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Assessment of Chemical Properties of Yellow-Fleshed Cassava (*Manihot esculenta)* Roots as Affected by Genotypes and Growing Environments

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ABSTRACT

The study evaluated the effect of genotype and growing locations on biofortified cassava root genotypes' chemical properties. Twenty-eight genotypes of biofortified yellow and white-fleshed cassava were planted across four geographical areas in Nigeria and harvested for two seasons. This research analyzed the chemical properties (DM, VC, TC, and CNP) of the samples using standard laboratory procedures. ANOVA, descriptive analysis, PCA, and HCA was carried out on the generated data using SAS software. Genotypes 94/0006 and 01/1273 had the highest and lowest DM values (31.9 and 18.3 g/100 g). Of the bio-fortified genotypes tested, 76% had VC values lower than that averaged by check samples. Genotype 01/1331 had the highest CNP value (33.1 mg/100 g), and 01/1115 had the lowest (5.7 mg/100 g). TC values in genotypes 1368, 01/1371, 01/1412, and 01/1277 were above 7.0 5 µg/g. Genotype and growing environment had a highly significant effect (P≤0.01) on the studied chemical properties. Genotype by location interaction influenced VC weakly (P≤0.05). Values of DM, VC, and CNP were environment-dependent, but TC was genotype-dependent. PCA compressed data to PC1, PC2, and PC3, accounting cumulatively for 89.1%, and cluster analysis (CA) grouped the genotypes into three groups based on similarities in their chemical properties. These findings are applicable in identifying the best-biofortified cassava genotypes in breeding programs that in the future can be applied by farmers.

INTRODUCTION

Cassava (*Manihot esculenta*) is a perennial crop originating in America and widely grown in Nigeria (Akinpelu, Amamgbo, Olojede, & Oyekale, 2011) with numerous uses as food, feed, and agro-allied/industrial raw material. Cassava is a starchy crop, easy to cultivate, that supplies year-round dietary food energy and is available (agriculturally and financially) to a large quota of the population (Maziya-Dixon, Dixon, & Adebowale, 2007). These qualities make it valuable to food security and livelihoods for prominent people in drought periods. Cassava generally has a high percentage of carbohydrates but low protein contents, vitamins, and lipids (Ayetigbo, Latif, Abass, & Müller, 2018). It is a highly resilient crop that can withstand stress from drought and poor dry soils while giving an acceptable outcome; therefore, it fits the survival crop's description. Cassava competes with other valuable crops such as maize, soybean, and vegetables, mainly in acid and low-fertility soils and low rainfall. Cassava could be over a wide range of edaphic and climatic conditions between 30°N and 30°S latitudes, growing in regions from sea level

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to 2300 m altitude (Akinpelu, Amamgbo, Olojede, & Oyekale, 2011; Ayetigbo, Latif, Abass, & Müller, 2018).

Chavarriaga-Aguirre et al. (2016) review the potential of using biotechnology in improving the quality of cassava. According to Singh, Praharaj, Singh, & Singh (2016), biofortification is a biotechnology method by which nutrients are bred or incorporated intrinsically into a food crop. It provides a lasting, cost-friendly, and viable way to curb micronutrient malnutrition, a global challenge of great importance. Biofortification serves to increase the concentrations and bioavailability of essential micronutrients in food. Yellow-fleshed cassava is a variety of the white-fleshed variety that has been bio-fortified with high concentrations of provitamin A carotenoids as a measure to provide the micronutrient in curbing vitamin A deficiency (VAD) within the at-risk population. Narayanan et al. (2019) carried out a study on cassava biofortification with iron and zinc. A study has also researched the effect of carotenoids biofortification as a mode of generating information to guide the deployment and valorization of provitamin A cassava in the feed and food industries (Atwijukire et al., 2019).

