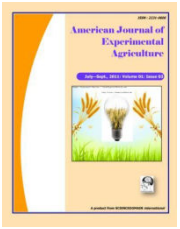

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Insights into the Physiological, Biochemical and Molecular Basis of Postharvest Deterioration in Cassava (*Manihot esculenta*) Roots

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Review Article

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ABSTRACT

Due to its favorable agronomic traits, tolerance to abiotic stresses and adverse environments, cassava is the most important source of dietary carbohydrates for 750 million people around the world, and is produced mainly by subsistence farmers in marginally agricultural land. Physiological postharvest deterioration (PPD) of cassava roots is an endogenous and complex process that restricts their storage potential to only a few days after harvest. This physiological phenomenon is one of the main constraints in cassava agriculture with an enormous impact on the cassava market chain. It is estimated that losses due to PPD in cassava production in Latin America and the Caribbean and in Asia reach 10% and 8%, respectively, whereas in Africa they reach 29%. Several years of research have been accumulating evidence to consider PPD as a wounding stress deficient process involving changes in enzymatic activity and oxidative stress. The primary symptoms, the development of dark bluish or brownish radial veins or streaks near xylem vessels of the root pith tissue, appear within 2-3 days after harvest and spread to the neighboring parenchyma tissues producing a more general browning discoloration throughout the root. Secondary post-harvest deterioration, often appears when the roots suffer moderate to severe damage at harvest and is mediated by a wide range of pathogenic microorganisms, Several strategies have been proposed to overcome the problem, but each alternative has its limitations due to the variable results, lack of objective and systematic methodology for PPD evaluation, applications not conducive for use at farmer-level, limited genetic variability or absence of genetic and biochemical information. The present review examines the socioeconomic impact of PPD, the physiological, biochemical and molecular processes occurring in the root during PPD, as well as the current and future alternatives to overcome the problem.

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Keywords: Cassava; Manihot; post-harvest deterioration; socioeconomic; Africa;

1. CASSAVA

Cassava (*Manihot esculenta* Crantz) is the most important source of dietary carbohydrates for 750 million people around the world, with its starchy root being the main harvested organ (Allen, 2002; Gleadow *et al*., 2009; Burns *et al*., 2011). Cassava is one of the few cultigens in the family Euphorbiaceae. The genus *Manihot* comprises 98 species spread throughout the Neotropics, 17 of which are native to North America and the others to South America (Rogers and Appan, 1973). There are more than 10,000 varieties of cassava with each having its own distinctive plant form, genetic structure, and adaptability to different environments. Cassava is a perennial, woody shrub that grows from about 1 m to about 3 m tall with the woody stems being topped by palmate, dark green or purplish leaves.

Due to its favorable agronomic traits, tolerance to abiotic stresses and adverse environments, cassava is produced mainly by small farmers in marginally agricultural areas. World cassava production has increased in recent years and is expected to increase further due primarily to both higher demand as human food and its value as raw material for industrial proposes, particularly in Africa (García and Dale, 1999; Nyerhovmo, 2004). However, the expansion of this crop is drastically restricted by the storage potential of the roots, which is limited to only a few days (Wenham, 1995). The short storage life of cassava roots is directly linked to an endogenous physiological process known as physiological postharvest deterioration (PPD), which is considered to be a complex procedure linked to enzymatic stress response to wounding (Beeching *et al*., 2002). This review examines the nature and symptoms of PPD, physiology, biochemical and gene expression changes occurring during PPD, socioeconomic impact of PPD, available methods to assess PPD as well as current strategies available to reduce PPD.

2. PHYSIOLOGICAL POSTHARVEST DETERIORATION (PPD)

PPD is the main cause of loss of root acceptability after harvest. This phenomenon has also been called primary postharvest deterioration or more generally vascular streaking, due the development of dark bluish or brownish radial veins or streaks near xylem vessels of the root pith tissue after harvest (Figure 1). These symptoms appear within 2-3 days after harvest and spread to the neighboring parenchyma tissues producing a more general browning discoloration throughout the root. Within this time period the taste of the root has also been altered to become more bitter (Booth, 1975; Wheatley, 1982; Plumbley and Richard, 1991).

Averre (1967) introduced the term vascular streaking or vascular discoloration as alternatives for describing PPD. Vascular streaking has two types which appear to occur independently (Kawano and Rojanaridpiched, 1983). The first type, VS-I (vascular streaking-I), is characterized by blue, black or brown streaks found as a ring around the inner part of the pith and is a physiological phenomenon independent of microbial activity (Data *et al*., 1982; Taniguchi *et al*., 1984). This first type tends to appear when the roots are not damaged by the harvest process (Kawano and Rojanaridpiched, 1983).The second type, VS-II (vascular streaking-II), is a blue-black pigmentation of vessels which commonly appears on or adjacent to microbial infected areas and is observed in the initial stages of microbial decay. VS II, also known as secondary post-harvest deterioration, often appears when the roots suffer moderate to severe damage at harvest (Kawano and Rojanaridpiched,

1983; Taniguchi *et al*., 1984). Secondary deterioration is mediated by a wide range of pathogenic microorganisms, and generally takes place when the roots have already become unacceptable due to primary deterioration (Booth, 1975).

