

A COMPARATIVE STUDY OF POST-HARVEST PHYSIOLOGICAL DETERIORATION OF CASSAVA (*MANIHOT ESCULENTA*) GENOTYPES: VISUAL VS. IMAGE-BASED SCORING METHODS

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ABSTRACT

Cassava (Manihot esculenta) is one of the most important staple crops for millions, contributing significantly to food security and economic growth, especially in sub-Saharan Africa. A major threat to cassava commercialization is postharvest physiological deterioration (PPD), a rapid phenomenon unique to the storage roots of cassava. Evaluation for PPD remains challenging and traditionally, the methods are subjective, relying on the visual analysis of deterioration. The main objective of this study was to determine the accuracy of two image analysis software for evaluating PPD onset and delay. Seven cassava accessions were evaluated for PPD tolerance, at 5, 10 and 15 days after harvest, using three independent methods; a standard visual analysis method and two image analytical software. No correlation was observed between the visual method and either of the image-based analysis software. According to the standard visual analysis NR090182 showed the least deterioration at 15 days after harvest.

Keywords: Post harvest physiological deterioration, Cassava genotype, Image analysis software,

1.1 INTRODUCTION

Cassava (*Manihot esculenta*), is an important staple crop and serves as a major source of carbohydrate for about 800 million people, worldwide (Patil *et al.*, 2015). The crops ability to remain viable for up to 3 years underground and its drought tolerance has made it an important food security crop during famine and armed conflicts (Rosenthal & Ort, 2012). Cassava is increasingly being considered an industrial crop, and in countries like Brazil and China its starchy roots are utilized for biofuel (Rabbi *et al.*, 2014). Global cassava production is estimated at 227.8 million metric tons with Africa accounting for about 61% (F.A.O, 2018). There are several factors inhibiting cassava production, but the short shelf life of its roots due to Post-harvest Physiological Deterioration (PPD) is a major inhibiting factor (Uarrota *et al.*, 2016). Described as an endogenous disorder unique to cassava storage roots, PPD is characteristically rapid, occurring within 3 days after harvest and reducing considerably the crops root palatability and commercial utilization (Reilly *et al.*, 2007). When the storage root is mechanically damaged, during harvest, a blue back discoloration extends from the wound site and spreads throughout. This discoloration is attributed to the oxidation of phenols, mediated by peroxidase (Xu *et al.*, 2013). An assessment of the economic impact by delaying PPD in cassava by several weeks revealed that in Nigeria alone, losses would be reduced by \$2.9 billion (Rudi *et al.*, 2010). Proven means of delaying PPD in cassava storage roots and largely maintaining their

quality include coating in paraffin wax, storing in plastic bags and freezing at 4°C (Zidenga *et al.*, 2012). But these techniques have not been widely adopted because they are expensive and impractical for such a low-cost commodity. The focus of some breeding programs in Africa, is developing cassava cultivars with delayed PPD onset. A robust method for assessing or scoring PPD tolerance in cassava cultivars is essential. For many years, scientists at the National Root Crop Research Institute (NRCRI) have employed the use of CIAT standard method (Wheatly & Gomez, 1985), which is scoring based on a visual scale. Studies have shown that because of the subjective nature of this method, PPD scores vary significantly (Zainuddin *et al.*, 2018). There is a need for a more objective quantitative method of assessing PPD tolerance and in this paper, we assess two image-based software, used for PPD tolerance evaluation.

2.1 MATERIALS AND METHODS.

2.1.1 Study Area

Seven cassava accessions from the Genetic Resource Unit (GRU) of the National Root Crops Research Institute, Umudike were evaluated for PPD onset at 5, 10 and 15 days after harvest (DAH). They included; COB-5-01, CR528-26, CW451-33, NR060251, NR090182, NR100417 and NR110490. The plants were grown within NRCRI (latitude 05°48' and longitude 07° 54'), which is located in the humid forest zone and is eight kilometres east of the state capital of Abia, Nigeria. The study area

occupies a land with an area of 1000 hectares and an altitude of 122m above sea level

Labouratory analysis (PPD initiation)

At twelve months after planting, roots were harvested and commercial sized roots of more than 18cm, with no mechanical damage were selected. The proximal and distal ends were cut till the remaining root section was at least 15cm long. This induces the onset of PPD. The distal end of the root was covered with PVC film to maintain the moisture content and inhibit the development of deterioration from that end of the root. Physiological deterioration developed only from the proximal end. The selected roots were stored at room temperature, away from sunlight, until the day of evaluation.

2.1.2Data (PPD evaluation)

On each day of evaluation, seven transverse sections at 2, 4, 6, 8, 10, 12 and 14cm were cut from the proximal end of a storage root (Figure 1a). Digital

images of the root slices were taken, 50 cm away, using a Canon camera under standard light conditions and without ultraviolet light exposure. Three independent methods were employed, to evaluate PPD onset and tolerance. They were: The standard CIAT method which is a visual scoring method while PPD Symptom Score Software written in Mat-Lab(Vanderschuren *et al.*, 2014) and the PPD analyser (Semi Automated), developed by the Artificial intelligence and Data Science Research Lab (mCrop) are both imaged based scoring methods. Briefly, the standard CIAT method is mostly based on a visual 0 - 10 scale, corresponding to increasing percentages of PPD symptoms (0 = 0% deterioration to 10 = 100% deterioration). Only the periphery of the parenchyma is evaluated by seven observers (Figure 1b) since the central tissues rarely deteriorate. For each accession, the scores from the observers were averaged and a score assigned.



