Biochemistry of Post-harvest Deterioration in Cassava Root Tubers

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Abstract

Cassava is one of the most important root crops in the world and provides food for more than 500 million people, especially in the lowland tropics. Within 48 hours post harvest the tuberous root undergoes biochemical changes, known as post-harvest physiological deterioration (PPD). Despite the economic importance of the crop, understanding of PPD is still limited. The aim of this project was to broaden knowledge on the cytology, histology and biochemistry of this process. The histological investigation revealed the occurrence of tyloses and occlusions in the vessels but no wound healing in the harvested tubers. Many secondary metabolites accumulate during the storage process. However, most of them could be correlated with the onset of microbial decay after about six days of storage. Only the accumulation of scopoletin, scopolin and hydrogen peroxide was found to occur during PPD.

Keywords: Cassava, hydrogen peroxide, Manihot esculenta, post-harvest deterioration, secondary metabolites, wound response.

Introduction

Cassava (*Manihot esculenta* Crantz), a member of the Euphorbiaceae, is a perennial shrub whose cultivation has spread throughout the lowland tropics from Latin America to Africa and Asia. It is grown principally for its large starchy root tuber. The roots provide the staple food for over 500 million people and in 1997 the world production was over 166 million tonnes. Cassava has the ability to grow on impoverished and marginal soils with the minimum of technological input. As a result it is often the food of the poor and can play a major role as a famine reserve crop.

However, it is increasingly being grown and processed as animal feed for export, or processed industrially into a range of products including starch (tapioca).

Within 48 hours after harvesting the root tubers of cassava suffer a wound-response and undergo biochemical changes, known as post-harvest physiological deterioration (PPD). This process renders them unpalatable and unmarketable. With increasing distances between farmers and markets due to increased urbanisation, PPD has become a major constraint to the development of cassava to farmers, processors and consumers. The process results in estimated losses ranging from 5 to 25% of the harvested roots. Therefore, research directed towards introducing resistance to PPD or delaying the response is considered a priority by international bodies such as the FAO and the cassava Biotechnology Network (CBN) (Wenham, 1995).

A multidisciplinary approach, combining general botanical, histological, biochemical, eco-physiological and molecular techniques is needed to broaden our understanding of PPD. This work will present results from our histological and biochemical investigations on PPD.

Materials and Methods

Cassava root tubers were either supplied by the Centro International de Agricultura Tropical (CIAT), Colombia or grown in the tropical greenhouses in Bath, UK. The identification and quantification of secondary metabolites as well as the detection and quantification of hydrogen peroxide during storage of the tubers were performed according to Buschmann et al. (2000a; 2000b). The histochemical localization of H_2O_2 was done by using the method described by Repka (1999). Light, fluorescence, scanning- and transmission-electron microscopy were performed according to Buschmann et al. (2000a, 2000c).

Results and Discussion

PPD is an abiotic stress response of the cassava roots (Beeching et al., 1999) and not due to microbial action (Averre, 1967), though microbial decay can set in subsequently. The first signs of PPD are seen as blue fluorescence under UV light (figure 2a) and a blue-black streaking of the vascular tissues (figure 1b). Subsequently the deterioration spreads to the adjacent storage parenchyma and the stored starch undergoes structural changes (Plumbley and Rickard, 1991). PPD is accompanied by an increase in respiration and ethylene biosynthesis, and the production of phenolic, terpenoid and other low molecular weight compounds (Uritani, 1998).

The activities of several enzymes, including phenylalanine ammonia-lyase, catalase, peroxidase and polyphenol oxidase increase during PPD (Campos and Carvalho, 1990). These compounds, enzymes and genes observed in cassava roots are all present and expressed during normal wound responses in other plant systems (Baron and Zambryski, 1995). Plant wound responses involve the production of signalling components that initiate the wound response, the preparation of the plant for potential microbial invasion, and wound repair that is followed by the inhibition of signals. These aspects of wound response are all present in the cassava root. However, the wound repair and the resultant down-modulation of the signals are incomplete, which results in a continuation of wound responses that spread throughout the cassava root tuber and are observed as PPD. Cassava cultivars exhibit ranges of PPD responses, indicating that there is a genetic component in the response and the chance for breeders and molecular biologists to use the genetic resource and to improve the crop (Wenham, 1995).

Histological investigations revealed a high number of occlusions and tyloses in the xylem vessels after 24 to 48 hours (figure 1c,d). The blue fluorescence of the inner tissues of the root tuber is due to the accumulation of fluorescent compounds in the apoplast of the xylem and subsequently spreading into the apoplast and cells of the parenchyma. The fluorescent compounds could be identified as hydroxycoumarins (Buschmann et al. 2000a).