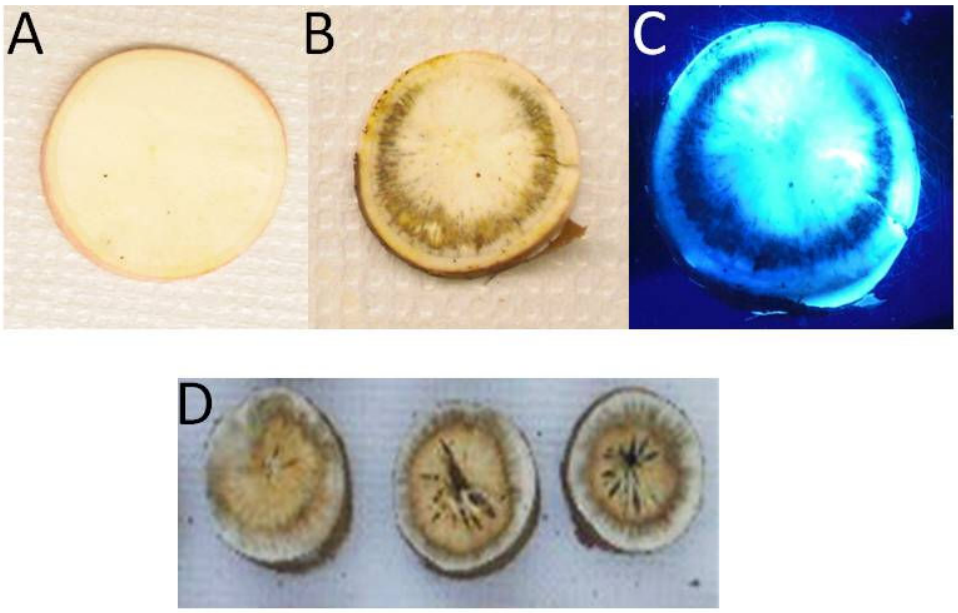

Figure 1: Postharvest deterioration in cassava root cross sections. A) No symptoms (1 day after harvest). B) Physiological postharvest deterioration (5days after harvest). C) Physiological postharvest deterioration (5days after harvest) under ultraviolet light. D) Secondary deterioration (15 days after harvest).

3. SOCIOECONOMIC IMPACT OF PPD

Cassava roots can be stored in the ground without harvesting for an extended period of time, making them a very secure food against famine (Cardozo and Souza, 1999). In traditional communities PPD is not a problem since cassava roots are harvested and consumed only as required. However, the growth of urban areas in developing countries has increased the distances between producers (who usually lack access to adequate postharvest treatments) and consumers as well as processors (Wenham, 1995; Reilly *et al*., 2004). PPD restricts the storage potential to only a few days, reducing the development of cassava as a commercial crop. Small-scale farmers suffer economic losses due to PPD by reduction in root quality while large-scale processors are affected by the risk involved in the reliable supply of cassava as a raw material (Wenham, 1995).

Reliable information is scarce to compute the actual losses worldwide due to PPD. Furthermore, it is not clear at which stage of the market chain (producer-trader-processor or consumer) the losses occur (Wenham, 1995). Available data often do not differentiate between losses in fresh roots and loss of processed products. It is estimated that postharvest losses in cassava production in Latin America and the Caribbean and in Asia reach 10% and 8%, respectively, whereas in Africa they reach 29% (FAO, 2000). In addition in some stages of the market chain the economic losses due to price discount can reach up 90% (Westby, 2002). The FAO reported that increasing the storage life of cassava roots to a

minimum of two weeks could have a substantial effect in on cassava utilization and solve an estimated 90% of the deterioration constraints associated with current cassava marketing and utilization practices (Oirschot *et al*., 2000). In Nigeria alone, the highest cassava producer worldwide, delaying of PPD for several weeks would reduce the economic losses by US$2.9 billion (Rudi *et al*., 2011).

4. FACTORS AFFECTING PPD IN CASSAVA ROOTS

Mechanical damage which takes place during harvesting is a critical factor in the rapid occurrence of PPD in cassava roots. The activation and intensity of cassava PPD is closely related with mechanical damages (Booth, 1975; Aristizabal and Sánchez, 2007). The first symptoms of PPD appear in areas where the root peel has been damaged (or removed) or in the proximal and distal ends of the root, which are the most susceptible zones to physical damage. PPD usually starts at the site of injury, making internal tissues more exposed to oxidation and microbial attack by pathogens responsible for rotting. Other factors to be considered are root shape, root length, presence of peduncles (which minimizes the exposure of root tissues to oxygen and thus roots with peduncles suffer less PPD), peel adherence and texture, soil compaction, and harvesting method (manual or mechanical) (Booth, 1975; Wheatley *et al*., 1983; Diamante, 1986; Torres-Ramos, 2001; Aristizabal and Sánchez, 2007).

Comparative evaluations of PPD among different cassava varieties and within the same variety has revealed a considerable variation in degree of development and severity of PPD (Plumbley and Richard, 1991; Buschmann *et al*., 2000a; Aristizabal and Sánchez, 2007), implying that PPD response is under genetic control but is also influenced by environmental factors (Iglesias *et al.,* 1994). For example, Kawano and Rojanaridpiched (1983), upon evaluating eight cassava cultivars in three different locations in Colombia, found a highly significant interaction between location and season suggesting that PPD was affected by the environment the roots are grown in. Highly significant interaction between clone and location suggested that cultivar selection was very important; however, the selection had to be conducted at each specific location.

