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Variation in Yellow Root Cassava (*Manihot esculentus* Crantz) Genotypes and Phenotypic Relationship for Selected Postharvest and Morphological Traits

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Abstract

This study evaluated the variation in yellow root cassava (*Manihot esculentus* Crantz) genotypes and phenotypic relationship for selected postharvest and morphological traits. The trial was established at the Njala Agricultural Research Centre experimental site, Njala, during 2017/2018 cropping season in a randomized complete block design with three replications. Findings showed that the higher the total carotene content (TCC) in yellow flesh cassava genotypes, the longer the rate of postharvest physiological deterioration (PPD). Genotypes TR-0051-TCC/17 and TR-0012-TCC/17 recorded higher TCC (18.9 µg/g and 13.6 µg/g) and longer rate of PPD (4.29 and 3.14), respectively. Genotypes TR-0051-TCC/17, TR-0016-TCC/17, TR-0028-TCC/17, TR-0012-TCC/17 and TR-0020-TCC/17 had the highest TCC values of 18.9 µg/g, 16.09 µg/g, 14.72 µg/g, 13.6 µg/g and 11.23 µg/g with corresponding higher color chart values of 6, 6, 6, 5, and 6, respectively. This suggests the direct dependence of TCC on the root parenchyma color intensity in yellow flesh cassava genotypes. Findings also show a direct relationship between morphological and postharvest traits in yellow flesh cassava genotypes that could be exploited for the genetic improvement of cassava for increased shelf life, nutrition and related quality traits, as well as conservation and utilization of the crop.

Keywords

Cassava, Variability, Regression, Correlation, Postharvest and Morphological

Traits

1. Introduction

Cassava (*Manihot esculenta* Crantz) is the sixth most important storage root food crop in the world [1]. The crop is the third most important source of carbohydrates in Africa [2] and the second most important staple crop in Sierra Leone [3]. The fresh cassava storage root yields can reach up to 50 - 82 metric tons per hectare, making it the highest-producing starchy staple [4] [5]. Moreover, the crop can be produced on marginal soil when other crops cannot have an economically viable yield. Cassava serves as food for more than 800 million people in the world [6], providing about 500 calories daily for over 70 million people [7]. Its starchy tuberous roots are a great all-year-round source of cheap calories in developing countries, where calorie deficit and malnutrition are prevalent [8] [9]. According to FAO [1], about 250 million people in sub-Saharan Africa (SSA) derive half of their daily calories from cassava. Cassava storage roots are useful for animal feed, industrial starch production and income generation for many small-scale farmers [10]. Cassava leaves and roots are available all year round [11], making it an important food security crop, even in drought-prone areas [7].

Cassava contains 25 mg of vitamin C, 40 mg of phosphorus and 50 mg of calcium per 100 g of fresh root [12]. The protein, riboflavin, thiamine and niacin contents in cassava are very low compared to other tuber crops, thus making it one of the highest sources of carbohydrates [13]. The carbohydrate content of cassava ranges from 64% to 72% starch (amylose and amylopectin). The starch present in cassava is structurally different from that found in cereal; in its branch chain length distribution, amylose content and its granular structure. Approximately 17% of sucrose is also found in cassava, predominantly in the sweet varieties, and limited quantities of fructose and dextrose have also been reported. The protein content is between 1% and 2%, with low essential amino acid profiles, particularly methionine, tryptophan and lysine. Furthermore, cassava possesses a high dietary fibre content (3.40% - 3.78% soluble, and 4.92% - 5.6% insoluble) [14] [15]. However, the technological processing of cassava, in the preparation of their derived food influences its composition [16].

Yellow root cassava has high levels of pro-vitamin A carotenoid and its consumption has been perceived as a sustainable approach for addressing Vitamin A deficiencies [17]. In cassava, intensity of yellow pigment in roots of some genotypes is strongly associated with β-carotene [18]. The β-carotene and other carotenoids (a dietary precursor of vitamin A) are known to be responsible for the yellow to orange coloration of the flesh of storage roots of some cassava varieties [19]. A delay of PPD onset has been reported in yellow-root cassava genotypes with high β [20] [21]. Vitamin A is essential for vision and immune competence, as well as for cellular differentiation, growth, and reproduction. The vitamin A

recommended dietary allowance (RDA) for adults (men and women) and children (4 to 9 years) is 0.75 and 0.3 to 0.4 mg/day retinol activity equivalents (REA)/day, respectively [22]. These dietary requirements are not adequately supplied by diets, especially in children, pregnant women, and the poor in some countries, including Sierra Leone. Biofortification is an approach that relies on conventional plant breeding and modern biotechnologies to increase the micronutrient density of staple crops, including cassava [23].

