STRATEGIES TO OVERCOME POST-HARVEST PHYSIOLOGICAL DETERIORATION IN CASSAVA (*MANIHOT ESCULENTA*) ROOT: *A REVIEW*

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ABSTRACT
Cassava is of global importance both as a food and energy source. Owing to the diversity of its utilization, adaptation and low input requirements, cassava not only provides a source of revenue for rural farmers, it also serve as an important source of raw material for many industries. Cassava roots are notorious for their short shelf life due to Post-harvest physiological deterioration (PPD) leading to significant economic losses. PPD - a rapid oxidative reaction that initiates in cassava roots within 24 to 48 hours after harvest, discolouring them, thereby renders them unmarketable and unpalatable. Secondary post-harvest deterioration often appears when the edible cassava roots suffer moderate to severe damage mediated by a wide range of pathogenic microorganisms. Documented strategies to delay PPD in cassava; include the use of improved storage techniques; conventional breeding; and genetic engineering to produce target changes in metabolism were extensively reviewed. The challenges posed by each strategy were highlighted.

Keywords: Cassava roots, post-harvest physiological deterioration, quality and tolerance

INTRODUCTION
Cassava is one of the most important food staples in the tropics and the fourth most important energy source (Alves, 2002). It has the highest potential production of calories per hectare per year compared to rice, maize and yam (Njoku *et al.*, 2014). It has the ability to tolerate poor soil and harsh climatic conditions and is generally cultivated by small-scale farmers as a subsistence crop in a diverse range of agricultural and food systems. Although cassava is a perennial crop, storage roots can be harvested from 6 to 24 months after planting (MAP), depending on the cultivar and growing conditions (El-Sharkawy, 2006). Roots can be left in the ground without harvesting for a long period of time, making it a security crop against famine.

World cassava production grew at an annual rate of 2.2% from 1984 - 1994 reaching 252 million tonnes in 2013 (FAO and IFAD, 2013). It is expected to continue growing at the same rate because of yield increases. Cassava has entered the modern market economy and there is still a growing demand for its use in processed food and feed products (Ceballos *et al*., 2008). Owing to the diversity of its utilization, adaptation and low input requirements, cassava often provides a source of revenue for rural farmers. It competes with alternative raw materials such as grains and sugarcane (Rosegrant, 2008). Packaged cassava and cassava flour are gaining greater acceptance in most markets. One of the potential uses of cassava is the starch market. According to the International Starch Institute, cassava starch production has grown globally between 1980 and 1997, from 16 to 35 million tonnes (FAO and IFAD, 2013). Thailand and Indonesia are the major suppliers of cassava to the world market, contributing 80% and 10% respectively, while the rest is provided by small exporters in Africa, Asia, and Latin America.

All parts of the cassava plant are utilized for food or feed but the storage roots are the most used product from cassava. The leaves are widely consumed in some regions of Africa, Asia and South America (Njoku *et al.,* 2013) as a source of protein, minerals, fiber, vitamins and essential amino acids (Ceballos *et al.,* 2008). The tender shoots and leaves are eaten as vegetables in many parts of Africa and they provide protein with a high content of lysine, minerals and vitamins. The storage root can be processed into various food products and starch for domestic consumption, local or foreign markets. The processed storage roots are also used to make bread, sugar, flour, laundry starch and alcoholic drinks confectioneries. The seed is processed for oil and seed cake and for formulating feed for livestock. The seed is also processed into medicinal products to cure skin diseases.

Despite its importance, several biotic and abiotic factors affect the relative efficiency of cassava to satisfy these needs. Notably among them is its short shelf life termed post-harvest physiological deterioration (Ceballos *et al*., 2008) which starts when the roots are harvested. As a result, farmers, processors and consumers suffer substantial losses during storage of the roots. Post-harvest physiological deterioration (PPD) is a rapid process that initiates within 24 to 48 hours after harvest (Beeching *et al*., 1998) and does not involve micro organisms. Subsequently, a microbial deterioration sets in 5 to 7 days later. PPD in cassava is an oxidative reaction that occurs when the storage roots are exposed to oxygen and when detached from the mother stem at harvest. It begins from the proximal end of the root and spreads towards the distal end. Roots of cultivars very susceptible to deterioration develop the characteristic PPD streak up to the distal end within 2-3 days. Roots with less susceptibility develop symptoms close to the proximal end of the section only, with little or no streaking at the distal end. PPD leads to induced respiration resulting in starch hydrolysis (Uritani *et al*., 1984).