Correlation between PPD and a few agronomic traits have been suggested, such as a positive correlation between desirable traits, root dry matter content and high starch content, and the degree of PPD (Plumbley and Richard, 1991; Sánchez *et al*., 2006; Aristizabal and Sánchez, 2007).Comparatively Oirschot *et al*., (2000) found a negative correlation between PPD and sugar/starch ratio, in contrast with Wheatley and Gomez (1985), who found no correlation between PPD and starch content. Furthermore, the level of carotenes in the roots seems to be negatively correlated with the level of PPD. The low susceptibility may be due to the antioxidant properties of the carotenoids which quench reactive oxygen species (ROS). ROS are involved in oxidative stress leading to PPD in cassava roots (Aristizabal and Sanchez, 2006; Sánchez *et al*., 2006; Chavez *et al*., 2007).

5. EVALUATION OF PPD

The method of evaluating PPD in cassava roots is a key step in experiments leading to better understanding of events taking place during PPD, such as mapping QTLs associated to PPD tolerance (Cortes *et al*., 2002; Rosero *et al*., 2010), changes in root metabolism during PPD (Uritani *et al*., 1983) and gene expression analysis in roots after harvest (Han *et al*., 2001). Several strategies have been used to evaluate the susceptibility of cassava roots

to PPD (Figure 2);(1) use of subjective visual scales using entire roots (Booth, 1972; Pino, 1979); (2) subjective analysis of biochemical and physiological changes in transverse sections of roots under ultraviolet (UV) light; (3) the Philippine Root Crop Research and Training Center (PRCRTC) method based on the severity of discoloration during PPD (Uritani *et al*., 1983); and (4) use of root tissue blocks and then scoring transverse sections of the root by visual inspection of the ring pattern of zone B formed in the root pith during PPD (Wheatley, 1982). In the last method, the distal and proximal ends of the roots are removed accelerating the PPD process; however, Morante *et al*. (2010) suggest that the use of entire roots is more realistic to evaluate the tolerance, as it simulates the conditions in which farmers and processors keep the roots until processing. All these strategies are based on subjective evaluations and a need exists to implement an objective quantitative and systematic phenotypic evaluation of PPD (Cortes, 1999; Han *et al*., 2001; Estevao, 2007).

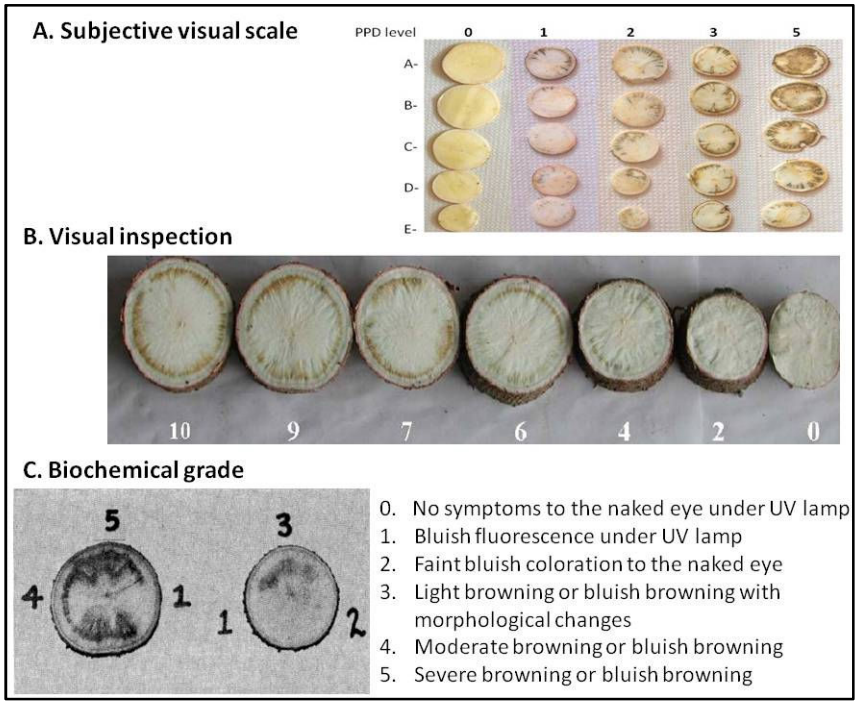

Figure 2: Methodologies to cassava PPD susceptibility assessment.
A) subjective visual scales in entire roots based in Booth (1972). B) visual inspection of the ring pattern of zone B (Wheatley, 1982); an entire root is cut at the proximal and distal ends to form a root tissue block of approximately 15 cm in length, the distal end is covered with a sheet of Parafilm and secured with a rubber band. The proximal end of the root is exposed during several days (3, 9 or 14). Seven slices 2 cm thick are made from the proximal to the distal end and a score corresponding of 0-10 (0=0%, 1=10%, 2=20%, etc.) is assigned to each slice. The mean PPD score for each block tissue is calculated by averaging the scores from seven slices. C) Numerical biochemical grade (Uritani et al., 1983).

Buschmann *et al*. (2000a) and Oirschot *et al*. (2000) have suggested the measuring of UV fluorescent compounds known as hydroxycoumarins as biochemical marker to assess the PPD susceptibility. Significant differences have been found in hydroxycoumarin accumulation among cassava varieties using High Pressure Liquid Chromatography,

however, these differences do not clearly correlate with PPD susceptibility. Salcedo *et al*. (2010) found similar results using field grown roots of 26 different varieties, where the accumulation of hydroxycoumarin measured as fluorescence emitted in transversal root sections did not correlate with basic visual symptoms of PPD in cassava roots. Interestingly, a promising alternative to evaluate PPD exists in the measurement of the sugar/starch ratio as described by Oirschot *et al*. (2000) who found a strong correlation between them. However, for this method to be widely accepted it need to be proven in different environmental and geographical conditions with different varieties of cassava.

