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Post-Harvest Physiological Deterioration of Cassava Roots (*Manihot esculenta* Crantz) During Storage Under Different Temperatures

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Abstract

Cassava storage roots are highly perishable owing to a phenomenon called post harvest physiological deterioration (PPD). PPD is thought to be result of failed wound response involving various biochemical pathways, reactive oxygen production, changes in phyto-hormones, oxidative enzymes and secondary metabolite production etc.. Storage temperature alter the biochemical processes in root tissue and hence three different storage temperatures such as low $(8\pm2^\circ\text{C})$, ambient $(28\pm4^\circ\text{C})$ and high temperature $(40\pm2^\circ\text{C})$ on the onset of PPD in five different varieties of cassava were studied. Root respiratory rate, oxidative enzymes such as catalase, peroxidase activities were studied for 1, 3 and 6 DAS and correlated with PPD scores. Storage temperatures influenced the respiratory rate as well as the activities of CAT and POX during storage. Varietal variations were noted for respiratory rate and enzyme activities with different storage temperatures. High temperature storage reduced the respiratory rate of roots compared to ambient temperature storage. CAT and POX activities were correlated positively during 1 and 3 DAS and negatively at 6 DAS. PPD positively associated with POX at 6 DAS. Storage of roots under high temperature (40°C) with high RH (80-90%) delayed the onset of PPD and extended shelf-life of cassava roots for a week.

Key words : Modified storage, cassava storage root, physiological deterioration, biochemical changes

Introduction

Cassava is a versatile food and industrial crop, cultivated throughout the tropics for its starch-rich storage roots. It is considered as a climate resilient crop and has potential to alleviate hunger in many parts of the world. It is the cheapest source of calories for both human consumption and animal feed (Tonukari, 2004). The estimated increase in global harvested area of cassava was 44% between 1980 and 2011, from 13.6 million to 19.6 million hectares respectively, which was the biggest percentage increase among the world's five major food crops (FAO, 2013). Cassava root is highly perishable and it deteriorates quickly (within 48 -72 hrs) after harvest and become unfit for both human and animal consumption and also for other industrial uses due to post harvest physiological deterioration (PPD) (Ravi et al. 1996; Saravanan et al. 2015).

PPD of cassava is recognized as one of the major constraints for expansion of its cultivation in many parts of the world (Wenham, 1995). The traditional practice of partial harvesting of roots in subsistence farming communities can delay the complete use of the roots per cassava plant, but the practice remains unfavourable in commercial fields. Furthermore, the remaining roots after partial harvest are subjected to a loss of starch content, decline of palatability due to the increase of fibre content and an associated increase in cooking time (Rickard and Coursey, 1981). Increasing the shelf-life of cassava storage roots is desirable not only to solve problems of utilization and marketing but also to facilitate the conversion of cassava from a traditionally famine reserve crop and rural food staple to a cash crop. PPD of fresh cassava roots restricts cultivation of this crop to nearby consumption areas, as the roots are to be transported from the farm to

urban markets and for industries for starch production and other value added products with minimal loss (Janssen and Wheatley, 1985; Wenham, 1995). Delays caused during transportation and processing decrease the quality of fresh roots by PPD and result in heavy losses and inferior quality products. Therefore, research directed towards introducing resistance to PPD, or delaying the onset of PPD symptoms, is considered a priority by international bodies such as the Food and Agriculture Organization of the United Nations (FAO) and the Cassava Biotechnology Network.