Various studies on white and yellow cassava, especially on the chemical compositions, have been done. Manano, Ogwok, & Byarugaba-Bazirake (2018) investigated five varieties in Uganda (three sweet, improved, high yields, mosaic-resistant, and two bitter types). The chemical components analyzed include the proximate, mineral, and antinutritional contents. Richardson (2013) studied the quality attributes, root yield, and nutrient composition of six varieties and determined proximate, sodium, and potassium contents. Oly-Alawuba & Agbugbaeruleke (2017) assessed the effects of processing on white and improved yellow varieties' chemical and sensory properties. They analyzed the proximate and mineral contents of these two selected varieties. All these studies determined the moisture content of the types.

Dry matter (DM) refers to the proportion of material left after the water designated as 'moisture content' has been removed. A higher DM result indicates a greater yield of material/unit g of fresh root. It is a valuable index in food and feeds production because of a higher quantity of all other nutritional components. Vitamins are micronutrients. Vitamin C (VC) or ascorbic acid is vital in boosting the immune system, mineral absorption, and numerous body metabolisms, including lowering blood pressure and protecting against oxidative tissue damage. Its deficiency is known to cause tissue and capillary weakness and scurvy (Ayetigbo, Latif, Abass, & Müller, 2018; Sudha & Reshma, 2017; Walingo, 2005).

The chemical defense system organization in plant and plant-insect interactions is related to cyanogenic glycosides' pivotal roles. Although not toxic on their own, the glycosides are potentially harmful when hydrolyzed into hydrogen cyanide. The toxicity is always measured by cyanogenic content, and it varies significantly in the different parts of the cassava plant. Cassava is generally known to have a high content of cyanogenic glycosides. It has been implicated in the bitter taste of roots which should not be eaten raw but only after processing. Cyanide toxicity can be reduced before cassava is consumed as food by employing one or a combination of appropriate processing techniques like peeling, slicing, boiling, fermenting, drying, baking, among others. Most bio-fortified yellow-fleshed variants contain more cyanogenic glucosides than most white-fleshed variants. Cyanide toxicity in humans has been reported to have many symptoms, such as rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhea, mental confusion, twitching, convulsion, and (in severe cases) death. These occur when the cyanide content exceeds the limit an individual can detoxify based on individual body weight. It is (on average) between 0.5 and 3.5 mg HCN per kg of body weight. However, children are particularly at risk because of their smaller body size (Ayetigbo, Latif, Abass, & Müller, 2018; Bolarinwa, Oke, Olaniyan, & Ajala, 2016).

Carotenoids are naturally occurring phyto-pigment compounds. They possess medicinal oxidative and anti-carcinogenic properties, exist in various colors, and are biochemically converted to vitamin A in the body. Deficiency is referred to as VAD conditions and includes developing visual and epidermal diseases (Jaswir, Noviendri, Hasrini, & Octavianti, 2011). Traditionally, root flesh is white, indicating the total absence of pigment and some phytonutrients (Ayetigbo, Latif, Abass, & Müller, 2018). Bio-fortified yellow-fleshed cassava has been developed as a 'remedy,' a variant with better quality, in alleviating micronutrient deficient conditions. Low cyanogenic potential (CNP), high DM, high total carotenoids (TC), and high VC are an added advantage to planters and end-users alike.

'Genotype' refers to the different species of a plant. Differences may be purely visual or chemical, e.g. flesh color, size, taste, culinary quality, and chemical/nutritional profile. Plants often produce varying samples of themselves depending on where they are planted. Cassava, in general, has environment-specific properties (Ayetigbo, Latif, Abass, & Müller, 2018). The genotype by environment (G×E) interaction is a routine plant breeding occurrence whereby significant differential genotypic responses were observed under varying environmental conditions (Akinwale, Akinyele, Odiyi, & Dixon, 2011). Cassava varieties often exhibit specific adaptation due to sensitivity to the G×E interaction in short and long-term crop performance experiments. The G×E interaction is of significant concern in plant breeding, making cultivar recommendations difficult since superior cultivars' choice varies with location (Maroya, Kulakow, Dixon, & Maziya-Dixon, 2012). This challenge can be addressed only by carrying out relevant studies on the essential chemical properties of biofortified cassava roots. Akinwale, Akinyele, Odiyi, & Dixon (2011) carried out a study to determine the possible effects of genotypic and environmental differences on DM, fresh root yield, and other related traits of 43 improved genotypes planted in three major agro-ecological zones of Nigeria. Another study amongst others is Benesi, Labuschagne, Dixon, & Mahungu (2004), who have checked G×E effects on native cassava starch quality and its potential in the commercial sector.