6. PHYSIOLOGICAL CHANGES UNDER PPD

In order to identify potential means by which to control PPD in cassava, it is important to understand the processes of deterioration from a physiological and biochemical focus (Hirose, 1986; Buschmann *et al*., 2000b). Overall, cassava postharvest physiological deterioration is considered as a complex abiotic wounding stress response (Westby, 2002; Beeching *et al*., 2002). Plant wounding induces the production of signaling components that initiate the plants' wound response. The initial wounding during harvest releases signal molecules that trigger protective or defensive responses locally as well as systemically. Such signal molecules are produced by the wounding itself (cell wall fragments, lipid peroxidation products), released from inactive precursors or synthesized *de novo.* Examples of metabolites produced *de novo* are jasmonic acid, salicylic acid, ethylene, system in or reactive oxygen species such as H_2O_2 (Beeching *et al*., 2002).The main responses of the plant to these signals are: (1) the production of defensive enzymes and secondary metabolites such as glucanases and chitinases, phytolaexins and antioxidants; (2) the synthesis of wound repair and sealing molecules (such as callose, lignin and suberin) and; (3) the insolubilization of hydroxyproline-rich glycoproteins (HRGPs) by H_2O_2 (Beeching *et al*., 2002).

In cassava wound repair does occur if the root remains attached to the plant (Richard, 1981; Plumbley and Richard, 1991; Reilly *et al*., 2004),thus illustrating that cassava can in fact repair and seal the wound site, removing the source of the signals and down-modulating the response, returning the plant to basal conditions. The problem lies once the root is detached, where althoughthe wound response is present, the healing process and the subsequent down-regulation of the signals are insufficient or too slow. As a result the production of wound response signals is not switched off, triggering a continual cascade of wound responses throughout the root which is observed as PPD. The cassava root is a storage organ for photosynthates and since it is not a propagule with reproductive function there is no biological need to repair wounds. It is thought that at some point during evolution cassava roots lost their efficiency in wound repair (Reilly *et al*., 2004).

The physiological changes occurring in cassava roots during PPD are considered to be mediated by enzymes due to the inhibition of the development of vascular streaking either by hot water treatment (53ºC for 45 min), storage root under water or storage under anaerobic conditions. Furthermore, when detached roots were treated with cycloheximide, a protein synthesis inhibitor, an inhibition of the visual symptoms of PPD and associated fluorescence was observed (Uritani *et al*., 1984; Ravi and Aked, 1996).

6.1 Oxidative Stress

Since Noon and Both (1977) showed that the anaerobic atmosphere and oxygen-depleted air inhibited the vascular streaking, PPD has been considered to be a product of an oxidative process. Further supporting this is the failure of bactericide and fungicide to inhibit PPD in cassava and the reduction of non-enzymatic antioxidants, *β*-carotene and ascorbate, during PPD in cassava (Iglesias *et al*., 1995, cited by Reilly *et al.,* 2004).

Peaks of ROS as well as increased activity of enzymes that modulate ROS have been detected during deterioration (Reilly *et al*., 2001). The reduction of molecular di-oxygen leads to reactive molecules which is termed ROS. Under stress conditions, in response to external stimuli there is a rapid production of $^{-}O_2$and H_2O_2, known as 'oxidative burst', which often occurs through the perturbation of electron transport chains of photosystems, mitochondria, peroxisomes and endoplasmic reticulum. The ROS generated during the oxidative burst facilitates plant defense through several complex and inter-connected roles such as: (1) cell wall strengthening; (2) induction of defense-related genes and; (3) triggering of host cell death.

At cellular physiological concentrations $^{-}O_2$and H_2O_2 are not toxic, however, at increased concentrations their toxicity arises due to their conversion to hydroxyl radicals (OH^{-}) through the iron-catalyzed Haber-Weiss reaction. Hydroxy radicals, which are highly reactive, affect cellular homeostasis resulting in DNA damage, lipid peroxidation and protein denaturation. Defenses have evolved in response to oxidative stress to detoxify ROS and/or to prevent further formation of highly damaging and reactive forms. These defenses can be enzymatic or non-enzymatic although enzymatic defenses have been predominantly studied including superoxide dismutase, catalases and peroxidases (Beeching *et al.,* 2002; Reilly *et al.,* 2004).

6.2 Accumulation of Hydroxycoumarins

The initial visual symptoms of PPD are accompanied by a rapid accumulation of fluorescent compounds in the parenchyma of the cassava root tissue. These compounds can be observed under UV and have been identified as hydroxycoumarins, which include scopolin(6-methoxy-7-hydroxy-coumaroyl-7β-D-glucoside), scopoletin (6-methoxy-7-hydroxy-coumarin) and esculin (6, 7-dihydroxycoumaroyl-6-β-D-glucoside). The main phenolic components produced during PPD are catechin and (+)-gallocatechin. Buschmann *et al*. (2000a) identified other compounds from cassava roots that may play a role in PPD such asleucoanthocyanins, cyanidin, delphinidin and 22 diterpenoid compounds. Hydroxycoumarins have been detected at higher levels in cassava root tissue blocks which contrasts with low levels detected in intact entire roots and no detectable levels in intact entire roots from pruned plants. The high levels of hydroxycoumarins produced in tissue blocks may be due partly to the wounding effect (Tanaka *et al*., 1984).