Several lines of evidence suggest that the PPD in cassava roots is an enzymatic, endogenous oxidative process. Initial symptom of PPD generally starts with a bluish grey to black vascular discoloration (commonly referred as vascular streaking) which quickly spreads to the parenchyma tissue. It was noticed that harvested roots accumulate colourless deposits which emits intense fluorescence under UV light, well before the actual visible symptoms in the storage tissue. Increases in the activities of polyphenol oxidase and peroxidase and other oxidative enzymes were also reported (Rickard, 1982; Rickard, 1985). PPD of cassava root is considered as a complex abiotic wounding stress response (Westby, 2002; Beeching et al. 2002; Zainuddin et al., 2018). Being a storage organ for photosynthates with no regenerative function as a propagule with reproductive function, cassava roots lack the biological need to repair wounds when detached from the plant (Reilly, et al., 2004). PPD symptom progresses from proximal end to distal part of roots after harvesting through intercellular signals transmitted from the sites of damage to other parts. Apart from the involvement of hormones like ethylene and signal molecule hydrogen peroxide, it remains widely unknown which other signalling molecules are involved in the wound response network and how their interplay affect PPD in cassava (Saravanan et al., 2016). Wounding of roots causes various physiological changes, including increased respiratory rate and water loss and triggers the production of signaling compounds, such as reactive oxygen species (ROS), jasmonic acid, salicylic acid and ethane (Liu et al. 2019a, 2019b). ROS production occurs rapidly in detached cassava roots: superoxide within 15 min, ¹O₂ within 3 or 4 h, H₂O₂ within 3-24 h and hydroxyl radical within 36 h of injury (Buschmann et al., 2000b; Reilly et al., 2004; Iver et al., 2010). A rapid increase in calcium ion (Ca^{2+}) in roots after wounding was proposed to play a significant role in the induction of the oxidative burst during the first stages of PPD onset (Djabou et al., 2017) and a crosstalk among calcium signaling, ROS, programmed cell death (PCD) and melatonin during PPD was also suggested (Hu et al., 2018).

Varietal variations for PPD development has been reported (Saravanan et al., 2015) and various factors including pruning of plants before harvest (Luna et al., 2021) and method of storage influences the storability of different cassava varieties (Ravi et al., 1996). Breeding for delayed PPD of roots and genetic engineering of cassava to extend the shelf-life of storage roots met with little success in the past. Modifying the storage conditions offer cheap and reliable ways to prolong the storability of roots. The simplest method that many farmers use is in-ground storage or staggered harvesting i.e. harvesting the crop when it is needed (Ravi and Aked, 1996). This is made possible by the fact that cassava does not have a distinct period of physiological maturity (Wenham, 1995). The harvesting window is thus flexible between 6-24 months depending upon requirement like human consumption or as animal feed or industrial purpose. Occasionally, cassava crop is retained in the field for 1 or 2 months after attaining maturity for better price or to increase the starch content in roots. This strategy has several disadvantages as prolonged in-ground storage may increase chances of pathogen infection (Westby, 2002). At the same time the roots become woody (fibrous) due to lignifications or spongy and there can be loss of quality (Lancaster and Coursey, 1984). Lastly, in-ground storage locks up land that can otherwise be productively used.

Cassava storage roots could be successfully stored using different storage methods (Ravi et al., 1996). Storage in clamp silos, where roots are piled up on a layer of straw in conical heaps weighing between 300 and 500 kg and covered with straw and soil with openings left for ventilation, has been found to be effective for four weeks (Ravi and Aked, 1996; Rickard and Coursey, 1981; Westby 2002). Box storage, with sawdust or coconut husk is also effective for four weeks (Westby, 2002). Moisture content of the sawdust requires careful control and lining the crates with plastic foil prevents drying out of the sawdust resulting in a storage period of 4-8 weeks (Ravi and Aked, 1996; Rickard and Coursey, 1981). Storage of cassava roots under cold conditions had been reviewed by Ravi et al., 1996. Low temperature storage is another strategy used to delay deterioration in cassava, although it is seldom practical for smallholder farmers. The most favourable temperature for storing fresh cassava is 3°C. At this temperature, the total weight loss after 14 days was 14% and was 23% after 4 weeks (Rickard and Coursey, 1981). Alternatively, roots, or pieces of root, can be stored frozen. Freezing changes the texture making it somewhat spongier, but the flavour is preserved (Rickard and Coursey, 1981). Roots stored at low temperature deteriorate faster when taken back to room temperature. Storage methods that cut off oxygen, such as storing in a water bath, are also practiced (Plumbey and Rickard, 1991). Since PPD in the fresh cassava roots after harvest, is an enzymatic process in response to wounding of the tissue and role of anti-oxidant enzymes in modulating the processes leading to PPD symptom development have been reported (Shang et al., 2021). The study was conducted to analyzed the effect of different storage temperatures on PPD development in roots of selected varieties of cassava and to understand the biochemical basis of the process through the changes in activities of anti-oxidant enzymes during the storage and PPD development.