Despite its importance, cassava production is fraught with a number of constraints, with its characteristic short shelf-life termed postharvest physiological deterioration (PPD), among key challenges affecting farmers, processors and consumers [24]. The PPD is an oxidative reaction that starts immediately after harvesting when the root is detached from the mother plant [25]. It starts from the central vascular bundles of the root, spreading to the adjacent storage parenchyma, and subsequently, the stored starch undergoes structural changes [24]. The roots deteriorate in 24 - 48 h after harvest, subsequently leading to changes in its color. Cassava roots are also bulky, containing approximately 65% water, which leads extensively to PPD [26] [27]. This rapid onset of decay has become an even greater problem with increased urbanization: markets are now at greater distances from cassava fields and processing can entail delays, making PPD a major source of post-harvest loss, especially in areas with less developed transportation networks [28]. The exact duration of cassava shelf life depends on the genotype, harvest practice, handling, and storage conditions. Some of the visible signs of PPD include vascular streaking with blue or black discoloration, rendering the roots unpalatable and unmarketable [24] [25].

Postharvest damage and spoilage cause substantial food waste and economic losses. The initiation and subsequent degree of deterioration are reported to be closely related to the presence of mechanical damage that is unavoidable and poor handling during harvest and transport of roots, respectively [29] [30]. Since PPD is physiological, determination of the physiochemical and functional changes in cassava roots during PPD helps in understanding the relationship between root quality and physiological deterioration. The PPD also results in market price reduction of a three to four-day old roots compared to the high selling price of the fresh roots. Eventually this encourages consumers to choose alternative supplies of carbohydrates, increasing dependency on other imported food.

Both traditional and modern techniques have been utilized to minimize PPD in cassava. Some of the traditional techniques utilized include leaving the roots unharvested in the soil after the period of optimal root development, until the roots can be immediately consumed, processed or marketed; 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; storage of cassava roots under in-field conditions such as in pits, clamps, trenches or boxes [31]. Commercial scale cassava production requires more efficient techniques rather than the traditional technique, which is considered as labor intensive, difficult to

manage and are not always completely effective [32]. Some of the modern techniques utilized to minimize PPD include: storage of fresh cassava roots in polyethylene bags after harvest, which prevents PPD up to 4 weeks by subjecting the root to high relative humidity inside the bag, which reduces transpiration and respiration [32]. Another technique is covering cassava roots with paraffin wax by dipping the root in paraffin wax (at a temperature of 55˚C - 65˚C 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 [33]. Storage for 2 weeks between 0˚C to 4˚C without any internal deterioration. The most favorable temperature for storing fresh cassava is 3˚C, 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 4˚C, the part of the root without decay is usually in excellent condition and is suitable for human consumption [31] [34].

Vitamin A deficiency (VAD) is a global public health problem that impacts millions around the world [35]. Vitamin A deficiency is a leading cause of morbidity and mortality, especially in young children, pregnant and lactating women. Food-based interventions focused on alleviating vitamin A deficiency in susceptible populations have advantages over-supplementation and fortification programs, especially in rural areas, because they can provide a sustainable source of variety of nutrients without the recurring transport and administration costs of these other methods [36].

Evaluation of yellow root cassava genotypes provides knowledge of the existing variability and phenotypic relationship among genotypes for selected postharvest and morphological traits. The existence of yellow-root cassava offers a different perception on nutritional benefits associated with the crop [37]. Enhanced content of β-carotene (provitamin A) in yellow root cassava [6] provides an opportunity to sustainably address vitamin A malnutrition through deployment of provitamin A cassava varieties where the crop is a major staple [38]. Biofortification of crops is one of the sustainable strategies to reduce vitamin A deficiency. Thus, the objectives of this study were to: 1) assess the parenchyma root color, total carotene content and postharvest physiological deterioration in yellow flesh cassava genotypes; and 2) determine the relationships among selected morphological and postharvest traits in yellow flesh cassava genotypes.