It is characterized by a bluish-brown or bluish-black discoloration of tissues along the peripheral vascular bundles of roots and spreads over to adjacent root parenchyma rendering the roots unmarketable and unpalatable. The wounding induces the production of signaling components that initiate the wound response (Beeching *et al*., 1998). This response is similar in other plants resulting in wound healing and survival; however, this is not the case for cassava roots. Instead of healing, it is observed as PPD (Beeching *et al*., 1998).

Estimated losses vary from 5 to 25% of harvested roots (Wenham, 1995). Most of the deteriorated roots end up as animal feed with a price reduction greater than 50%. In Colombia where 1.3 million tons are consumed fresh, approximately 10% is lost due to PPD with a reduction in price from US$ 80 per tonne (fresh market) to US$ 40 (animal feed), generating a loss of about US$ 5 million (Wenham, 1995). Similar losses have been reported for Africa where PPD accounts for 7 to 90% discount on three day old cassava in Tanzania (Personal communication). It also results in reduction in starch quality and quantity (Uritani 1984) which indirectly leads to reduction in cassava root utilization as a raw material for the starch industry. These problems have negative impacts on the rural and national economies.

For instance, in Ghana cassava is mainly used for *fufu*, however, roots with visible signs of deterioration is not used for *fufu* but rather peeled, chipped and sun-dried to produce *kokonte.* Although this is time-consuming and a strenuous process, the price of *kokont*e is low compared to equal quantity of fresh cassava and also considered as inferior food that is only consumed by the poor. It is also difficult to pound and has less desirable elasticity than *fufu* prepared from fresh roots (Personal communication with Dr Ruth Thompson). Deteriorated cassava roots are considered to have poor eating and processing quality (Rickard *et al*.,

1992). It takes longer to cook and they have an unpleasant bitter flavor and an unattractive off colour. G*ari* processed from deteriorated roots also has lower and less desirable swelling properties than that produced from fresh roots.

PPD is a serious problem confronting not only farmers but to a large extent processors and consumers alike. To avoid this problem, the storage roots have to be consumed or processed immediately after harvesting. However, this is only practical on small scale basis. On a larger scale, poor roads, time and distance between harvesting and processing play a critical role in the distribution and marketing of cassava making PPD a more serious problem. Characteristics of major cassava growing communities in Nigeria include poor roads, inappropriate means of transport and a badly organized distribution system. As a result, fresh cassava can only be marketed over long distances only if there is well-developed road system which ensures that time of transportation can be kept to a minimum and the roots delivered when they are still fresh.

Techniques currently used in delaying PPD for the export market includes exclusion of oxygen by storing and transporting the roots in plastic sacks, coating with paraffin wax and freezing. These have been adopted in developed countries with little success due to the increased cost; however, they are not practical in developing countries. In Africa, farmers have developed traditional methods to delay deterioration. Roots are either stored in pits or clamps or left in the soil until they are consumed, processed or marketed. With this practice, the land is occupied and it is unavailable for further production, reduction in dry matter and palatability declines as roots become more fibrous. Cooking time also increases (Wheatley and Gomez, 1985). These techniques are labour intensive and are not always effective. Due to these problems, research towards delaying PPD is a major priority to minimize the risk of yield losses.