Materials and Methods

Root treatments and PPD evaluation

The cassava roots were harvested carefully (8-10 months after planting) with minimal damage to them and were washed thoroughly in tap water to remove the soil and other dirt adhering to them. The roots were surface dried kept for 15-20 minutes and taken for experimental purpose to study the effect of different storage temperatures on PPD. The experiment was conducted with roots of five cassava varieties viz., Sree Athulya, Sree Jaya, Vellyani Hraswa, Kalpaka and Sree Padmanabha were kept at, i) room temperature in steel rack, ii) at 40°C for high temperature treatment and iii) in refrigerated condition at $8 \pm 2^{\circ}$ C. The roots were kept for a week and PPD development was studied. Sampling was done with three roots for each treatment at 1, 3 and 6 days of storage.

For scoring of PPD expression, the roots were randomly chosen and cut into three equal parts transversely from proximal end to distal end and visually scored in a scale of 1 to 5 following Salcedo et al., (2010) with modifications. The PPD scores were given as no damage (score 1), upto 25% damage (score 2), 26-50% damage

(score 3), 51-75% (score 4) and fully damaged root slice (score 5). The mean PPD score for each root was calculated by averaging the scores for the 3 transverse sections.

Determination of starch and total sugar

Starch was determined by titrimetric assay using potassium ferricyanide with methylene blue as indicator of end point of titration following the method by Moorthy and Padmaja (2002). Dried cassava root tissue (2g) was sliced and cut into small cubes of approximately 0.5 cm³ and taken in 100ml flask. The root samples were extracted with 20ml 80% ethyl alcohol for overnight. The extract was filtered (Whatman No. 1) and collected separately for sugar estimation. To the residue 20 ml 2N HCl (Hydrochloric acid) was added and taken in a flask and kept on hot plate at 100 °C for 30min. The content was cooled and volume was made up to 100ml with double distilled water. The starch was completely hydrolyzed by treatment with 2N HCl while the non-reducing sugars were converted into reducing ones. Analysis of both the components was done in a similar manner, based on the number of reducing groups. The supernatant was directly used for starch estimation.

To a 100 ml flask, 10 ml of Potassium ferricyanide (1%) was pipetted out followed by 5 ml of NaOH (2.5 N) and the contents were mixed thoroughly. The flask was kept over the flame for boiling. When the reagents began boiling, the flame was lowered and 3 drops of dilute methylene blue was added. The solution turned to bluegreen. The starch hydrolysate was taken in a 2 ml blow pipette during starch estimation and added drop by drop to the boiling reagent, while for sugar estimation, the sugar extract was taken in a 10 ml blow pipette (since the titre value will be 5-8 ml). The nearing of end point was indicated by change of colour from blue-green to violet. A few more drops were added carefully, to reach the end point, which was indicated by the rapid disappearance of the violet colour. At this stage, the titre reading was noted, Titrations were repeated for each of the aliquots and starch content and total sugars were estimated based on the calculation as given below,

Total Starch;

Volume of potassium ferricyanide X Makeup volume X 0.9 X 100

Titre volume x Weight of sample x 1000

Root respiration measurement

Root respiratory rate was recorded for intact roots using LI-7000 soil respiration system (Li-Cor Inc. Lincoln, USA) by covering the chamber bottom air tight. Intact roots of cassava were weighed and kept in the measuring chamber and the respiratory CO_2 flux (μ mol CO_2 per kg) was measured for three minutes at 15s interval. Three replicate measurements were made for each variety.