This research evaluates the impact of genotype and location on the selected chemical properties of yellow-fleshed cassava genotypes. Thus, the information from this study will help the breeders and farmers to identify the best genotypes for each agroecology or across agroecology with optimal carotenoids and VC and low cyanide contents.

MATERIALS AND METHODS

Twenty-five (25) genotypes of yellow-fleshed storage root from an Advanced Yield Trial (AYT) and three check varieties with white roots (30572, TME 1, and 91/02324) collected from multi-location field trials at four different research farms [Ubiaja (forest-savanna), Zaria (northern Guinea savanna), Ibadan (forest-savanna transition) and Mokwa (southern Guinea savanna)] of the International Institute of Tropical Agriculture, Nigeria. During the rainy season in July 2005 and 2006 cropping seasons, the trial began using the randomized complete block design (RCBD) with four replicates and grown under rain-fed conditions. There is no fertilizer or herbicide application during the experiment, and weeding was done manually when necessary. The storage roots were harvested 12 months after planting (MAP). Only the two middle rows per plot were harvested and roots processed were collected from only two replications, but each replication was analyzed in duplicate in the laboratory.

Sampling Method

The sampling method described by HarvestPlus (2005) was adapted for use in sampling and sample preparation for analysis of the roots. First, three storage roots of different sizes (large, medium, and small) were selected, washed thoroughly with potable water to remove dirt and sand particles, and air-dried on a clean concrete surface. Next, the roots were peeled manually using a stainless-steel knife, rinsed with de-ionized water to remove contaminants and cut longitudinally (from the proximal to the distal end) into four equal parts. Next, two opposite sections from each root of each genotype were taken, combined, manually chopped into small pieces, and mixed thoroughly. This study chose a batch of samples for analysis.

Chemical Analysis

Dry matter (DM) Determination

The method described by AOAC (2005) was adopted to analyze the DM for each genotype. A prepared sample of 100 g weight was oven-dried at 105°C for 24 hours, and DM was calculated as follows:

$$\mathrm{DM\ (g/100\ g)} = \frac{\text{weight of dried sample}}{\text{weight of fresh sample}} \times 100 \quad \text{.................1)}$$

Vitamin C (VC) Determination

Ten grams of the sample was immediately extracted with 40 ml of the meta-phosphoric-acetic acid solution by blending in a Warring blender for 3 minutes (Blender 8010G; Model HGBTWTG4 was used here). The resulting slurry was then filtered, and an aliquot of the juice extract was titrated with standard indophenol dye solution until a constant color change (AOAC, 2005; Doka, El Tigani, & Yagi, 2016).

$$VC\left(\frac{mg}{100}g\right) = \frac{B \times F \times \text{volume of extracting solvent used}}{\text{weight of sample} \times \text{volume of filtrate used}} \times 100 \ldots 2)$$

Where: B = difference between average titer values of the sample solution and blank; F = weight of ascorbic acid equivalent to 1.0 ml indophenol standard solution in mg.

Cyanide Potential (CNP) Determination

Cyanide potential (CNP) was quantified using the enzyme method described by Maziya-Dixon, Dixon, & Adebowale (2007). Extracts were made from fresh roots with cold orthophosphoric acid. Reduced temperature and acidity served to minimize the evaporative loss of hydrogen cyanide (HCN), hinder cyanogenic glucosides' degradation, and stabilize cyanohydrins. In addition, these extracts were treated with the excess exogenous enzyme (linamarase), and the consequent increase in pH > 5.0 produced cyanide ions quantified by spectrophotometry.