Scopoletin is not usually present, or has very low levels, in fresh roots. However, within a few hours after harvest its concentration increases (Aristizabal and Sánchez, 2007). Wheatley and Schwabe (1985) and Buschmann *et al*. (2000a) found an increase of scopoletin during the first 24 to 48 hours after wounding. The first increase of scopoletin was followed by a decline and a second, comparatively smaller increase at 4 to 6 days after harvest. Uritani *et al*. (1983) found that scopoletin is produced initially, followed by the production of scopolin and esculin. The amount of scopoletin was maximal 20 hours after harvest, while scopolin and esculin accumulation were maximal approximately 40 hours after

harvest. Wheatley and Schwabe (1985) demonstrated that roots from plants pruned a few days prior to harvest, which did not deteriorate rapidly, responded to exogenously applied scopoletin by rapid onset of PPD. Comparatively, storing of pruned roots in the absence of oxygen negated that response. This suggests that pruning may be resulting in a reduction of internal scopoletin levels, while oxygen depletion (or curing) leads to a loss of a scopoletin precursor or inactivation of the enzymes involved.

These findings led to the assumption that scopoletin may be directly involved in PPD but the precise role is still unclear (Richard and Gahan, 1983; Ravi and Aked, 1996). Though scopoletin has never been shown to diffuse into the xylem vessels, Wheatley (1980 cited by Ravi and Aked, 1996) suggested that scopoletin is oxidized to black pigments in the process and thereby contribute to occluding material. Wheatley and Schwabe (1985) suggest that the peroxidase-mediated oxidation of scopoletin results in the blue/black vascular streaking observed as PPD. Supporting this is the presence of all required components of the reaction, namely scopoletin, H_2O_2, and peroxidase enzyme activity, near the root xylem parenchyma vessels where vascular streaking symptoms occur (Reilly *et al*., 2004).

6.3 Respiration

The propensity to develop vascular streaking is cultivar dependent and has been associated not only with varying levels of scopoletin, but also with varying rates of wound respiration and wound ethylene production (Brench, 2003). According to Hirose (1986) roots injured by removal of cortex or periderm have higher rates of respiration rates than intact roots after harvest. Respiratory rates of injured roots reached their highest rates on the first day of storage while respiratory rates of the intact roots and roots with minimal periderm removed reached their highest rates on the second day. The respiration rates then decreased gradually until the fourth day when the rates began to increase again, reaching their maximum approximately on the fifth day. These two peaks of increased respiratory rates are thought to be due to wound respiration induced at harvest and biochemical changes induced by the development of PPD, respectively, since the rapid development of PPD on day 5 -6 coincide with the occurrence of the second respiration rate peak. Eight days after harvest the respiratory rate decreased to levels similar to time of harvest. These differences in respiratory rates in roots under PPD, particularly at one day after harvest, were also found to be cultivar dependent (Hirose *et al*., 1984a).

Pruning of the cassava plant a few weeks prior to harvest is one of the most effective and low-cost measures to reduce PPD although the cellular mechanism involved is still unclear (Hirose *et al.,*1984b).Hirose *et al.* (1984a) found that, one day after harvest, intact roots from pruned plants showed a lower respiration rate compared with unpruned plants. The same behavior was observed when pruned and unpruned tissue blocks were compared one day after harvest. However, Hirose (1986) showed that the respiratory rate of the roots from pruned plants was higher than the roots from unpruned plants, which is contradictory with the previous report.

6.4 Metabolic Compartmentalization

There are three main parts in the cassava root pith: the A part (outermost parenchymatous tissue which is approximately 2 - 3 mm thick and is adjacent to the peripheral cortex); the B part (the intervening tissue which is approximately 7 - 8 mm thick between the A and C parts); and the C part (the innermost tissue which is approximately 16 - 18 mm thick) (Figure

3) (Uritani *et al.,* 1984). Uritani *et al*. (1984) and Kojima *et al*. (1983) showed that the primary deterioration, classified by brownish discoloration or vascular streaking, occurs in the B part which does not contain xylem vessels. This shows that the parenchymatous cells in the B part may have different characteristics from those in the A and C parts. Uritani *et al*. (1983) further reported the simultaneous production of secondary metabolites (including coumarins and phenols) and enzymes responsible for the production of these secondary metabolites in all three parts. But in most cases the production of secondary metabolites was more abundant in the B part.

Tanaka *et al*. (1984) found that enzymatic activities (phenylalanine lyase, peroxidase, and acid invertase) and coumarin accumulation in intact roots of pruned plants were lower compared to intact roots of unpruned plants. In the B part (the site of physiological deterioration) of the roots of unpruned plants, all enzymes tested showed the maximal activity compared with the other parts. This increase in activity in the B part of intact roots of unpruned plants seemed to be directly related with PPD, because enzymatic activity in all parts (A, B and C) of root tissue blocks from roots of pruned and unpruned plants were very similar. This leads to the conclusion that in block tissues from roots the effect of wounding could be masking the effect of PPD.