Determination of protein

Protein concentrations were estimated according to Bradford's method using bovine serum albumin as standard (Bradford, 1976). The commercial protein assay reagent (5X - Bradford reagent) was diluted to a working solution (1:5) with distilled water. Standards of known concentrations in the range of 0-50 μ g protein (bovine serum albumin BSA) were prepared and 5 μ l of the protein standards and of the samples were added to 1 ml of the diluted assay reagent. The samples were mixed by vortexing briefly and allowed to incubate for 15 minutes. Absorbance of the samples was then measured at 595 nm against a blank containing no proteins and the readings noted. A standard curve of absorbance versus micrograms of protein was prepared and the concentration of protein in the samples determined from the curve.

Measurement of antioxidant enzyme activities

Sample preparation for enzyme assays. Five gram fresh cassava root sample was homogenized in 20 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA (ethylenediamine-tetraacetic acid) and 1% insoluble polyvinylpyrrolidone. The extract was centrifuged at 8000 rpm using Sorvell table top refrigerated centrifuge at 4°C for 10 minutes and the supernatant was stored in minus 20°C until for enzyme analysis.

a. Assay of catalase:

Catalase (EC1.11.1.6) activity was measured following Aebi(1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and enzyme extract. Catalase was assayed at 25°C following the decomposition of H_2O_2 by the decline in the absorbance at 240 nm (E=26.6 mM⁻¹cm⁻¹) using Lamda 50 (M/S Perkin Elmer, USA) UV-Vis spectrophotometer. Two technical replicates were carried out for each standard and experimental sample. Samples were standardized using the total protein content to

account for differences in protein extraction efficiency between samples.

b. Assay of guaiacol peroxidase:

Peroxidase activity (EC1.11.1.7) was measured according to the method described by Lin and Kao (1999). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 20 mM H_2O_2 , 0.05% guaiacol, and the enzyme extract. Peroxidase activity was measured as the rate of the oxidation of guaiacol at 470 nm. The molar extinction coefficient of tetraguaiacol (26.6 mM⁻¹cm⁻¹) was used in calculating the enzyme concentration. One unit of peroxidase was defined as the amount of enzyme that caused the formation of 1 mM of tetraguaiacol per minute.

Statistical analysis

All statistical analyses including ANOVA and correlation studies were carried out using R Studio statistical package (R Studio, 2019).

Results and Discussion

Morphological parameters like fresh root weight, root length, root girth of five selected cassava varieties are presented in Table 1. Root characters significantly differed for the varieties. However, the root dry matter (DM) content of the varieties did not vary significantly even though the starch content differed among them. Mean fresh root weight was highest for landrace Kalpaka (1235 cm) with maximum root girth (24.7 cm) whereas, Sree Athulya had the longest roots. The dry matter content varied from 35% to 40% among the varieties studied, however DM of individual tubers within the same variety varied widely and hence difference in DM was not significant for the varieties studied. The starch content of Sree Padmanabha and Sree Athulya were higher comparable to other varieties.