2. Materials and Methods

2.1. Experimental Site

The study was established at Njala Agricultural Research Centre (NARC), Njala, during 2017/2018 cropping season. Njala is located on an elevation of 50 m above sea level on latitude 8˚6'N and longitude 12˚6'W of the equator. Njala experiences distinct dry and wet seasons. The rainy season starts from April to November and the dry season starts from October to May. The mean monthly air temperature ranges from 21˚C to 23˚C for greater part of the day and night, especially during the rainy season. The land cover of the experimental site is predominantly

secondary bush and consists of well-balanced mixture of sand, clay, and humus.

2.2. Planting Materials, Layout, Design and Management

The experimental materials utilized in this study were stem cuttings of 10 introduced cassava genotypes including TR-0020-TCC/17, TR-0034-TCC/17, TR-0016-TCC/17, TR-0008-TCC/17, TR-0024-TCC/17, TR-0028-TCC/17, TR-0012-TCC/17, TR-0051-TCC/17, TR-0027-TCC/17 and TR-0029-TCC/17. The experiment was laid out in a randomized complete block design with three replications. About 10 stem cuttings per genotype, each measuring 30 cm long, were planted on a 10 m long ridges at 1 m × 1 m spatial arrangement. Hand weeding was done regularly with no applications of fertilizers, pesticides and/or herbicides.

2.3. Data Collection

The morphological traits comprising above-ground traits (color of stem exterior (CSex), width of leaf lobes (WLL), shape of central leaflet (SCL)) were evaluated at 6 MAP; and below-ground traits (root parenchyma color) at 12 MAP based on the descriptor of cassava described by Fukuda *et al.* [39]. Root parenchyma color was determined using a 1 to 9 scale where 1 = white, 2 = light cream, 3 = cream, 4 = light yellow, 5 = yellow, 6 = deep yellow, 7 = orange, 8 = pink and 9 = pinkish. At harvest (12 MAP), 5 g healthy fresh storage roots was taken out from each fresh storage roots for the determination of total carotenoid content (TCC), while two fresh storage roots were used for postharvest physiological deterioration (PPD) after harvesting, and a fresh storage root for root color during harvesting. The distal and proximal portions of each fresh storage root were cut. The central sections of the fresh storage roots were used for PPD determination. The individual sample roots were assessed for PPD using the method of Wheatley and Gomez [29] with slight modifications. The fresh storage roots were prepared and stored for 7 days instead of 3 days as follows: 1) two commercial-sized roots (minimum length 12 cm) were randomly selected to represent each clone; 2) about 1 cm from both the proximal and distal ends was cut off; the cut-off sections was covered with cling film; 3) the storage roots were stored under ambient conditions for seven days; 4) after 7 days, 2 cm transversal slices starting from the proximal end were made; 5) scoring for each slice was done on a scale of 1 - 10, corresponding to the percentage of the cut surface showing discoloration (with 1 = 10%, 2 = 20%, …, 10 = 100%) as described by Salcedo *et al.* [40]; and (vi) average of the seven slices per root was done to represent the deterioration of the storage root for final analysis.

For the TCC determination, yellow flesh storage roots were harvested and placed on a transparent plastic bag, moved to the laboratory and kept under room temperature for at least 10 min. The roots were then washed to remove dirt, peeled, cut into smaller pieces and grind to finer particle sizes. Each grind sample was mixed with de-ionised water (5 g grind cassava mass: 20 ml water) and placed in a tube. A 5 g/ml of the mixture was extracted and injected into the reagent tube

and allowed to settle for few min, before analysis using an I-check instrument. The TCC was determined using the formulas below.

$$\text{Diffusion Factor} = \text{Vol. of surley/weight of grind cassava mass} \quad (1)$$

$$\text{TCC} = \text{Diffusion Factor} \times \text{Reading obtained from I-check instrument.} \quad (2)$$

The volume of surley is a constant (25 ml).

2.4. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using General Linear Model procedure (PROC GLM) of SAS version 9.4 [41]. The treatment averages were compared using the (SNK) at the level of 5% of probability. Statistical analyses for column charts and scattered plots were performed using Excel 2010. The statistical relationships among selected variables were determined through correlation and regression analysis. The total variations in the dependent variable explained by the independent variables were evaluated through the coefficient of determination (R^2) [42].