Total Carotenoids (TC) Determination

For TC's determination, three plants per clone were harvested, and three roots of different sizes were randomly selected per clone and placed in labeled polythene bags. The roots were washed with tap water and then air-dried on a chux-lined surface, peeled with a stainless-steel knife, and rinsed in de-ionized water. Samples (transverse sections) were collected from the roots' proximal, middle, and distal portions. The sections were quickly rinsed in de-ionized water, removed with plastic forceps, and chopped into small pieces. The chopped pieces from each root were mixed and rinsed promptly in de-ionized water. All samplings were done under subdued light and were immersed in liquid nitrogen and packed in polythene sample bags. They were then stored in a deep freezer at –80°C. Total carotene content was determined by spectrophotometric as described in the HarvestPlus Handbook for Carotenoids Analysis (Rodriguez-Amaya & Kimura, 2004).

$$TC(\mu g/g) = \frac{A_{total} \times volume\ of\ extract \times 10^4}{A_{1\ cm}^{1\ \%} \times weight of sample} \ldots\ldots\ldots\ldots\ldots 3)$$

Where: = A_{total} absorbance reading; $A_{1\ cm}^{1\ \%}$ = absorption coefficient recommended for mixtures (2500).

Statistical Analysis

Data analysis was done using the Statistical Analysis System software (version 9.4, 2001) to analyze variance (ANOVA) and descriptive statistics. Means were separated using Fisher's protected least significant difference (LSD) test at $P<0.05$. In addition, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were also applied.

RESULTS AND DISCUSSION

Table 1 shows the goodness of fit statistics for the model used to analyze the variance of the chemical properties of genotypes from four different growing environments of varying agroecology. The observed R^2 of 0.9 (except for VC with R^2 of 0.8) indicates that 90% of the data were used for the regression model and explains the variability observed for the chemical properties. The mean square error (MSE) of the chemical properties ranged from 0.8 for TC to 29.6 for CNP, and the root means squared error (RMSE) ranged from 0.9 for TC to 5.4 for CNP. The smaller the MSE, the closer to the line of best fit, indicating the estimator's quality. For instance, TC is seen to be closest to the best line of fit. The regression models were used for the analysis of the variance.

Table 1. Goodness of fit statistics for all parameters across four locations

	DM	VC	CNP	TC
Observations	224	224	224	224
DF	81	81	81	81
R²	0.9	0.8	0.9	0.9
Adjusted R²	0.7	0.5	0.7	0.8
MSE	11.5	28.1	29.6	0.8
RMSE	3.4	5.3	5.4	0.9

Remakrs: DF = Degree of Freedom; MSE = Mean square error; RMSE = Root means square errors; DM = Dry matter ; VC = Vitamin C; CNP = Cyanide Potential; TC = Total Carotenoids

Table 2 shows the mean squares of ANOVA for DM, VC, CNP, and TC of the genotypes. Considering the p-value of the F statistic from the ANOVA table and the significant level of 5%, it could be observed that the explanatory variables' information is significantly better than that of a basic mean. The mean squares, genotype, and location bring vital information to explain all the dependent variables' variability and imply that they had a highly significant effect (P≤0.001) on the studied chemical properties. However, genotype*location interaction did not give vital information to explain the variations in the chemical properties except for VC, showing a weak significant effect (P≤0.05).

The location influences DM, CNP, and VC among the explanatory variables, while genotype is the most influential for TC. The observation implies that environmental factors are critical for the variations observed for all parameters except TC, where genotype plays a significant role in its variability. Genotype, location, and genotype*location interaction were highly significant (P<0.001) for fresh root yield, number of roots per plot, and percentage DM as studied by Akinwale, Akinyele, Odiyi, & Dixon (2011), indicating genetic variability between genotypes by changing the growing environments. In similar research, Tsegaw (2011) observed significant G×E interaction for DM in potato genotypes tested. Also, Haynes, Clevidence, Rao, Vinyard, & White (2010) reported significant G×E interaction for some of the carotenoid components tested for the potato plant. In this study, the DM, VC, and CNP contents are environment-dependent, TC is genotypic dependent. These findings are vital in breeding cassava varieties with high DM, TC, VC, and low CNP.