The respiratory CO₂ flux was measured for intact cassava roots after 1, 3 and 6 days after storage and presented in Fig. 1. Root respiratory flux (R_{res}) was higher in roots stored at ambient temperature compared to high or low temperature. Among the varieties studied, Vellayani Hraswa and Sree Jaya showed higher respiratory rate of 99.6 and 90.1 μ mol min⁻¹ kg⁻¹ fresh root respectively, under ambient/room temperature storage at 6 DAS. Sree Athulya was consistently had low respiratory CO₂flux under all three temperature regimes. Combined

| Variety | Mean fresh | Root length | Root girth (cm) | Dry matter | Starch | | |
|------------------|-------------------|--------------------|---------------------|--------------------|--------------------|--|--|
| variety | | Root lengui | Root gir tir (CIII) | 5 | | | |
| | weight of | (cm) | | content (%) | content (%) | | |
| | roots (g) | | | | (Dry weight basis) | | |
| Vellayani Hraswa | 768 ^b | 34.3 ^{bc} | 20.7 ^b | 35.4 ^{NS} | 80.2 ^{ab} | | |
| Sree Athulya | 866 ^b | 37.8° | 15.8ª | 40.3 ^{NS} | 85.4 ^c | | |
| Kalpaka | 1235 ^c | 32.6 ^b | 24.7° | 37.8^{NS} | 79.8ª | | |
| Sree Jaya | 412ª | 31.2 ^b | 14.2ª | 35.6 ^{NS} | 84.5 ^{bc} | | |
| Sree Padmanabha | 523ª | 26.4ª | 15.0ª | 35.5^{NS} | 86.0 ^c | | |

Table 1. Root characters, dry matter and starch content of selected cassava varieties



Fig.1. Respiratory CO₂flux of storage roots of cassava varieties as affected by temperature. Results are average of three samples.

respiratory rate of all the varieties averaged 40.0, 56.5 and 63.5 μ mol min⁻¹ kg⁻¹ during 1, 3 and 6 DAS respectively under ambient storage (Fig 2). Whereas, mean R_{res} of all the varieties was the lowest under low temperature stored roots with average values of 18.4, 16.8 and 19.3 μ mol/min/kg during 1, 3 and 6 DAS. R_{res} values varied widely among the varieties in the ambient temperature storage at 3 and 6 DAS compared to other storage temperatures. Respiratory rate during storage increased under both ambient and high temperature from 1 to 6 days in most of the varieties studied except Sree Padmanabha which showed nearly 30% decrease in CO₂flux at 6 DAS under high temperature.

Catalase activity was measured in the roots at 1, 3 and 6 DAS is presented in Fig. 2. CAT activity modulated in cassava roots over the storage period as well as storage temperature. Temperature had a marked influence on catalase activity. Maximum catalase activity was recorded at 1DAS in all the varieties studied irrespective of temperature regime. Highest CAT activity of 56.32 units



Fig. 2. CAT activity measured in the roots of cassava after 1, 3 and 6 days after storage as affected by temperature regimes.

mg⁻¹protein was observed in variety, Kalpaka under low temperature and the least (3.06 units mg⁻¹ protein) was observed in Sree Padmanabha roots stored under high temperature at 3 DAS. CAT activity decreased drastically during storage under all temperature regimes. Under ambient temperature storage, Sree Padmanabha had the highest reduction (~70%) of CAT activity at 3 DAS compared to 1 DAS. Catalase activity was highest in roots stored at low temperature followed by ambient/room conditions. Roots had the lowest CAT activity when stored at high temperature.

Peroxidase activity (POX) in root tissue of cassava showed a clear increasing trend from initial storage to later stages (Fig. 3). The POX activity in cassava varieties significantly increased from 1 to 6 DAS. Highest peroxidise activity was recorded in the variety Sree Padmanabha at 6 DAS with ten-fold increase (8.8 ± 1 unit per mg protein) compared to POX activity at 1 DAS. The increase in POX activity was less prominent in Kalpaka compared to other varieties studied. Low temperature storage markedly



Fig. 3. Peroxidase activity measured in the roots of cassava after 1, 3 and 6 DAS as affected by temperature regimes.

affected the POX activity with minimal alteration compared to other coregimes.

PPD scores of roots under different storage temperatures significantly varied. Storage temperature had influenced the metabolic processes in roots which resulted in lower PPD symptom development in low and high temperature regimes (Fig. 4). Significantly higher scores for all the varieties was under ambient storage, whereas roots stored under high temperature had low vascular streaking compared to ambient as well as low temperature. Cassava varieties significantly differed for the PPD scores under low temperature during the initial period (3 DAS) with lowest PPD score in Kalpaka.