The shelf-life of cassava can be increased to two weeks (FAO, 2013) either by conventional breeding or biotechnology which could enhance cassava utilization and reduction of PPD by 90%. Evaluation of the visual symptoms of PPD in various cultivars of cassava shows that differences exist in their susceptibility to deterioration (Iglesias *et al*., 1996). These differences have provided breeders with the opportunity to use the genetic variability to improve the crop. This variability to PPD has not been adequately explored in Nigeria. Efforts by scientists to improve the crop have resulted in cassava varieties with PPD tolerance. Currently, four different sources of PDD tolerance have been identified (Morante *et al*., 2010). These include an interspecific hybird, irradiated materials (where one of the genes involved in PPD was silenced), high-carotene clones (antioxidant properties of carotenoids protects the roots from PPD) and finally, spontaneous mutation that was observed as waxy-starch (amylose-free) (Ceballos *et al*., 2008). This information can be utilized in Nigeria to improve farmers' cultivars. This work is a critically review of the importance, constraints, and prospects of genetic improvement for delayed post-harvest physiological deterioration on the edible cassava root.

THE PROBLEM OF PPD IN CASSAVA

Cassava roots, unlike any other tuber crop, suffer a remarkably short shelf life due to a physiological deterioration. The extent of deterioration depends upon the degree of mechanical damage by roots as well as the genotype. It occurs in two phases, primary and secondary deterioration. The primary deterioration starts from the central vascular bundles of the root, spreads to the adjacent storage parenchyma and subsequently stored starch undergoes structural changes (Plumbley and Rickard, 1991). This is known as post-harvest

physiological deterioration of which the visible signs are vascular streaking with a blue or black discoloration rendering the roots unpalatable and unmarketable (Beeching *et al*., 1994). This initial deterioration is physiological and biochemical and does not involve microorganisms. The secondary deterioration is due to infection with microorganisms leading to fermentation and softening of the root tissue (Wenham, 1995). PPD is much more important economically than secondary microbial deterioration because the visible colouration of the root is used as an indication of its cooking quality and probably the taste making the crop difficult to sell.

Wounded Plants and PPD

Wounded plants induce the production of a signaling mechanism that initiates a wound response. Plants response to wounds in three interrelated forms (Bennett and Wallsgrove, 1994). These are production of signals, defensive compounds and enzymes and wound repair. Signals produced either act locally at the wound site or systemically. These signals are produced either as a direct consequence of the wounding (such as membrane peroxidation products or cell-wall fragments) or are induced by the wounding (such as jasmonic acid, abscisic acid, salicylic acid, systemin or hydrogen peroxide). Other signals that act systemically prepare the plant for the extension of wounding or pathogen invasion (these include systemin, electrical and hydraulic signals). The second phase is the production of defensive enzymes and molecules that help in the protection of the plant against pathogens or the effects of wounding. These enzymes include glucanases and chitinases that attack components of microbial cell walls and secondary metabolites such as phenolics that act as antimicrobials (e.g. phytoalexins) or anti-oxidants (Han *et al*., 2001).

Wound repair occurs via the synthesis of suberin and lignin from phenolic components, callose synthesis, the insolublisation of hydroxyproline-rich glycoproteins by hydrogen peroxide, and the formation of a wound meristem (Han *et al*., 2001). This repair leads to the sealing of the wound and subsequently, inhibition of the production of the signals triggering the wound response and a return of the plant to normal development. Branch pathways from general phenylpropanoid metabolism lead into all these aspects of the wound response (Dixon and Paiva, 1995). Although these aspects of the wound response are found in the cassava root, the wound repair and the resultant down- modulation of the signals are inadequate leading to a continuous cascade of wound responses that spread throughout the cassava root. This incomplete wound response is observed as PPD (Beeching *et al*., 1998). It is an abiotic stress response, typical of wound responses in other plant systems (Dixon and Paiva, 1995). Although these responses result in wound healing and survival in other plants, this is not the case in cassava. The storage roots are not propagules, but rather serve primarily as repositories of carbohydrate for the plant. In contrary to the detached root, wound repair does occur if the root remains attached to the plant (Mwenje *et al*., 1998).