Table 3 shows the descriptive statistics of the chemical properties of the fresh yellow-fleshed roots from all four growing environments. The DM ranged from 13.0 to 39.6 g/100 g with a mean value of 25.2 ± 6.4 g/100 g; TC ranged from 0.7 to 9.4 µg/g with a mean value of 5.2 ± 2.2 µg/g; CNP values ranged between 0.1 and 57.6 mg/100g with a mean value of 12.5 ± 9.5 mg/100g, and VC ranged from 8.3 to 48.1 mg/100 g with a mean value of 24.4 ± 7.7 mg/100 g. DM results of white-fleshed cassava samples reported by Montagnac, Davis, & Tanumihardjo (2009), Oly-Alawuba & Agbugbaeruleke (2017), and Richardson (2013) are higher (averaged at 36.47, 36.58, and 40.30% respectively) than those obtained in this study. However, Maroya, Kulakow, Dixon, & Maziya-Dixon (2012) result for yellow-fleshed cassava was lower (21.0%). It was due to genetics and environmental effects and the different methods of analysis. In contrast, cassava's average DM content of the studied genotypes was higher than that reported for potatoes (23.27%) by Tsegaw (2011). Haynes, Clevidence, Rao, Vinyard, & White (2010) reported significant G×E interaction for some of the carotenoid components tested for potato plants and a value averaged at 1.01- 5.11 µg/g fresh weight.

Montagnac, Davis, & Tanumihardjo (2009) reported lower values for VC, although Oly-Alawuba & Agbugbaeruleke (2017) reported higher values. Manano, Ogwok, & Byarugaba-Bazirake (2018) said that CNP values for a bitter variety of Ugandan cassava were four times more than those averaged in this study. However, values lower than in the current study were reported for sweet Ugandan and yellow- and white-fleshed cassava varieties (Manano, Ogwok, & Byarugaba-Bazirake, 2018; Oly-Alawuba & Agbugbaeruleke, 2017). Atwijukire et al. (2019) reported that TC increased with an increase in root pigmentation intensity. Thus white-fleshed cassava generally has lower TC than bio-fortified varieties, as expected. The mean TC value obtained is comparable to those reported by Maroya, Kulakow, Dixon, & Maziya-Dixon (2012) and Montagnac, Davis, & Tanumihardjo (2009).

Table 2. Mean squares of analysis of variance for chemical properties of yellow-fleshed cassava across four locations

Source	DF	Mean squares			
		DM	VC	CNP	TC
Genotype	27	112.1***	140.95***	232.40***	31.68***
Location	3	1246.0***	950.82***	2204.91***	8.85***
Genotype*Location	81	15.6	47.57*	38.90	1.40*

Remarks: DF = Degree of Freedom; DM = Dry matter; VC = Vitamin C; CNP = Cyanide Potential; TC = Total Carotenoids; ***, **, * = Significant at P≤0.001, P≤0.01 and P≤0.05, respectively

Table 3. Descriptive statistics of data on the chemical properties of yellow-fleshed cassava across four locations

Variable	Obs.	Obs. with missing data	Obs. without missing data	Min.	Max.	Mean± SD
DM (g/100 g)	448	0	224	13.0	39.6	25.2 ± 6.4
VC (mg/100 g)	448	0	224	8.3	48.1	24.4 ± 7.7
CNP (mg/100 g)	448	0	224	0.1	57.6	12.5 ± 9.5
TC (µg/g)	448	0	224	0.7	9.4	5.2 ± 2.2

Remarks: DM = Dry matter; VC = Vitamin C; CNP = Cyanide Potential; TC = Total Carotenoids; Obs. = Observations, Min. = Minimum, Max. = Maximum, SD = Standard Deviation

Yellow-fleshed genotype 94/006 had the highest score across the four locations with a DM value of 31.9 g/100 g (Table 4), higher than in white-fleshed genotypes (91/02324, TME1, and 30572) used as checks. The DM values of statements were 29.8, 30.9, and 29.1 g/100 g, respectively. Highly significant differences (P<0.05) were observed among the DM values that may be a result of inherent differences in the genetic makeup of the plant clones (Getie, Madebo, & Seid, 2018). Fifteen out of the 28 genotypes investigated had DM values above a mean value of 25.2 g/100 g. That implies most (53.57%) had excellent DM values and compared favorably with the local checks used as control. This is one of the characteristics that drive the adoption of cassava varieties by both farmers and processors. However, genotype 01/1273 had the lowest DM of 18.3 g/100 g. Maroya, Kulakow, Dixon, & Maziya-Dixon (2012) obtained lower values for DM in genotypes 01/1371 (20.9 g/100 g) and 01/1368 (22.7 g/100 g) for which Oly-Alawuba & Agbugbaeruleke (2017) obtained a much higher value, i.e., 41.15 g/100 g. DM values more heightened than in the current study were reported for genotypes 95/0379, 96/1089A, and 91/02324 (chk) (Akinwale, Akinyele, Odiyi, & Dixon, 2011).