Pearson correlation coefficient calculated for root respiratory rate, enzyme activities like CAT and POX and PPD score at different storage periods. There were



Fig. 4. PPD scores of cassava roots after 3, 6 and 9 days after storage in different temperature regimes.

significantly positive correlation between root respiratory flux at 3 and 9 DAS to the CAT and POX activities among the cassava varieties. However, root respiration did not show significant correlation with PPD, however at 3 and 6 DAS, there was a positive correlation between root respiratory rate and PPD symptom development. There were significant positive correlation for activities of both CAT and POX enzymes and PPD at 6 and 9 DAS of storage (Table 2).

The physiological and biochemical changes in detached cassava root lead to PPD. It is evident from the fact that cassava root indeed heal after wounding if the roots remain attached to the plants (Plumbley and Richard 1991; Reilly et al. 2004). However, the roots lose their down regulation of wound induced changes and subsequent repairing mechanism once they are detached from the plant. Unlike other tuber crops, cassava storage root lacks the active apical or intercalary meristem and hence wound healing and repairing of wounded tissue does not take place. The physiological processes during PPD are controlled by various enzymes and inhibition of those enzymes can alter the PPD progress. Treatments such as hot water treatment (53°C for 45 min), storage root under water or storage under anaerobic conditions and treatment with cycloheximide (a protein synthesis inhibitor) had resulted in inhibition of the visual symptoms of PPD and associated fluorescence (Uritani et al. 1984; Ravi and Aked, 1996). Several types of PPD avoidance storage techniques had been employed by cassava consuming communities around the world. Amazonian Indians successfully stored fresh cassava roots traditionally by burying them in the soil. This practice is also followed in Kerala, India but to a very limited extent. In Mauritius, fresh cassava roots were stored in straw-lined trenches up to 12 months period (Booth and Coursey, 1974). The roots can also be coated with a loam paste to attain a storage ability of 4 - 6 days (Rickard and Coursey, 1981).

In the present study, root respiratory CO_2 flux was the highest in roots stored at ambient conditions during the storage period of 6 days. Among the varieties studied, Vellayni Hraswa and Sree Jaya had maximum respiratory activity of 99.3 and 90.1 μ mol min⁻¹ kg⁻¹ fresh root respectively at 6 DAS under ambient/room temperature. Sree Athulya consistently had low respiratory CO_2 flux under all three temperature regimes. The increase in root respiratory rate during storage indicated the active cellular

| Para meter | CAT1 | CAT3 | CAT6 | POX1 | POX3 | POX6 | PPD-D3 | PPD-D6 | PPD-D9 | Res-D1 | Res-D3 | Res-D6 |
|------------|--------|--------|--------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
| CAT1 | 1.00 | | | | | | | | | | | |
| CAT3 | 0.87* | 1.00 | | | | | | | | | | |
| CAT6 | 0.91* | 0.89* | 1.00 | | | | | | | | | |
| POX1 | 0.29* | 0.39* | 0.35* | 1.00 | | | | | | | | |
| POX3 | -0.24 | -0.25 | -0.33* | 0.23 | 1.00 | | | | | | | |
| POX6 | -0.55* | -0.59* | -0.59* | 0.10 | 0.32 | 1.00 | | | | | | |
| PPD-D3 | 0.34* | 0.17 | 0.39* | 0.20 | -0.15 | 0.14 | 1.00 | | | | | |
| PPD-D6 | 0.54* | 0.30* | 0.49* | 0.26* | -0.04 | 0.11 | 0.74* | 1.00 | | | | |
| PPD-D9 | 0.46* | 0.19 | 0.41* | 0.03 | -0.04 | -0.03 | 0.67* | 0.79* | 1.00 | | | |
| Res-D1 | -0.21 | -0.40* | -0.22 | 0.13 | 0.42* | 0.29 | 0.24 | 0.20 | 0.14 | 1.00 | | |
| Res-D3 | -0.16 | -0.41* | -0.22 | -0.16 | 0.37* | 0.07 | -0.00 | 0.08 | 0.21 | 0.73* | 1.00 | |
| Res-D6 | -0.16 | -0.36* | -0.23 | -0.16 | 0.33* | -0.01 | -0.03 | -0.01 | 0.16 | 0.71* | 0.91* | 1.00 |