3. Results and Discussion

3.1. Influence of Parenchyma Root Color, Total Carotene Content, Postharvest Physiological Deterioration (PPD) on Yellow Cassava Genotypes

Table 1 presents values for root parenchyma color, postharvest physiological deterioration and total carotene content (μg/g) of the various genotypes studied. Generally, postharvest physiological deterioration and total carotene content significantly ($P < 0.05$) varied among yellow root cassava genotypes (**Tables 1-3**). Genotypes TR-0034-TCC/17 (8.42%) and TR-0016-TCC/17 (7.28%) had the highest PPD of storage roots, while genotypes TR-0051-TCC/17 and TR-0016-TCC/17 recorded highest TCC of 18.9 μg/g and 16.09 μg/g (**Table 1**) and slower rate of PPD of 4.29 and 3.14, respectively (**Table 1**), indicating that, the higher the TCC the longer the postharvest life (slower rate of PPD) on yellow cassava genotypes. Genotypes TR-0020-TCC/17, TR-0008-TCC/17, TR-0028-TCC/17, TR-0027-TCC/17, TR-0024-TCC/17 and TR-0012-TCC/17 showed medium rate of deterioration with a range of 4.28% - 5.57% and are classified as normal deteriorations. The results give guidance to breeding efforts for improved shelf life of yellow flesh cassava storage roots. This result agrees with that of Sánchez *et al.* [18], who reported a higher tolerance to PPD in genotypes with high carotenoid contents. Similarly, PPD has been found to be correlated with the content of b-carotene since Morante *et al.* [26] observed a less susceptibility to PPD for the genotypes with high level of b-carotene compared to those with less level of b-carotene. Similarly, it supports the view of Morante *et al.* [27], who reported that cassava roots with high carotenoid content in their roots tended to have lower incidence of PPD; and Sánchez *et al.* [18] found that high-carotene roots had reduced onset of PPD by only 1 or 2 days. Chávez *et al.* [43] also showed that an inversely correlation between light yellow parenchyma color of roots associated

to high amount of carotenoid content and delaying of PPD. Findings of the present study support the view that the inherent genetic differences among genotypes significantly contribute to the wide variation in PPD [40]. The highest deterioration observed at the proximal could be attributed to the unavoidable detachment of root at proximity to the parent stock, which caused wounding.

Table 1. Fresh root parenchyma color, total carotene content and postharvest physiological deterioration as affected by genotypes.

Genotype	Rootparenchyma color	Color chat score	Postharvest physiological deterioration	Total carotene content (µg/g)
TR-0012-TCC/17	Yellow	5	3.14 i	13.6 0d
TR-0051-TCC/17	Deep yellow	6	4.29 g	18.90 a
TR-0008-TCC/17	Cream	3	5.57 c	9.38 j
TR-0020-TCC/17	Deep yellow	6	4.43 f	11.23 f
TR-0029-TCC/17	Yellow	5	3.29 h	9.88 i
TR-0027-TCC/17	Light yellow	4	4.86 d	10.25 h
TR-0016-TCC/17	Deep yellow	6	7.29 b	16.09 b
TR-0034-TCC/17	Cream	3	8.43 a	11.73 e
TR-0024-TCC/17	Cream	3	4.57 e	10.53 g
TR-0028-TCC/17	Deep yellow	6	5.57 c	14.71 c

Means in column with the same letter are not significantly different at $P > 0.05$ (SNK).

Table 2. Analysis of variance for postharvest physiological deterioration as affected by genotypes.

Source	DF	SS	MS	F-value	Pr > F
Model	9	74.605	8.2895	82895.5	0.0001
Error	20	0.002	0.0001		
Corrected Total	29	74.607			

$R^2 = 0.999993$, CV = 0.194401, Root MSE = 0.010, Mean = 5.144.

Table 3. Analysis of variance for total carotenoid content as affected by genotypes.

Source	DF	SS	MS	F-value	Pr > F
Model	9	258.131	28.681	100000	0.0001
Error	19	0.00185	9.7E−05		
Corrected Total	28	258.132			

$R^2 = 0.999993$, CV = 0.078572, Root MSE = 0.0098675, Mean = 12.558621.