Biochemical mechanisms of PPD

Biochemical changes during PPD include increases in respiration (Hirose, 1986), mobilisation of starch to sugars and changes in lipid composition. There is also the increase of enzymes (Tanaka *et al*., 1983) and phytohormones (Hirose *et al*., 1984), occurrence of a wound-induced oxidative burst (Reilly *et al*., 2001), and the accumulation of secondary metabolites (Wheatley and Schwabe, 1985) which precedes the discolouration (Wheatley and Schwabe, 1985). Associated with the discolouration, coloured occlusions and tyloses are formed from the xylem parenchyma that block adjacent xylem vessels. The activity of various enzymes including dehydrogenases, peroxidases, catalases, phenylalanine ammonia lyase (PAL) and phenol oxidase increase (Hirose, 1986). Ethylene, a phytohormone, is

considered to play a role in coordinating wound and senescence responses in plants (Ecker and Davis, 1987). Secondary metabolites which accumulate during PPD include diterpene, flavan-3-ols, catechin, catechin gallate and gallocatechin and the hydroxycoumarins; esculin, esculetin, scopolin and scopoletin. These are derived from the phenylpropanoid pathway and are localized in the apoplast of the parenchyma. Several of these metabolites show anti-microbial and or anti-oxidant activity (Buschmann *et al*., 2000).

Strong evidence has been obtained for the importance of reactive oxygen species (ROS), enzymes and compounds that modulate ROS in the PPD response. ROS is any compound (e.g. hydrogen peroxide) that is synthesized actively by the plant as a response to stress or as a component of defense against pathogen attack. This stress-related synthesis can be very rapid and is part of a process called "oxidative burst". A reaction occurs between anionic peroxidases, scopoletin and hydrogen peroxide and this produces an insoluble blue-black precipitate, which explain much of the discolouration.

At the molecular level, the development of PPD is evidently a complex phenomenon involving multiple components. Cycloheximide inhibition of protein synthesis is an indication that PPD is an active, rather than a degenerative, process involving change in gene expression and protein synthesis. Analyses of expressed genes show altered regulation of proteins and enzymes involved in signal transduction, reactive oxygen species (ROS) modulation, phytohormone synthesis, senescence and programmed cell death (PCD) responses. It also shows synthesis of anti-microbial, antioxidant or other defensive compounds, and the formation of other compounds that are involved in the synthesis of cell wall components, as well as proteins that are themselves components of cell walls (Reilly *et al.*, 2004).

PHENOTYPING OF CASSAVA FOR PPD

Reliable estimates of PPD are obtained by sampling a large number of root, usually ten or twenty roots are sampled per clone. Results are then expressed as the mean of percentage deterioration of all individual roots.

Equipment used for PPD Phenotyping

Some of the equipment used for PPD laboratory screening and scoring are PVC film, Scissors, Stainless steel knife, Wooden or Plastic or steel shelves**,** table, etc.

Methodology

Procedure 1 (Wheatley's method)

Select commercially sized roots with a minimum length of 18 cm, without mechanical damage and with no pre-harvest rotting visible. Cut the proximal and distal roots ends, ensuring the remaining root section is at least 15 cm long after cutting of the ends. Cover the distal end of the root with PVC film in order to maintain moisture content of the distal end and inhibit the development of deterioration from this end of the root. Physiological deterioration therefore develops only from the proximal end where loss of tissue moisture occurs (Fig. 1).

Store roots on wooden, plastic or steel shelves at 21-28°C and 70-80% relative humidity in a place protected from direct sun, rain and rodents, but exposed to air. Evaluate root section after three days of storage, sufficient time for physiological deterioration to develop but insufficient for microbial deterioration. The evaluation is carried out as follows:

- Cut transverse slices 2, 4, 6, 8, 10, 12 and 14 cm from the proximal end, giving a total of seven slices.

- Assign values from 0% to 100% PPD based on the extent of physiological deterioration on the surface of each slice of the root. Usually, only the periphery of the parenchyma is considered, since the central tissues rarely deteriorate.
- Divide this part of the root surface into 10 sections and estimate deterioration in each section.
- Each of the seven slices is scored and the mean for the entire root calculated. The mean for all 10 or 20 roots can then be calculated.

Procedure 2 (Booth's method):

Select ten commercially sized roots with a minimum length of 16 cm. Store the intact roots on wooden shelves at 21-28°C and 70-80% relative humidity. Evaluate roots seven days after storage by cutting them into transverse slices 2, 4, 6, 8, 10, 12 and 14 cm from the proximal end, giving a total of seven slices. Score each slice by assigning values from 0-100% based on the extent of physiological deterioration on the surface of each slice. Each of the seven slices is scored and the mean for the entire root calculated. The mean for all 10 roots can then be calculated.