Figure 1: (A) Transverse slices of 2, 4, 6, 8, 10, 12 and 14cm were cut from the proximal end of the root. (B) seven observers evaluated and scored cassava slices for PPD symptoms.

For the PPD Symptom Score Software (PPD S), three slices were selected from the seven, representing the distal, central and proximal regions of the root. Each image was manually marked with a red polygon covering the affected area on the slice. The software then converted the colour image into a

grey value image and determined its histogram (Figure 2a). The range of the grey value interval, covering 95% of the occurring grey values, was normalized with the 97.5% quantile to generate a PPD score, which ranges in theory from 0 to 1.

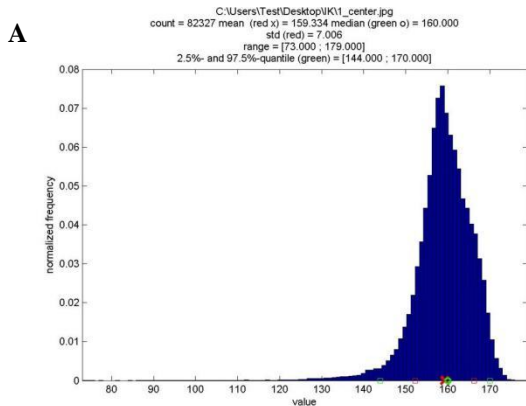


Figure 2: (A) Histogram of grey values generated from PPD Symptom Score Software, used to score for PPD; (B) PPD score generated from the PPD analyzer (Semi-automated)

Images of the seven transverse sections of a cassava accession was uploaded to the PPD analyzer (PPD A) generating an image with the affected areas highlighted and a PPD score, in percentage, at the top right (Figure 2b).

2.1.3 Statistical analysis

The PPD values were expressed in percentages. The data were logit transformed before analysis and back-transformed using expit transformation. Data were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's HSD test at 5% probability. The assumptions of ANOVA including: Shapiro-Wilk test for normality of residuals and Levene test for equality of variance were conducted. All statistical analysis was carried out using an open source R environment version 3.6.2.

Anova model

$y_{ij} = \alpha_i + \varepsilon_{ij}$, where $\varepsilon_{ij} \sim N(0, \sigma^2)$ are independent and $\sum \alpha_i = 0$; $\mu = \bar{Y}_{..} = \sum_{i,j} \frac{y_{ij}}{n}$; $\hat{\alpha}_i = \bar{Y}_{i.} - \bar{Y}_{..}$

Shapiro-Wilk test

The Shapiro-Wilk test statistic W is defined as:

$$W = \frac{(\sum_{i=1}^n a_i y_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

where W is bounded by 0 and 1, and the expected value of W converges to 1 for Gaussian input data as the sample size is increased. The expected value of W becomes smaller as the input signal becomes non-Gaussian (Yap & Sim 2010).

Levene Test

$$F = \frac{\sum_{j=1}^k n_j (\bar{z}_j - \bar{z})^2 / (k-1)}{\sum_{j=1}^k \sum_{i=1}^{n_i} (z_{ij} - \bar{z}_j)^2 / (n-k)}$$

The Levene test rejects the hypothesis that the variances are equal if $F > F_{(k-1, n-k, \alpha)}$ where $F_{(k-1, n-k, \alpha)}$ is the upper critical value of the F-distribution with $k-1$ and $n-k$ degrees of freedom at a significant level of α (Levene, 1960). A correlation analysis was also conducted to determine the relationship of PPD analyzer and the PPD symptom score software against the standard CIAT method.

3.1 RESULTS AND DISCUSSION

The diagnostic checks of analysis of variance at 5, 10 and 15 days after harvest are presented in Table 1. The normality check was done on the residuals at 5, 10 and 15 days and the corresponding p-values ($p > 0.05$) suggest that the residuals are drawn from a normal population. Similarly, homogeneity of variance test ($p > 0.05$) suggest that there exists equality of variance among the sub-groups. Cassava genotypes play a significant role in the levels of tolerance to PPD, with dry matter and carotene content being two major characteristics that allow and inhibit deterioration. Previous studies have reported that 5 days after storage, metabolic changes associated with PPD occur (Salcedo *et al.*, 2010).