Interestingly, when the A, B and C parts of roots were separated and incubated individually, although the biochemical changes were induced in all three parts, PPD took place only in the B part (Uritani *et al.,* 1983). This result showed that the biochemical changes in the B part lead to the induction of PPD and those in A and C parts do not participate directly in the induction of PPD.

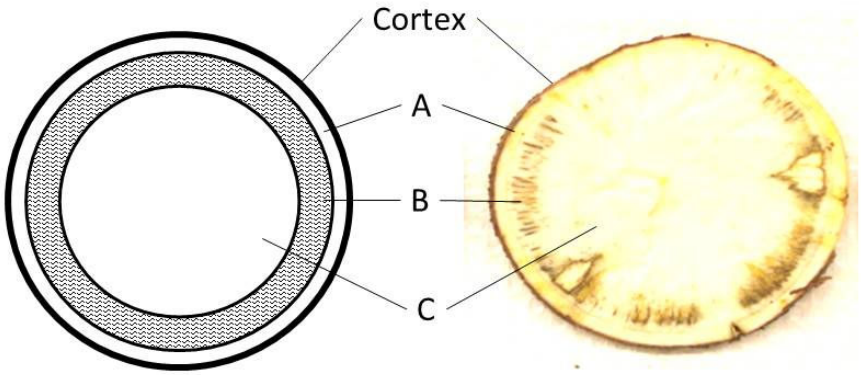

Figure 3: Cassava cross section showing the metabolic zones identified by Uritani *et al*. (1984a). Left: Illustrative diagram. Right: Cassava root cross section.

6.5 Ethylene

Although Plumbley and Richard (1991) showed that auxins could enhance root deterioration, of the phytohormones it has been ethylene that has been found to influence the onset of PPD by altering the respiratory pathway while also changing the activity of the peroxidase enzyme (Plumbey *et al*., 1981; Hirose, 1986). Using transverse slices of cassava roots, Hirose (1986) found that ethylene production began after 15 - 16 hours and then increased gradually to reach a maximum at 1 day, after which the ethylene production decreased gradually until ceasing at 44 hours after incubation began. Differences in rates of ethylene accumulation were also found among cassava accessions. When ethylene production in root

slices with and without deterioration was compared, the former produced 4-fold higher levels of ethylene. Comparatively, no clear differences in ethylene production were observed between the root tissue blocks taken from pruned and unpruned plants (Hirose *et al*., 1984b).

Ethylene production was also compared in four histologically different parts of the cassava roots (cortex, A, B and C parts), where Hirose *et al*. (1984b) found that each part produced ethylene at different rates, varying among cassava accessions. Interestingly, compared to the other parts of the roots, the B part did not produce significantly more ethylene being the primary area of deterioration,

6.6 Other Metabolites involved

One to two days after harvest there is a rapid increase in total sugar content of roots accompanied by a decline in starch content and dry weight. Furthermore, water loss accelerates PPD suggested by the significantly lower fresh weight of the roots from unpruned plants compared with those of pruned plants. The development of cracks due to dehydration in the roots of the unpruned plants, coupled with high sugar content, encourages the entry of microorganisms leading to subsequent decay of the root (Booth, 1975; Maini and Balagopal, 1978; Ravi and Aked, 1996).

Accumulation of cyanogenic glucosides, a decrease in linamarase activity (a key enzyme in cyanogenic glucoside breakdown and release of HCN) and a slight decline in HCN content under PPD has also been reported (Maini and Balagopal, 1978; Kojima *et al.,* 1983; Westby, 2002). This reduction of linamarase activity and HCN is not uniform throughout the cassava root as there is evidence of a radial gradient from the cortex to the C part in terms of cyanide content and linamarase activity (Kojima *et al*., 1983).

Gloria and Uritani (1984) found that a decrease in β-carotene is also correlated with severity of PPD especially in the B and C parts and maybe related to the production of lipoxygenase or a kind of β-carotene-bleaching enzyme in parallel with the appearance of PPD. It would be useful to study this mechanism, considering the loss of β-carotene observed in both the B and C parts of the roots, where PDD occurrence is induced and not induced, respectively.

Oxidation of polyphenols may also play a significant role in PPD. Padmaja *et al.* (1982) evaluated the total phenol content and the activity level of polyphenol oxidase in six cassava varieties. In all varieties a decrease in total phenols was observed after 2 -3 days of storage, followed by a slight increase on the fourth day of storage. There was a concomitant increase in polyphenol oxidase after 2 days of storage. Padmaja *et al*. (1982) hypothesized that brown (or bluish brown) color is caused by disorganization of the cellular membrane system in the B part of the root which leads to the free oxidation of phenols such as (+)-catechin by polyphenol oxidase. The polyphenols may be enzymatically oxidized to highly reactive quinones, which form a colored complex with amino acids and other micro-molecules, which are deposited in the vascular bundles. These studies indicate that polyphenol oxidase would be a key enzyme associated with vascular deterioration of cassava roots. However this hypothesis was challenged by Richard and Gahan (1983) who did not detect the production of quinones in cassava roots undergoing PPD.

7. STRATEGIES TO OVERCOME PPD

There have been three main approaches to overcome the PPD in cassava; (1) the use of improved storage techniques; (2) conventional breeding; and (3) genetic engineering to produce target changes in metabolism (Westby, 2002).