Procedure 3 (Image Analysis method)

In this procedure, roots from field or greenhouse grown 10-12 month-old plants of cassava are harvested and the roots were sliced into 5-mm-thick slices. The root slices are placed on Petri dishes containing filter papers presoaked with 2-5 mL of distilled water to prevent drying. The sliced roots are then randomized and stored at a constant temperature of between 27- 29°C in the dark. For each time point, three replicates consisting of two root slices each are sampled. Samples are frozen in liquid nitrogen after incubation for 0, 6, 12, and 24 h. The root slices are then photographed under standard light conditions with a Nikon D700 camera. Image analysis are performed using the PPD Symptom Score Software written in MatLab (The Mathworks). Photographs of root probes are first interactively marked with a polygon in the image. The software then converts the color image into a gray value image and determine its histogram. To obtain a robust PPD score that is insensitive to changing illumination conditions, and outliers, the width of a binary histogram are used to calculate the number of occurring gray values. The range of the gray value interval, covering 95% of the occurring gray values, are normalized with the 97.5% quantile to generate a PPD score, which ranges in theory from 0 to 1. (Kunttu et al., 2003). Because the root slice method triggers rapid and homogenous PPD symptoms, a saturation of the PPD symptom score between 48 and 72 h after harvest would be observed. The inner 50% PPD value is automatically calculated based on the gray values occurring in the inner 50% surface area of the initially selected root area.

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).

Storage Techniques

Traditional 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 (Wenham, 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 (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. For example, 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. 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, 1976; 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).

Modern Techniques

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 (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 relative humidity inside the bag which reduces transpiration and respiration. The use of polyethylene bags for storage while being transported long distances is now being widely adopted in West Africa and 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 (Aristizabal and Sánchez, 2007). Paraffin wax works probably by cutting of the influx of oxygen needed for oxidative respiration into the harvested root.

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 3ºC but 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 (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. 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).

Conventional breeding for PPD tolerance

Breeders have made considerable efforts in improving cassava yield and resistance to biotic and abiotic stresses through conventional breeding. However, lack of resistant genes in existing germplasm, high heterozygosity, poor flowering and the outcrossing nature of cassava limits the success of breeding, especially for a quantitative trait such as PPD (Jennings 2002). Unfortunately, some desirable traits are often recessive and genetically linked to undesirable ones making trait separation difficult. There is a strong genetic link between PPD and high dry matter content (Jennings & Iglesias, 2002), making breeding for PPD tolerance via conventional means a difficult challenge.

Nevertheless, there is an indication that it is possible to break the linkage between high dry mater content and PPD (Iglesias et al., 1996). Screening of numerous cassava varieties and improved lines for nutritional and agronomic traits has provided a solid platform for cassava breeding (Chávez *et al*., 2005). Four different sources for PPD tolerance have been identified (Morante *et al*., 2010). The first source was from the wild specie, *M. walkerae,* a native to the United States. A second source was induced by mutagenic levels of gamma rays which putatively silenced one of the genes involved in PPD. A third source was a group of high-carotene clones. It is postulated that the antioxidant properties of carotenoids protects the roots from PPD. Finally tolerance was also observed in a waxy-starch (amylose-free) mutant. It is expected that tolerance to PPD co-segregated with the starch mutation and is not a pleiotropic effect. PPD is highly influenced by the environment, this makes scoring for minor differences difficult (Rodríguez, 2001). Although breeding and genetic experiments can provide useful information to help solve the problem of PPD, potential biotechnological input such as the development of molecular markers will also make progress.

