPD
- Including up-regulation of genes involved in:
  - reactive oxygen species (ROS) turnover
  - signal transduction or perception
  - senescence
  - stress responses
  - ion, water or metabolite transport
  - cell-wall metabolism & remodelling
  - biosynthesis and metabolism
  - transcription and translation



Are reactive oxygen species (ROS) central to PPD?

- Superoxide detected within 15 min of harvest, declining by 8 – 10 hrs
- Superoxide detected histochemically in root parenchyma with intense staining in cambium just below cortex
- H_2O_2 detected within 3 hrs, peaking at 24 hrs.





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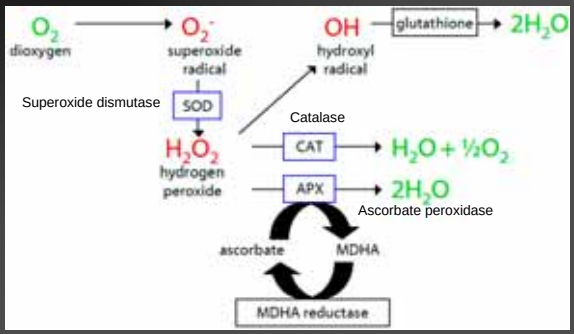
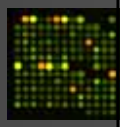
The multiple functions of ROS:

- As signalling molecules
- During development. E.g.:
  - lignin biosynthesis
  - insolubilisation of hydroxyproline-rich glycoproteins (HRGPs) in cell walls
- In cell death. E.g.:
  - cellular damage – photo-oxidative damage, herbicides
  - programmed cell death - hypersensitive response (HR)
- Function depends largely on species, concentration & localisation
- Plants possess anti-oxidant defence systems

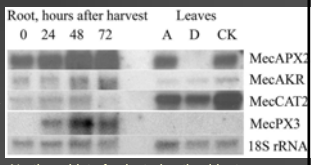
Plant anti-oxidant defences:



Genes involved in ROS turnover during PPD:



- cDNA microarrays showed significant increase in expression of:
  - catalases – MecCAT1 & MecCAT2
  - ascorbate peroxidase – MecAPX2
  - secretory peroxidase – MecPX3
  - thioredoxin peroxidase
  - thioredoxin-like protein
  - metallothionein
  - quinone-oxidoreductase
  - aldo/keto reductase – MecAKR
  - early light induced protein



Northern blot of selected anti-oxidant genes

Anti-oxidant defence during PPD:

- Increased activity/expression of:
  - catalase - root parenchyma
  - peroxidase – epidermis, cortex and xylem at harvest, during PPD more extensive and spreads through root parenchyma, esp. xylem parenchyma
  - CuZn superoxide dismutase remains at low levels during PPD
- Accumulation of ROS-scavenging secondary metabolites during PPD, including scopoletin which forms blue/black precipitate with H_2O_2 and peroxidase
- Conclude that:
  - ROS are generated during PPD
  - anti-oxidant defence enzymes & compounds are synthesised
  - but response is not sufficient to contain ROS-induced damage & restore homeostasis
  - therefore, ROS & their modulation are central to PPD

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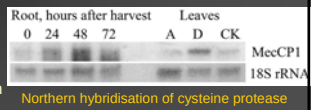
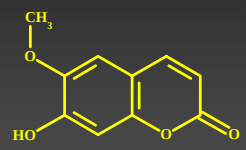
Is PPD a senescence phenomenon?

- Senescence / programmed cell death (PCD) / apoptosis
  - it is an active process involving the controlled death of cells
  - i.e. there are apoptotic genes that trigger cell death
  - and anti-apoptotic genes that prevent cell death
- cDNA microarrays show
  - up-regulation of:
    - cysteine protease – proteolytic enzyme, mediator of signal transduction &/or effector of PCD
    - class IV chitinase – hydrolysis of chitin, role during PCD
  - down-regulation of:
    - cystatin-like protein – inhibitor of cysteine protease activity
    - translationally controlled tumour protein – anti-apoptotic in mammalian systems; e.g. human cancer cells



Northern hybridisation of cysteine protease

Remember scopoletin?



- Accumulates during PPD
- Exogenous application accelerates PPD
- Scopoletin induces apoptosis in cancer cells
- Does it play a similar role in cassava PPD?

Life Sci. **77**:824; Planta Med. **72**:862

Conclusions:

- ROS & the reactions in which they are involved are central to PPD
  - but are they a cause or a consequence?
  - or do they play several roles?
- Senescence / PCD / apoptosis is also involved in PPD
  - but again: cause or consequence?
- Are we seeing a ROS-mediated PCD?

Tentative hypothesis:

- Initial oxidative burst of superoxide functions as a signal
- Which triggers an apoptotic response
- Of which the accumulation of ROS and scopoletin are major parts
- Leading to oxidative events & ultimately to cell death
- This hypothesis is testable

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