7.1 Storage Techniques

Traditional marketing and storage systems have been adapted to avoid root perishability but currently there is no general technique to store and preserve cassava roots commercially (Aristizabal and Sánchez, 2007). These adaptations include processing centered in proximity to the areas of production to ensure a daily supply of raw material, processing into storable forms (through sun drying, fermentation, etc.) at the farm level and the common practice of trading of small quantities of roots (Weham, 1995; Westby, 2002). A common way of avoiding root losses due to PPD is to leave the roots unharvested in the soil after the period of optimal root development, until the roots can be immediately consumed, processed or marketed. Cassava roots are known to last in soil up to three years. This strategy has disadvantages because large areas of land are used by the standing crop, unavailable for additional agriculture production. Furthermore, even though the roots may increase in size they become more woody and fibrous, decreasing palatability and increasing the cooking time, respectively, if left longer than the optimal harvest time of 10-12 months after planting. Another negative effect occurring due to extensive in-field storage of cassava roots is their increased susceptibility to attack by pathogens as well as the reduction of extractable starch (Wenham, 1995; Ravi *et al.,* 1996).

Another traditional practice to overcome PPD is pruning, which consists of the removal of all leaves and stems of the cassava plant approximately 40- 50 cm above the soil level approximately 2-3 weeks prior to harvest. Pruning has been associated with the reduction in the time of onset of PPD compared to unpruned plants (Tanaka *et al*., 1984; Plumbley and Richard 1991). There are other traditional practices involving the storage of cassava roots under in-field conditions such as in pits, clamps, trenches or boxes, but these methods are after root harvest (Booth, 1977). For example, Balagopal and Padmaja (1985, cited by Ravi *et al*., 1996) reported a novel low-cost method for extending the shelf life of fresh cassava roots by using pits in sandy soils. Although this method prolonged shelf life for more than two months, the roots became very sweet and had poor cooking qualities leading to its only use as cattle feed. These traditional methods are based on the process of curing, a common method for enhancing the storage life of other root crops (Booth, 1975). Curing relies on the fact that at relatively high temperatures (25 to 40ºC) and high relative humidity (RH; 80 to 85%) wounds produced by harvest are healed faster thus limiting deterioration (Booth, 1975; Ravi *et al*., 1996). The use of traditional techniques are not widespread nor adopted on a commercial scale as they are considered rather labor intensive, difficult to manage and are not always completely effective (Wenham, 1995; Ravi *et al*., 1996). Since cassava is a relatively low-cost staple food, it cannot normally support the cost of sophisticated techniques for better storage. These traditional techniques can result in extending cassava root shelf-life but are somewhat disadvantageous due to the investment required, convenience, and availability of materials (Ravi *et al*., 1996; Oirschot *et al*., 2000). As cassava becomes a more industrial commodity modern techniques, such as the use of polyethylene bags, waxing and deep freezing are being applied commercially. The application of these more modern techniques is very limited considering the conditions under

which much of the world's cassava is grown (Ingram and Humphries, 1972, cited by Booth, 1975; Ravi *et al*., 1996).

The technique of cassava root storage in polyethylene bags after harvest prevents PPD up to 4 weeks by subjecting the root to high RH inside the bad which reduces transpiration and respiration (Ravi *et al*., 1996). The use of polyethylene bags for storage while being transported long distances is now being widely adopted in South America but successful conservation depends on the quality of the roots (with minimal damage), protection from sunlight, treatment with fungicide, and packing within three hours after harvest (Ravi *et al*., 1996). A more common modern method of limiting PPD is covering cassava roots with paraffin wax by dipping the root in paraffin wax (at a temperature of 55-65ºC for a few seconds) after treatment with fungicide. Use of wax has been reported to prolong shelf-life of cassava roots up to 2 months (Ravi *et al*., 1996; Aristizabal and Sánchez, 2007). Cassava roots can also be stored for 2 weeks between 0 to 4ºC without any internal deterioration. The most favorable temperature for storing fresh cassava is 3ºCbut after 4 weeks microbial infection takes place and will increase with subsequent storage time. However, even after 6.5 months of storage between 0 to 4ºC, the part of the root without decay usually is in excellent condition and is suitable for human consumption (Ravi *et al*., 1996; Oirschot*et al.,* 2000). At temperatures above 4ºC roots develop the PPD symptoms more rapidly and have to be discarded after 2 weeks of storage (Ravi *et al*., 1996). Alternatively, entire roots or more usually pieces of root can be stored frozen under deep-freeze conditions in polyethylene bags and the roots were quite palatable after thawing, although some sponginess was present, and was able to be kept for a further 4 days (Averre, 1967). This technique is used at a commercial scale in many Latin American countries such as Brazil, Colombia, Costa Rica and Puerto Rico (Ravi *et al*., 1996).

7.2 Conventional Breeding

Though improved storage techniques have more immediate impact, the level of the improvement is limited by the roots' inherent perishability (Westby, 2002). Breeding and genetic modification are long-term strategies to tackle PPD.