Molecular breeding for PPD tolerance

PPD is a complex polygenic trait which is hereditable but with a strong environmental interaction (Cortes *et al*., 2002). One strategy that could facilitate a breeding program for PPD tolerance is marker-assisted selection (MAS). Specific genes involved in PPD tolerance have been identified, characterized and their expression evaluated (Reilly *et al*., 2001). The correlation between PPD and these genes will allow indirect selection in breeding programs. Attempts have been made to identify quantitative trait loci (QTL) associated with PPD. Ten putative QTLs have been identified, however, the correlations were not strong (Cortes *et al*., 2002). It is therefore important to develop populations using parental lines with distinctly contrasting PPD responses which will be used to identify stronger QTL's. Key candidate genes, enzymes, compounds, and metabolic pathways that modulate PPD response have been identified. These can be manipulated via genetic modification experiments to ascertain their response to PPD. Genes for the biosynthesis of carotenoids and other antioxidants are also

good candidates that can be cloned and used in transgenic cassava plants in order to determine their exact contribution to PPD tolerance.

Transgenic breeding for PPD tolerance

Biotechnology presents a tool that provides quick gene introgression into crop plants. Genes isolated from other sources have been successfully inserted into cassava genome (Ihemere *et al*., 2008). The expression of various inserted genes into the cassava genome, which had resulted in increased disease resistance, modified starch quality, reduced level of cyanogenic glucosides (Sayre, 2011), and extended shelf life of cassava roots (Thro *et al*., 1996), demonstrates the ability of genetic engineering to bring about rapid cassava crop improvement.

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). 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). 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.

CONCLUSION

Cassava roots are notorious for their short shelf life due to Post-harvest physiological deterioration leading to significant economic losses. PPD - a rapid oxidative reaction that initiates in cassava roots within 24 to 48 hours after harvest, discolouring them, thereby rendering unmarketable and unpalatable was extensively reviewed. Documented strategies to delay PPD in cassava; include the use of improved storage techniques; conventional breeding; and genetic engineering to produce target changes in metabolism. The challenges posed by each strategy were x-rayed.

REFERENCES

Alves, A. A. A. 2002. Cassava botany and physiology. In: Hillocks, R. J., M.J. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, production and utilisation.* CABI. International, Oxford. pp. 67-89.

Aristizabal J, Sanchez T (2007). Technical guide for the production and analysis of cassava starch. Bulletin of Agriculture Services of the FAO 130 Rome Italy, p. 134.

Beeching J. R, Reilly K, Gómez-Vásquez R, Li H, Han Y, Rodriguez M. X, Buschmann H, Taylor N, Fauquet C, Tohme J (2002). Post-harvest physiological deterioration of cassava. In: M. Nakatani and K. Komaki (eds) 12th Symposium of the International Society for Tropical Root Crops: Potential of root crops for food and industrial resources. Tsukuba, Japan, pp. 60–66.

Booth R. H (1976) Storage of fresh cassava (Manihot esculenta). 1. Post-harvest deterioration and its control. Exp Agric 12: 103–111

Bradbury J. H (1988). The chemical composition of tropical root crops. Asian Food J., 4: 3-13

Buschmann H, Reilly K, Rodriguez MX, Tohme J, Beeching JR (2000) Hydrogen peroxide and flavan-3-ols in storage roots of cassava (Manihot esculenta Crantz) during postharvest deterioration. J Agric Food Chem 48: 5522–5529

Ceballos H, Iglesias AC, Perez JC, Dixon A (2004). Cassava breeding: opportunities and challenges. Plant. Mol. Biol., 56: 506–516.

Ceballos, H., T. Sanchez, K. Denyer, A.P. Tofino, E.A. Rosero, D. Dufour, A. Smith, N. Morante, J.C. Perez, and B. Fahy. (2008). Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). J. Agric. Food Chem. 56:7215–7222.

Chavez A. L, Sanchez T, Ceballos H, Rodriguez-Amaya D. B, Nestel P, Thome J, Ishitani M (2007). Retention of carotenoids in cassava roots submitted to different processing methods. J. Sci. Food Agric., 87:388-393.

Cortes D. F, Relly K, Okogbenin E, Beeching J, Iglesias C, Tohme J (2002). Mapping wound-response genes involved in post-harvest physiological deterioration (PPD) of cassava (Manihot esculenta Crantz). Euphytica, 128: 47-53.