Genotype TR-0051-TCC/17 recorded the highest TCC (18.90 µg/g), followed by TR-0016-TCC/17 (16.09 µg/g), TR-0028-TCC/17 (14.72 µg/g), TR-0012-TCC/17 (13.6 µg/g), and TR-0020-TCC/17 (11.23 µg/g), while TR-0008-TCC/17

had the least value of 9.38 μg/g (Table 1). The high TCC values correspond with high color chart values of 6, 6, 6, 5, and 6, respectively. Findings indicate direct relationship between TCC and root parenchyma color intensity of yellow flesh cassava genotypes. This result agrees with Sánchez *et al.* [18], who reported that, white or creamy flesh cassava genotypes do not have appreciable amount of carotene content in root tissues as compared to high carotene levels observed in deep yellow-fleshed varieties. Findings also corroborate the view of Howe *et al.* [44], that beta carotene is the predominant carotenoid in yellow root cassava accessions. The efficacy of vitamin A from bio-fortified cassava matches those obtained from food supplements and can adequately maintain vitamin A status in consumers. Graham and Rosser [45] and Hess *et al.* [46] noted that cassava genotypes with improved pro-vitamin A carotenoids have added advantage due to synergistic effects of these carotenoids with zinc and iron bioavailability.

3.2. Relationship Among Postharvest Traits of Yellow Cassava Genotypes

A moderate positive and high significant correlation (r = 0.67709, p = 0.03219) between root color and total carotene content indicates that, as root color intensity increases, total carotene content also increases (Table 4). Chávez *et al.* [7], also reported that the yellow color intensity of pulp is closely related to carotenoid content in cassava storage roots. Carvalho *et al.* [47], Njoku *et al.* [48] and Welsch *et al.* [49] reported the biotechnological approaches utilized for provitamin A content enhancement in cassava storage roots that complement previous selection efforts to identify naturally occurring varieties with roots enriched in carotenoids.

Table 4. Pearson correlation coefficients among postharvest physiological deterioration, total carotene content and root color.

	Postharvest physiological deterioration	Total carotene content	Root color
Postharvest physiological deterioration	1		
Total carotene content	−0.0149	1	
	0.9675		
Root color	−0.3497	0.67709	1
	0.3219	0.0315	

R^2 = 0.999993, CV = 0.078572, Root MSE = 0.0098675, Mean = 12.558621.

The stepwise regression of showing relationship between total carotenoid content and root color accounted for 54.1% of total variability in TCC (R^2 = 0.54; p = 0.05) (Figure 1). The result implies that the remaining percent variability is possibly attributed to environmental error. Findings suggest that the deeper the root color the more carotene contents a genotype contains. The results are expressed in fresh rather than dry basis in order to have a direct idea of the nutritional

potential of each genotype when consumed fresh. Processing methods used to make popular cassava food products such as gari and fufu have been shown to reduce b-carotene concentrations of bio-fortified cassava by 30% for gari preparation and 65% for fufu preparation [50].

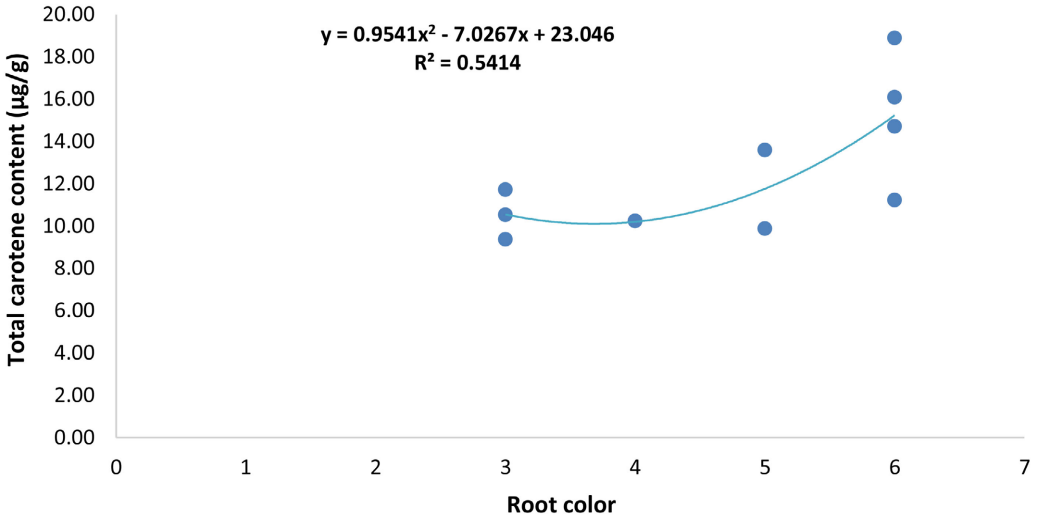

Figure 1. Scattered plots showing relationship between total carotenoid content and root color.