The lowest value for VC was in genotype 01/1663 (16.2 mg/100 g), and the highest was in 30572 (chk) (31.9 mg/100 g). Genotype 01/1646 had the highest VC content amongst the bio-fortified varieties with a value of 30.8 mg/100 g. Half of the genotypes assessed (50%) were found to have values above the mean value of 24.4 mg/100 g (Table 3). Nineteen of the 25 bio-fortified genotypes tested (76%) had VC contents lower than that averaged by check samples. The value for checks averaged only 28.4 mg/100 g, while that for the bio-fortified cassava averaged 24.0 mg/100 g. Thus, significant differences exist between values obtained for the genotypes (P<0.05). Montagnac, Davis, & Tanumihardjo (2009) reviewed values between 14.9 and 50 mg/100 g for ascorbic acid in cassava.

The cyanide content, which could be measured as CNP, is a critical anti-nutrient of cassava and makes the crop toxic for consumption unless processed. Therefore, Breeders aim for low cyanide levels and always screen for low CNP value, making genotype 01/1115the most probable variety for consideration. A total of 21 genotypes (75%) investigated had CNP values above the mean value of 12.5 mg/100 g. Genotype 01/1331 had the highest CNP (33.1 mg/100 g), and 01/1115 showed the lowest value of 5.7 mg/100 g. Also, genotypes 01/1115 and 01/1277 had CNP values below the check genotype (TME1) with the lowest (6.6 mg/100 g). The checks 91/02324 and 30572 had CNP values above the mean value, and this confirms the observation that most of the genotypes showed CNP values that compare well with the local checks used in this study. The means separation for CNP content of all the genotypes shows a significant difference at P<0.05. Oly-Alawuba & Agbugbaeruleke (2017) obtained a lower CNP value (3.42 mg/100 g) for 01/1368.

Table 4 presents the TC content of cassava clones at the four locations in Nigeria. Seventeen genotypes (60.71%) showed higher TC values above the mean value (5.2 ± 2.2 µg/g), and all the yellow-fleshed genotypes had higher TC than the check genotypes that ranged from 0.9 to 1.0 µg/g. Genotypes 1368, 01/1371, and 01/1412, 01/1277 showed TC contents above 7.0 µg/g, while 96/1089A had the lowest(2.6 µg/g). These genotypes are promising lines selected for further evaluation for release as bio-fortified cassava for alleviating VAD conditions. It is essential to point out that 01/1368, 01/1371, and 01/1412 have been officially released in Nigeria since 2011, as reported by Maroya, Kulakow, Dixon, & Maziya-Dixon (2012), who obtained TC values different from this study for genotypes 01/1368 and 01/1371. The genotypes showed highly significant differences in their TC values at a 5% significance level. Careful consideration of the desired chemical composition's mean values indicates that genotype 95/0379 is outstanding across all four locations and could be a good selection for advancement.