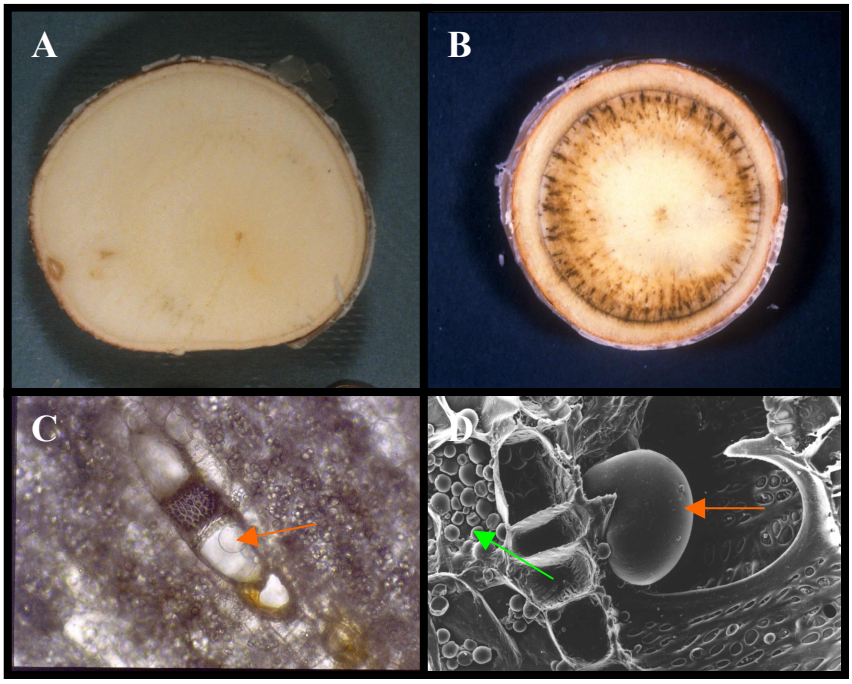

Figure 1: Cross sections of a cassava (MCOL 22) root tuber. A. Non-deteriorated fresh cut tuber. B. Tuber after four to five days. C. Light microscopic image (cross section, 400x) of a deteriorated tuber showing brown occlusions in the vessels and tyloses (orange arrow). D. Scanning electron-microscopic image of a deteriorated tuber; Xylem vessels with tyloses (orange arrow) and parenchymatic cells with starch granules (green arrow).

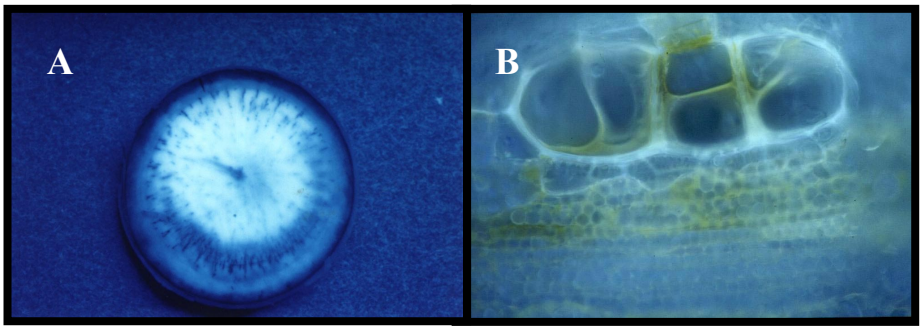

Figure 2: Cross sections of a deteriorated cassava root tuber (MCOL 22). A. Bright blue fluorescence under UV light (366nm). B. Fluorescence microscopic image showing fluorescence in the apoplast of the vessels and parenchyma.

The detailed analysis of secondary metabolites and enzyme activities revealed a more detailed view of the biochemical processes involved in PPD (Buschmann et al., 2000a; b). The authors showed that many of the compounds (e.g. (+)-gallocatechin; figure 3) that had been described in

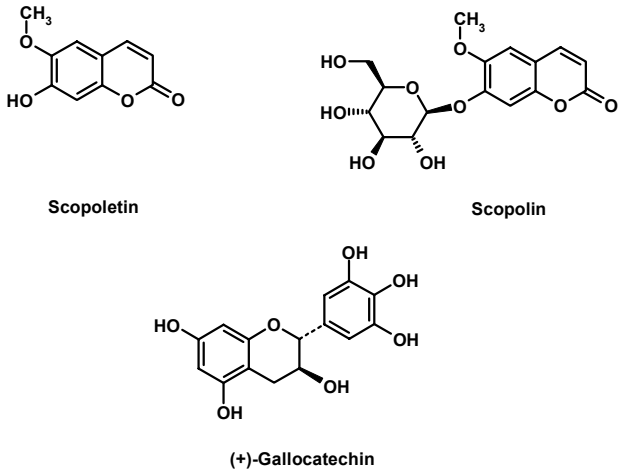

Figure 3: Structures of phenolic compounds identified from cassava root tubers.

the literature as related to PPD proved to be more related to responses to microbial decay (Buschmann et al., 2000b). Only the accumulation of scopoletin (figure 4) and hydrogen peroxide (figure 5) as well as the increasing activities of enzymes (e.g. peroxidase) could be related to the physiological deterioration process. As shown in figure 2b and 5b scopoletin and H_2O_2 accumulate in the same compartments. We postulate the existence of a scopoletin specific peroxidase in the apoplast, similar to the enzyme described by Edwards et al. (1997), that produces the black-blue precipitate that we recognise as vascular streaking during PPD (figure 1b).