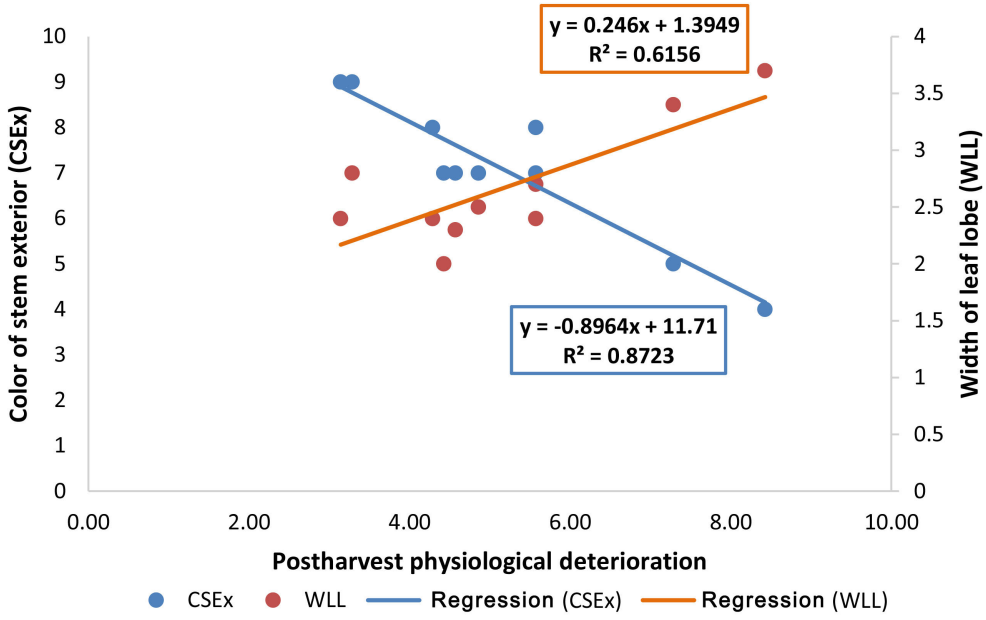

Figure 2. Scattered plots showing effect of postharvest physiological deterioration on breeding traits (CSex = color of stem exterior and WLL = width of leaf lobes).

A positive correlation between the delay of PPD onset and carotenoid concentrations of yellow flesh varieties of cassava has been previously reported [20]. Moreover, Beyene *et al.* [51] found a transgenic line with the highest levels of carotenoids, but displayed no detectable PPD after 10 days of storage. Genotypes with low PPD or delayed PPD trait were less susceptible to microbial deterioration

compared to those with high PPD. This was also reported by Reilly *et al.* [52], who opined that the extent of PPD damage and speed of symptom development in roots depends on the genotypic as well as the environmental conditions.

The correlation among postharvest traits is useful for planning a breeding program that is aimed at developing genotypes with desirable shelf life or low rate of PPD. Generally, the stepwise regression of postharvest physiological deterioration indicated that color of stem exterior (CSex) contributes more to variability relative to width of leaf lobes (WLL) (**Figure 2**). The postharvest physiological deterioration and CSex accounted for 87.2% ($R^2 = 0.8723$; $p = 0.05$), while the regression of postharvest physiological deterioration on WLL accounted for 62.6% of total variability ($R^2 = 0.6256$; $p = 0.05$) in the yellow flesh cassava genotypes (**Figure 2**). The result implies that the remaining percent variability is possibly attributed to environmental error.