El-Sharkawy, M.A. (2006). International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. Photosynthetica 44: 481–512.

FAO 2013. Food and agriculture organizations statistics database. FAO, Rome. http://faostat.fao.org/. Accessed Oct 2013.

Fregene M, Angel F, Gomez R, Rodriguez F, Chavarriaga P, Roca W, Tohme J, Bonierbale Halsey M. E, Olsen K. M, Taylor N. J, Chavarriaga-Aguirre P (2008). Reproductive biology of cassava Manihot esculenta Crantz and of tuberous roots of cassava Manihot esculenta Crantz. Ann. Bot., 86: 1153-1160.

Hiroshe S, Data E. S, Quevedo M. A (1984). Changes in respiration and ethylene production in cassava roots in relation to post-harvest deterioration. ln: Uritani I, Reyes ED (eds) Tropical root crops: postharvest physiology and processing, Japan Scientific Societies Press. Tokoyo, Japan, pp. 83-89.

Ihemere U, Siritunga D, Sayre RT (2008) Cassava. In C Kole, TC Hall, eds, Compendiumof Transgenic Crop Plants. 7. Transgenic Sugar, Tuber and Fiber Crops. Wiley-Blackwell, West Sussex, UK, pp 177–198

Kunttu, L., Lepisto, L., Rauhamaa, J., and Visa, A. (2003). Binary histogram in image classification for retrieval purposes. In Journal of WSCG, No. 1, WSCG'2003, February 3-7, 2003 (Plzen, Czech Republic: Copyright UNION Agency, Science Press), pp. 269–273.

Nelson JC. (1995) QGene: a computer package for the marker-based analysis of populations, user manual. *Http://Www.Qgene.Com*.

Njoku, D.N., Vernon, G.E., Offei, S.K., Asante, I.K ., Egesi, C.N. and Danquah, Y (2014). Identification of Pro-vitamin A Cassava (*Manihot esculenta* Crantz) Varieties for

Adaptation and Adoption through Participatory Research, *Journal of Crops Improvement, pp 212-120.*

Oirschot Q, O'Brien GM, Dufor D, El-Sharkawy MA, Mesa E (2000). The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. J. Sci. Food Agric., 80: 1866-1873.

Plumbley, R.A., Richard, J.E. (1991). Post-harvest deterioration of cassava. Tropical Science, 31, 295-303.

Ravi, V., Aked, J., Balagopalan, C. (1996). Review on tropical root and tuber crops I. Storage methods and quality changes. Critical Reviews in Food Science and Nutrition, 36, 661-709.

Reilly K, Berna D, Cortes DF, Gomez-Vazquez R, Thome J, Beeching J (2007). Towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration. Plant Mol. Biol., 64:187–203.

Reilly K, Yuanhuani H, Thome J, Beeching J (2001). Isolation and characterization of a cassava catalase expressed during post-harvest physiological deterioration. Biochim. Biophys. Acta, 1518: 317-323.

Rickard J. E (1982). Investigation into post-harvest behavior of cassava roots and their response to wounding. Thesis Ph.D. Biochemist University of London.

Rosegrant, M. W. (2008). Biofuels and grain prices: Impacts and policy responses. Sustainable solutions for ending hunger and poverty. Washington D. C. International Food Policy Research Institute, 1-4.

Sanchez T, Chávez AL, Ceballos H, Rodriguez-Amaya DB, Nestel P, Ishitani M (2006). Reduction or delay of post-harvest physiological deterioration in cassava roots with higher carotenoid content. J. Sci. Food Agric., 86: 634–639.

Sayre R, Beeching JR, Cahoon EB, Egesi C, Fauquet C, Fellman J, Fregene M, Gruissem W, Mallowa S, Manary M, et al (2011) The BioCassava plus program: biofortification of cassava for sub-Saharan Africa. Annu Rev Plant Biol 62: 251–272

Tanaka Y, Data ES, Hirose S, Taniguchi T, Uritani I. (1983) Biochemical changes in secondary metabolites in wounded and deteriorated cassava roots. *Agric. Biol. Chem.* **47**: 693-700.

