# TOWARDS A MODEL FOR POST-HARVEST PHYSIOLOGICAL DETERIORATION (PPD) OF THE CASSAVA STORAGE ROOT

Reilly K [1], Gomez-Vasquez R [1], Han Y [3], Cortes D. F . [2], Gbadegesin A.M. [1] and Beeching J.R. [1]

[1] University of Bath, Bath, BA2 7AY, U.K. [2] CIAT, Cali, Colombia. [3] Plant Science Division, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, U.K.

## INTRODUCTION

Post - harvest physiological deterioration of cassava storage roots has been described since the 1920's and seriously hinder marketability and storage of the crop. It can occur within 24 – 48 hours after harvest, and is initially observed as blue / black streaking of the xylem tissues. During the 1970's it was demonstrated that such primary deterioration occurred *via* endogenous processes within the root rather than *via* microbial deterioration. It is an active process which can be inhibited by cycloheximide, as well as by low $O_2$ treatments, high or low temperature storage and pre-harvest pruning. Several authors have suggested that oxidative stress and wound defence responses may play a role in the deterioration process [1, 2]. We have isolated and sequenced a number of target cDNA clones from a root PPD cDNA library, and have used molecular and biochemical approaches to characterize processes which occur during PPD.

## RESULTS

### • Cassava Storage Roots Exhibit a Wound Induced Oxidative Burst

Production of superoxide anion ($O_2^-$) occurred within 15 minutes of root injury, and declined to low levels 6 to 10 hours later. Hydrogen peroxide ($H_2O_2$) was produced within 3 hours of injury, peaking 24 – 27 hours later before declining. Hydrogen peroxide was localised primarily to the packaging parenchyma surrounding the xylem tissues.


**Figure 1:** *In situ* detection of $O_2^-$ production in wounded cassava storage roots according to May *et al.* (1996) [3]. Superoxide production is indicated by a purple reaction product. ∇ = root 1 ▽ = root 2 ▯ = root 3.

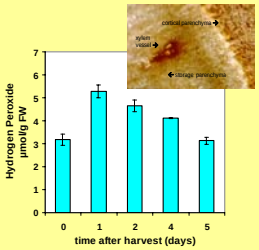


**Figure 2:** Measurement and localization (inset) of $H_2O_2$ production in injured storage roots. Hydrogen peroxide production is indicated by a brown reaction product.

### • Isoforms of Peroxidase and Catalase, but not Superoxide Dismutase are Up-Regulated

A range of root peroxidase isoforms were detected by IEF PAG, several of which showed up-regulation during PPD. A cationic peroxidase (MecPX1) isolated from a root cDNA library showed strong up-regulation. Four root superoxide dismutase isoforms were detected and showed little change in expression. Likewise a cDNA probe MecCuZnSOD showed little change. However, a root catalase cDNA transcript (MecCAT1) was up-regulated during PPD.

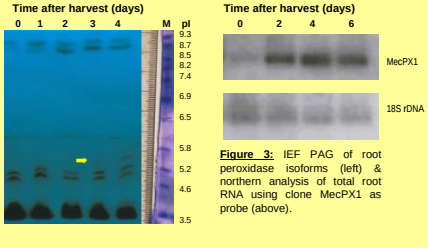


**Figure 3:** IEF PAG of root peroxidase isoforms (left) & northern analysis of total root RNA using clone MecPX1 as probe (above).

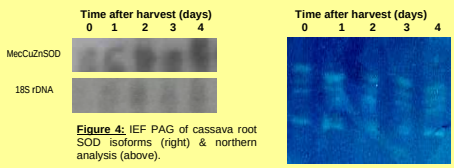


**Figure 4:** IEF PAG of cassava root SOD isoforms (right) & northern analysis (above).

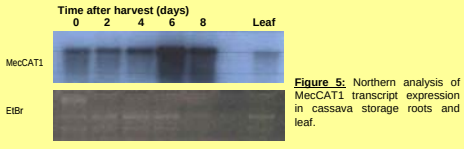


**Figure 5:** Northern analysis of MecCAT1 transcript expression in cassava storage roots and leaf.

### • Defence and Senescence Related Genes are Up-Regulated

Transcript expression of defence related genes including PAL (MecPAL1) – a key entry point to phenylpropanoid metabolism; a hydroxyproline rich glycoprotein (MecHRGP); and an aspartic protease (MecASP1) and cysteine protease inhibitor (MecCPI1) which have been implicated in programmed cell death responses in other plants; were also up-regulated during PPD.

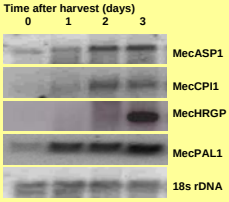


**Figure 6:** Northern blot analysis of defence and putative senescence associated genes using cassava cDNA clones isolated from a root cDNA library.

### • Possible Signalling Components?

To date Northern blot analysis has indicated that MecCAT1, MecPX1 and MecPAL1 are up-regulated by ethylene treatment. Ethylene is produced within 6 hours following wounding of the cassava storage root and has been proposed to modulate the response [4]. MecPAL1 is up-regulated by $H_2O_2$; and catalase MecCAT1 is up-regulated in roots from pruned plants. Such plants show reduced susceptibility to PPD.

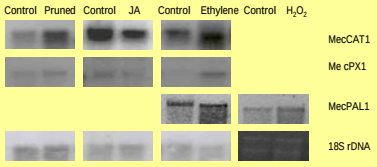


**Figure 7:** Northern analysis using MecPX1, MecCAT1 and MecPAL1 hybridisation probes. Pruning treatment = plants were pruned 2 weeks prior to harvest. Ethylene treatment = 0.02% ethephon for 24 hours. $H_2O_2$ treatment = 500mM for 24 hours. JA treatment = 500µm MJA for 24 hours.

### • A Putative Model for Processes Occurring during PPD

**Figure 8** Schematic model representing processes that may be occurring during post-harvest physiological deterioration of the cassava storage root. Genes or proteins known to be expressed during PPD are indicated in blue text. Those known to be up-regulated are indicated in orange boxes, those known to be down regulated are indicated in blue boxes. Possible interactions between components in the model are indicated by pink arrows. Unknown components are indicated with a question mark. Possible points where treatments known to inhibit PPD may act are indicated in red. Of the secondary phenolic compounds known to accumulate in the root after injury, those which are capable of acting as antioxidants are marked with an asterisk. Genes which are expressed in the later stages after injury (after 48 hours) are indicated towards the base of the figure on a dark grey background.

## CONCLUSIONS

• A rapid transient production of $O^{2-}$ and $H_2O_2$ occurs after injury of cassava storage roots. ***A possible signalling role?***

• $H_2O_2$ is initially primarily localised to the xylem tissues. ***Implications of this localisation for PPD?***

• Genes associated with oxidative stress, defence and senescence are up-regulated during PPD. Ethylene and $H_2O_2$ may play a role in modulating gene expression.

• Catalase is strongly up-regulated in pruned (less susceptible) plants. ***A possible defensive role via scavenging of $H_2O_2$?***

## ACKNOWLEGEMENTS

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