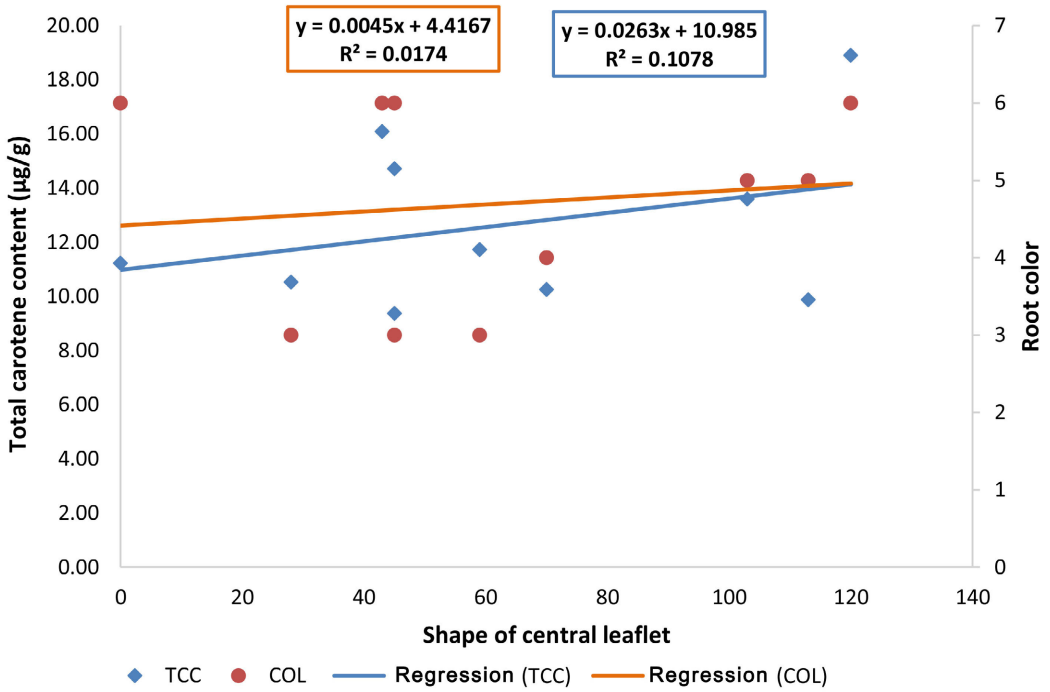

Figure 3. Scattered plots showing effect of shape of central leaflet on total carotene content and root color.

The stepwise regression of shape of central leaflet on total carotene content; and shape of central leaflet on root color indicated that TCC contributes more to variability relative root color (**Figure 3**). The shape of central leaflet on total carotene content accounted for 10.9% ($R^2 = 0.1078$; $p = 0.05$), while the regression of shape of central leaflet on root color accounted for 1.7% of total variability ($R^2 = 0.0174$; $p = 0.05$) in the yellow flesh cassava genotypes (**Figure 3**). The result implies that the remaining percent variability is possibly attributed to environmental error. The results confirm that there are useful relations for selected breeding traits within the collection that could be indicative of a broad range of useful genotypes which could be exploited for postharvest characterization on yellow flesh cassava.

This is in line with, Njoku *et al.* [47]. Cassava germplasm with elevated b-carotene content has been identified and is currently being developed using conventional breeding strategies to address VAD in SSA [50]. Development of cassava storage roots with increased b-carotene through breeding or biotechnology, as described here, offers a viable solution to address a major nutritional challenge in SSA.

The regression between root color and postharvest physiological deterioration accounted for 12.2% ($R^2 = 0.1222$; $p = 0.05$) (Figure 4). The result implies that the remaining percent variability is possibly attributed to environmental error.

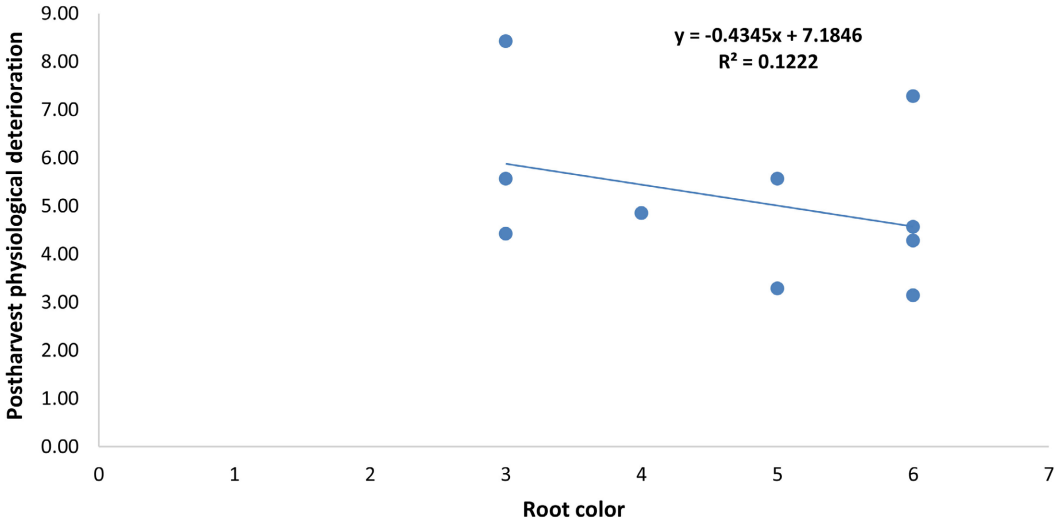

Figure 4. Scattered plots showing relationship between postharvest physiological deterioration and root color.