Conventional breeding potentially could produce cultivars with resistance to PPD by using recurrent selection methods. Improvement through breeding in cassava has unique problems such as low seed set, erratic flowering regime leading to difficulty in coordinating flowering times between two varieties, as well as geographically specific flowering of a particular cultivar. Furthermore, the multigenic nature of the favorable characteristics of cassava results in the requirement of tremendous efforts to incorporate additional agronomical traits into cassava cultivars without changing the favorable characteristics of the parent genotypes (Westby, 2002). Use of conventional breeding to alleviate PPD in cassava faces other challenges such as high heterozygosity and the low natural fertility of cassava (Zhang, 2002), the lack of genetic variability for resistance to PPD (Ceballos *et al*., 2004) and the presence of an inverse relationship between PPD and cassava root dry matter (Estevão, 2007). However, PPD analysis of F_1 from crosses of susceptible and moderately resistant cassava clones, estimates heritability of 0.64 for PPD (64% of PPD variation is due to genetic variation). These findings suggest that inheritance of post-harvest root deterioration is at least partially controlled by additive and quantitative factors rather than qualitative ones (Kawano and Rojanaridpiched, 1983). Cortes *et al.* (2002) and Estevão (2007) linked quantitative trait loci (QTLs) influencing PPD of cassava to molecular markers, finding ten and three putative QTLs, respectively, which explain between 5 and13% of the phenotypic variance of PPD.

Though advances have been made, conventional breeding has been unsuccessful in generating cassava cultivars with improved shelf-life (Taylor *et al*., 2004). The only apparent source of significantly delayed PPD has been the identification of an interspecific hybrid between cassava and *M. walkerae* named CW 429-1 (Blair *et al*., 2007; Estevão, 2007; Morante *et al*., 2010). This hybrid displays no visible signs of deterioration after 15 days post-harvest.

7.3 Genetic Engineering

Since genetic engineering can transfer new traits to cassava varieties without altering other desired traits, genetic modification to overcome PPD by using molecular techniques is considered most appropriate to solve PPD in cassava. However, there is a lack of concrete information available about the genes involved in the biochemical pathways associated with cassava PPD (Westby, 2002; Taylor *et al*., 2004). Recent strategies have focused on the reduction of reactive oxygen species (ROS) by the over expression of the cyanide-insensitive mitochondrial enzyme alternative oxidase (AOX) from *Arabidopsis* in cassava roots (Sayre *et al.,* 2011). This strategy resulted in the production of transgenic plants exhibiting delayed of PPD of approximately three weeks. Additional strategies are also being explored such as the over expression of ROS metabolizing enzymes (e.g., catalase, SOD, ascorbate peroxidase) or the accumulation of ROS quencher metabolites such as β-carotene, which has been reported to extend the storage life up to 4 weeks (Sayre *et al.,* 2011). Though these strategies show promise, further studies to understand the biochemistry, molecular biology, and genetics of PPD in cassava need to be carried out in order to provide new information on the genes involved in key pathways to develop potential strategies for PPD control (Westby, 2002; Taylor *et al*., 2004). With the rapid growth in the technology associated with the production of transgenic cassava plants, the only limiting factor is the availability of target genes. But the completion of the cassava genome sequencing project (Cassava Genome Project, www.phytozome.net/cassava) combined with the ongoing gene annotation projects are likely to rapidly increase the availability of target genes related to PPD for manipulation using transgenic technologies.

8. GENE EXPRESSION ANALYSIS DURING PPD

PPD is an extremely complex phenomenon involving multitude of components such as ROS modulation, phytohormone synthesis and breakdown, regulation of molecules involved in signal transduction, synthesis of defense molecules, responses related to programmed cell death, synthesis of cell wall components, etc. As a result a myriad of genes involved in PPD response in cassava and many molecular studies using different techniques have been conducted to better understand this phenomenon at the level of gene expression (Reilly *et al*., 2004).

By using a cDNA AFLP approach, Huang et al. (2001) isolated 70 transcript derived fragments (TDFs) related with the regulation of gene expression during PPD. Based on the sequence homology of these TDFs, they catalogued 24% as metabolism, 22% stress/wounding, 12% signal transduction, 8% development, 6% programmed cell death and 28% unknown. Reilly *et al*. (2007), identified 72 genes with significantly altered expression using microarrays and a cDNA library of cassava varieties with early and late PPD. In addition, a subset of 21 storage-root-wounding-specific transcripts, which potentially are PPD specific, was identified by a comparison between the expression in harvested storage roots and unwounded and wounded leaves. These transcripts (and their respective genes)

can potentially be the key tools necessary not only to further understanding the nature of PPD in cassava but also to eradicate PPD (or delay the onset of PPD) through genetic engineering.

9. CONCLUSIONS AND FUTURE DIRECTIONS

Despite the enormous importance as an essential part of the daily diet for millions of people, cassava remains a low-cost subsistence crop farmed primarily by small-scale farmers in developing countries, especially in the tropics. In areas where cassava is cultivated a significant amount of crop production is lost due to post-harvest losses, aggravating hunger and poverty (FAO, 2009).In the case of cassava, PPD is the third most important constraint for farmers after phytosanitary problems and low yield (Aerni, 2006). A solution leading to the reduction of PPD, which is subsistence-farmer-friendly, would greatly impact not only food security but also environmental pollution and climate change since less land, water and energy will be necessary for cassava production, handling and transport to both consumers as well as processors.

Thus research on PPD in cassava, both basic and applied, should focus on long-term strategies such as improved varieties through conventional breeding and genetic transformation. Also of basic importance is the development of an appropriate universal method to evaluate PPD in cassava that is both practical and quantitative. Such a uniform tool would benefit scientists worldwide to better associate phenotypic tolerance to PPD with results from molecular and cellular experiments. In addition, studies using novel techniques will be necessary to better understand genes and metabolic pathways involved in PPD in cassava.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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