Thro AM, Beachy RN, Bonierbale M, Fauquet C, Henry G, Henshaw G G, Hughes M A, Kawano K, Raemakers CJJM, Roca W, Schopke C, Taylor N, Visser RGF (1996). International research on biotechnology of cassava (tapioca, *Manihot esculenta* Crantz) and its relevance to Southeast Asian economics: a cassava biotechnology network review. Asian J. Trop. Biol. 2:1-30.

Uritani I, Data ES, Villegas RJ, Flores P, Hirose S (1983). Relationship between secondary metabolism changes in cassava. Agric. Biol. Chem., 47: 1591–1598.

Wenham, J.E. (1995). Post-harvest deterioration of cassava. A biotechnology perspective. FAO Plant Production and Protection Paper 130.NRI/FAO. Rome., pp90.

Westby, A. (2002). Cassava utilization, storage and small-scale processing. In: Hillocks, RJ., Thresh, J.M., Bellotti, A.C. (Eds.) Cassava Biology, Production and Utilization CABI publishing Oxfordshire, UK., pp 480.

Wheatley C, Lozano C, Gomez G (1982). Deterioration and storage of cassava roots. In Cassava: Research, Production and Utilization, Cassava Program, International Center for Tropical Agriculture, Cali, Colombia.

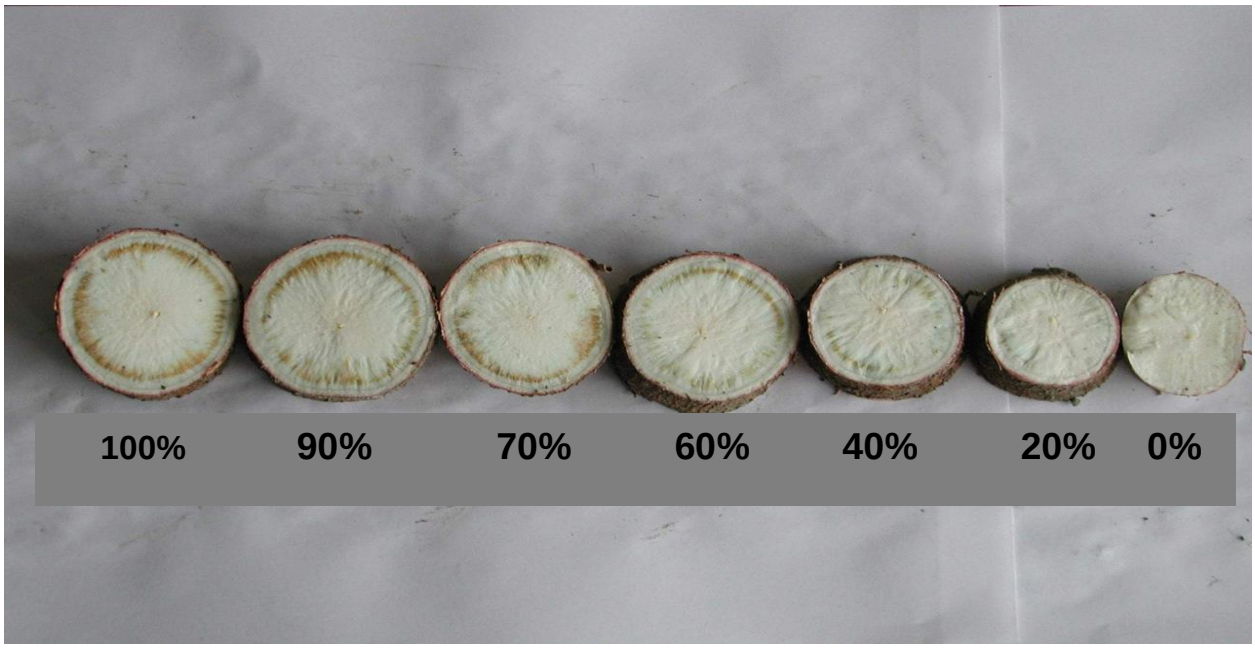

Fig.1: Common scoring pattern of cassava roots for delayed post-harvest deterioration.