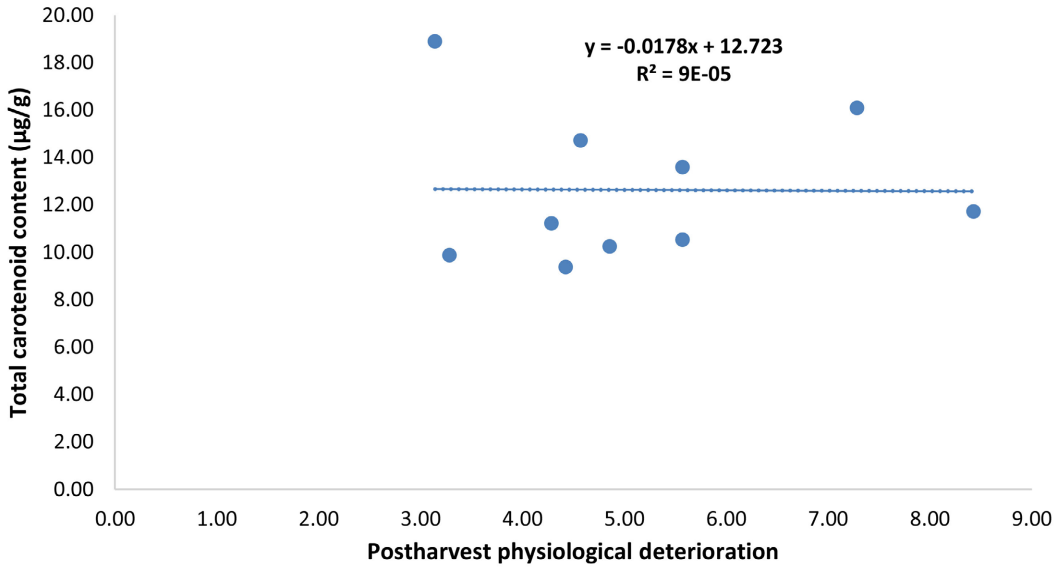

Figure 5. Scattered plots showing regression of total carotenoid content on postharvest physiological determination.

A negative and non-significant correlation ($r = -0.01488$, $p = 0.9675$) was recorded for that of TCC and PPD (Table 2), indicating that the rate of PPD is non-

dependent on the amount of TCC present on yellow flesh cassava. The regression between postharvest physiological deterioration and total carotene content accounted for 0.0001% (R^2 = 0.00009; p = 0.05) (**Figure 5**). The result implies that the remaining percent variability is possibly attributed to environmental error.

These findings complement previous selection efforts to identify naturally occurring varieties with roots enriched in carotenoids [50] [52].

4. Conclusion

This study demonstrates that the higher the total carotene content (TCC) in yellow flesh cassava genotypes, the longer the rate of postharvest physiological deterioration (PPD). Genotypes TR-0051-TCC/17 and TR-0012-TCC/17 recorded higher TCC (18.9 µg/g and 13.6 µg/g) and longer rate of PPD (4.29 and 3.14), respectively. Genotypes TR-0051-TCC/17, TR-0016-TCC/17, TR-0028-TCC/17, TR-0012-TCC/17, and TR-0020-TCC/17 had the highest values for TCC (18.9 µg/g, 16.09 µg/g, 14.72 µg/g, 13.6 µg/g and 11.23 µg/g) with a corresponding higher color chart value (6, 6, 6, 5, and 6), respectively. This suggests the direct dependence of TCC on the root parenchyma color intensity in yellow flesh cassava genotypes. Findings also show a direct relationship between morphological and postharvest traits in yellow flesh cassava genotypes that could be exploited for the genetic improvement of cassava for increased shelf life, nutrition and related quality traits; as well as conservation and utilization of the crop.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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