Evaluation started 5 days after harvest (DAH) and to determine tolerance, roots were further evaluated for 10 and 15 DAH. As seen in Table 2, 5 DAH, visual assessment (CIAT method) showed significant differences ($p > 0.05$) among the cassava accessions with respect to degree of PPD deterioration. CR528-26 and NR100417 gave the highest PPD deterioration at 35.73% and 38.93% respectively while COB-5-01 and NR090182 had the least PPD deterioration at approximately 2%. For PPD S there was significant differences among the accessions while there was no significant difference for PPD A. COB-5-01 had the lowest degree of PPD deterioration for all of the methods on day 5. On day 10, CR528-26 gave the highest degree of deterioration with the CIAT and PPD S methods, while NR100417 had the highest deterioration score with the PPD A method. There was no significant difference among the accessions. On the last day of evaluation (15 DAH) there was high significant difference among the cassava accessions for the CIAT and PPD S methods, with CR528-26 giving the highest degree of deterioration at 52.97% and 41.73% for each method, respectively. For the PPD A method, CW451-33 gave the highest PPD score at 50.7%.

Effort has been put into improving the nutritional quality of cassava, especially β -carotene (precursor to Vitamin A) enrichment. Vitamin A is required for the proper functioning of vision and the immune system, of which many cassava consumers lack (Akinwale *et al.*, 2010). Besides from the nutritional benefits of β -carotene, studies reveal that high levels of carotenoids in cassava roots delay PPD. This may be attributed to the antioxidant properties of carotenoids that halt ROS production (Xu *et al.*, 2013). It is important to note that the yellow storage root of some cassava genotypes is attributed to high levels of carotenoids. Several studies have also revealed verifiable links between carotenoids and dry matter content in cassava. Akinwale *et al.* (2010) reported a negative correlation between dry matter and total carotene/ β -carotene content. Even in sweet potato, negative correlations have also been observed between the two very important agronomic traits (Rukundo *et al.*, 2013). The accession NR090182 appeared to have a yellow flesh colour, indicating the presence of carotenoid and might also explain why it showed the highest PPD delay 15 days after harvest.

3.1.1 Validation

At 5, 10 and 15 days after harvest, PPD analyzer method significantly overestimated the average values of CIAT standard with approximately 180%, 41% and 18% respectively.

Similarly, at 5DAH, PPD Symptom Score Software method overestimated the values of CIAT standard with approximately 38% while at 10 and 15 DAH, PPD Symptom Score Software method

underestimated the average PPD values of CIAT standard by 0.52% and 8.3% respectively. Hence, PPD Symptom Score Software method offers a better average estimate than the PPD Analyzer. These contrasting results among the three methods were confirmed when correlation analysis showed low

association (Table 3). This finding agrees with Andres et al (2010) that visual inspection remains the preferred method for evaluating. He compared the subjective visual assessment with a florescent accumulation of hydroxycoumarins and found it to be less reliable for evaluating PPD delay.

Table 1: Analysis of variance (ANOVA) assumption test outputs

Shapiro-Wilk Test		Levene Test	
W	p-value	F-statistics	p-value
0.93242	0.07098	10.411	0.3176
0.94145	0.12031	1.3547	0.2784
0.96013	0.35062	0.8428	0.5512

Table 2: PPD evaluation scores of 7 cassava accessions, 5, 10 and 15 days after harvest, using the three methods of evaluation

Cassava accessions	CIAT standard			PPD Analyser			PPD Symptom Score Software		
	5DAH	10DAH	15DAH	5DAH	10DAH	15DAH	5DAH	10DAH	15DAH
COB-5-01	2.15	21.61	26.43	18.10	39.38	25.41	14.34	25.60	26.92
CR528-26	35.73	46.18	52.97	45.48	32.90	39.25	25.76	34.03	41.73
CW451-33	7.46	27.77	31.48	43.37	34.48	50.70	17.95	29.57	29.79
NR060251	7.84	19.11	20.53	44.73	47.19	38.98	17.65	22.17	24.55
NR090182	2.86	15.43	3.45	52.16	32.97	43.94	28.83	31.41	20.56
NR100417	38.93	23.50	25.60	56.21	39.79	34.08	24.00	21.66	28.01
NR110490	12.45	33.63	53.84	41.66	37.35	20.29	20.06	21.81	24.85
HSD	31.116	NS	21.322	NS	NS	NS	NS	NS	NS
Average	15.35	26.75	30.61	43.10	37.72	36.09	21.23	26.61	28.06
Image_{avg} / CIAT_{avg} (%)				280	141	118	138	99.48	91.70

HSD:Honestly Significant Difference. Genotype with the same alphabet do not differ significantly ($p > 0.05$). Alpha = 0.05

Table 3: Correlation analysis between both image-based software and the CIAT standard method

Correlation Coefficient	CIAT vs PPD analyser			CIAT vs PPD Symptom Score Software		
	5DAH	10DAH	15DAH	5DAH	10DAH	15DAH
R	-0.2672	-0.1090	-0.056	0.3736	0.1454	0.4538

This suggests that neither image-based software are reliable tools for evaluation of PPD tolerance in cassava.

4.1 CONCLUSION

Cassava storage roots is an essential part of the daily diet for millions of people and income generation. However, its potential as food and industrial crop is still limited by PPD. The study of PPD mechanism response is important in increasing our molecular knowledge and for their potential in developing an effective approach to control PPD losses. Significant variation among the cassava accessions for PPD

tolerance was observed but there is low association between the two image base methods and the visual CIAT standard method. NR090182 showed the most delayed PPD onset.

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