Table 2. Pearson correlation co-efficient for cassava root respiratory flux, activity of oxidative enzymes and PPD intensity during storage

processes during storage. The flux of skeletons required for various metabolic processes inside the cell are met from starch hydrolysis and changes in the enzymatic activities like POX and PAL corroborate the findings. Altered membrane peroxidation might increase permeability and leakage of lytic enzymes from cellular compartments, thus increasing catabolic activity and respiratory rate as well. Method such as clamp storage of cassava (Richard and Coursey, 1981) resulted in altered physiology of cassava roots and increased the shelf-life in effect. In such storage system, a conical pile of 300-500 kg of fresh cassava roots was seated on a circular bed of straw and covered with straw. The whole unit was covered with soil to a thickness of 10 - 15 cm. With this storage system, acceptable levels of loss (upto 20 per cent) were achieved for periods of up to 2 months. In this type of storage system injured roots tended to undergo a woundhealing response that prevent vascular discoloration or reversed it. This 'curing' was correlated with a resistance to discoloration by application of exogenous scopoletin (Wheatley and Schwabe, 1985). However, clamp storage performed poorly during the hot season. The temperature inside the clamp easily reached 40°C, and heavy losses resulted even after 1 month of storage (Booth and Coursey, 1974). Cassava roots packed in boxes containing adsorbent material such as sawdust (Rickard and Coursey, 1981) had low PPD. The relative humidity inside the box was found to be critical for a successful storage. When the humidity was too high, deterioration due to bacteria and fungi rapidly caused damage to roots. If the humidity was too low, vascular deterioration was not prevented. Packing cassava roots in polyethylene bags successfully preserved the roots for about 2 months (Ravi et al., 1996). However, complete loss of the stored roots occurred as a result of microbial deterioration. Treating the roots with fungicides retarded the onset of spoilage (Rickard and Coursey, 1981). High temperatures above 40°C as well as low temperatures below 10°C had positive effect on the duration of storage. Cold storage of cassava roots can also prevent and delay PPD. When kept below 4°C, cassava roots did not show internal discoloration (Rickard and Coursey, 1981). Low temperatures extend the storage ability of cassava roots by delaying the rotting which occurs rapidly at normal storage temperatures. Experiments have shown that most favorable temperature for the storage of fresh cassava roots is 3°C. When roots stored at this temperature, the total loss after 14 days amounted to 14% and after 4 weeks it was 23% (Rickard and Coursey, 1981). But microbial damages can happen during storage if appropriate temperature regime is not maintained during storage. A bluish mould occurs on the surface of the roots at higher storage temperatures ($> 33^{\circ}$ C) and the flesh of the roots turns brownish. Cassava roots could be kept satisfactorily under deep-freeze conditions but textural changes might result. Deep freezing of cassava is not recommended primarily due to its high-cost involved (Ravi et al., 1996). High temperature storage linked to modulation of root respiratory flux, reactive oxygen scavenging enzymes like CAT and POX and hence exhibited the causal relationship with PPD expression.

The findings will help to identify ways to increase shelf life of cassava roots. Storing fresh cassava roots at relatively high temperature (40°C) immediately after harvest with high humidity favorably altered the anti-oxidant enzymes and helped in enhancing the shelf-life compared to ambient temperature.

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