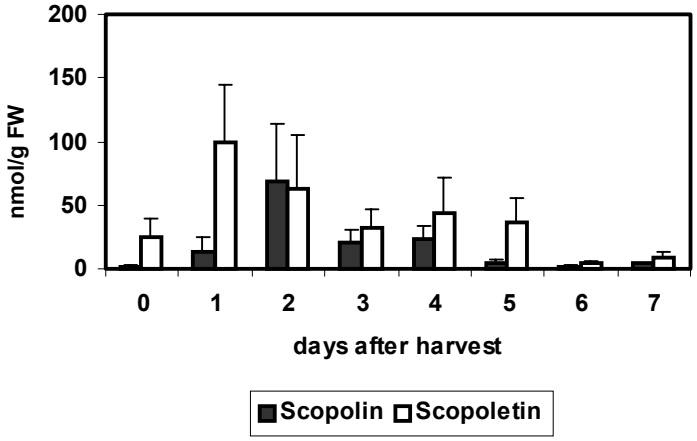

Figure 4: Quantification of scopolin and scopoletin accumulation in cassava root (cultivar MCOL 22) slices that had been stored over a time period of seven days. The columns represent the mean of four different root tubers and taken from different plants (mean ± SD).

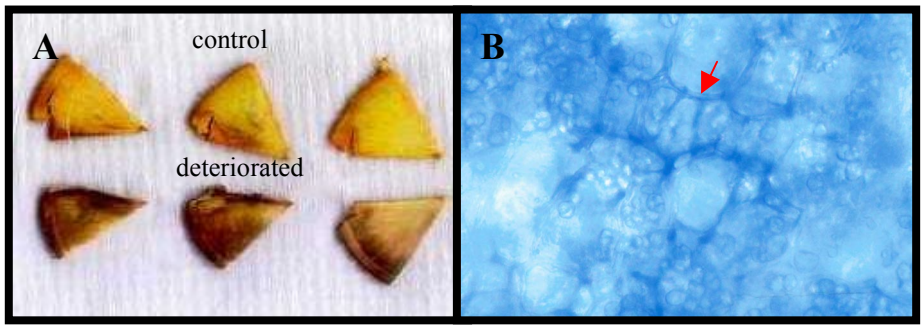

Figure 5: Detection and localization of hydrogen-peroxide in deteriorated cassava root tubers. A. Detection of H_2O_2 after infiltration with DAB. B. Localization of H_2O_2 in the apoplast (arrow) of parenchymatic cells by means of KI/starch stain.

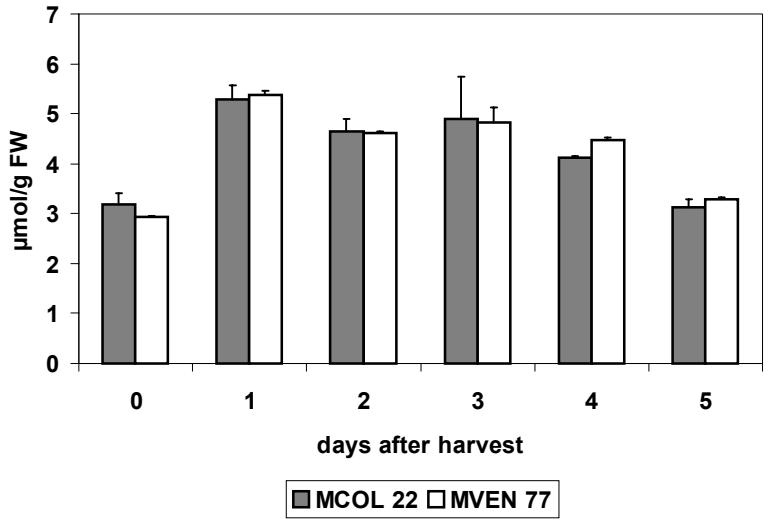

Figure 6: Quantification of free hydrogen-peroxide in cassava root slices of two different cultivars (MCOL 22 [highly susceptible] and MVEN 77 [low susceptible]) and over a storage period of five days. Each column represents the mean of three different roots per cultivar taken from different plants (mean ± SD).

It seems that the differences in PPD responses between the cultivars are not due to the accumulation time but to the amount of synthesised scopoletin (Buschmann et al. 2000a). However, the biosynthesis and regulation of scopoletin and reactive oxygen species in cassava roots undergoing PPD is still unknown and needs to be investigated. As well there is the need for a comparison of PPD responses of different susceptible cultivars as well as a more detailed study of the histological and biochemical processes directly at the wound surface.

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