Table 4. Mean value of chemical properties of yellow-fleshed cassava genotypes across four locations (N=224)

Genotype name	DM (g/100 g)	VC (mg/100 g)	CNP (mg/100 g)	TC (µg/g)
01/1404	26.3 ^{abcdefg}	25.2 ^{abcde}	17.1 ^{bc}	6.4 ^{abcde}
01/1368	26.1 ^{abcdefg}	28.2 ^{abcd}	10.1 ^{bcd}	7.8 ^{a}
01/1380	26.1 ^{abcdefg}	29.4 ^{abcd}	15.7 ^{bcd}	4.1 ^{fghi}
01/1224	26.4 ^{abcdefg}	22.2 ^{abcde}	15.1 ^{bcd}	6.6 ^{abcd}
01/1646	29.7 ^{abc}	30.8 ^{ab}	9.9 ^{bcd}	4.8 ^{efgh}
94/0006	31.9 ^{a}	28.9 ^{abcd}	12.5 ^{bcd}	3.3 ^{hi}
98/2132	26.8 ^{abcdefg}	24.7 ^{abcde}	15.4 ^{bcd}	5.4 ^{defg}
90/01554	27.7 ^{abcdef}	26.3 ^{abcde}	14.1 ^{bcd}	3.6 ^{hi}
01/1206	28.4 ^{abcde}	29.7 ^{abc}	9.6 ^{cd}	3.8 ^{ghi}
01/1371	23.0 ^{defghi}	23.0 ^{abcde}	11.3 ^{bcd}	7.3 ^{ab}
01/1649	23.0 ^{defghi}	22.3 ^{abcde}	15.5 ^{bcd}	6.2 ^{abcde}
94/0330	25.1 ^{bcdefgh}	29.1 ^{abcd}	11.7 ^{bcd}	4.0 ^{fghi}
01/1331	20.9 ^{ghi}	20.1 ^{cde}	33.1 ^{a}	6.6 ^{abcd}
01/1335	20.5 ^{ghi}	21.2 ^{bcde}	20.3 ^{b}	6.4 ^{abcde}
95/0379	27.6 ^{abcdef}	28.8 ^{abcd}	7.9 ^{cd}	5.5 ^{cdef}
01/1412	24.2 ^{cdefghi}	22.8 ^{abcde}	9.1 ^{cd}	7.0 ^{abcd}
01/1235	24.5 ^{bcdefghi}	23.6 ^{abcde}	11.8 ^{bcd}	5.9 ^{bcde}
96/1089A	29.9 ^{abc}	25.9 ^{abcde}	8.1 ^{cd}	2.6 ^{ij}
01/1277	22.9 ^{defghi}	21.3 ^{bcde}	6.1 ^{d}	7.2 ^{abc}
01/1442	20.4 ^{ghi}	21.0 ^{bcde}	14.5 ^{bcd}	6.1 ^{abcde}
01/1610	19.4 ^{hi}	19.3 ^{de}	14.3 ^{bcd}	6.5 ^{abcd}
01/1663	21.6 ^{fghi}	16.2 ^{e}	9.7 ^{cd}	6.7 ^{abcd}
01/1115	22.0 ^{efghi}	20.7 ^{bcde}	5.7 ^{d}	6.2 ^{abcde}
01/1413	22.0 ^{efghi}	20.6 ^{cde}	8.3 ^{cd}	6.1 ^{bcde}
01/1273	18.3^{i}	17.8 ^{e}	9.6 ^{cd}	6.3 ^{abcde}
91/02324(chk)	29.8 ^{abc}	28.1 ^{abcd}	12.6^{bcd}	0.9 ^{k}
TME 1(chk)	30.9 ^{ab}	25.3 ^{abcde}	6.6 ^{d}	1.0 ^{jk}
30572(chk)	29.1 ^{abcd}	31.9 ^{a}	14.4^{bcd}	0.9 ^{k}
Pr> F(Model)	***	***	***	***
Pr> F(Genotype)	***	***	***	***

Remarks: DM = Dry matter; VC = Vitamin C; CNP = Cyanide Potential; TC = Total Carotenoids; ***, **, * = Significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively; chk = check; Means with different alphabetic superscript within the same column are significantly different.

Table 5 shows the mean values of the chemical properties of yellow-fleshed genotypes based on each location. Cassava in Ubiaja had the highest DM contents; the lowest averaged DM was 20.5 g/100 g in Mokwa. Lower DM values imply less solid content per unit g of harvested roots. Thus, producers in Ubiaja should anticipate a higher yield of solid content than those in any of the other locations. Dry matter contents varied significantly across all four test locations. No significant difference existed between the content of VC in Ubiaja and Mokwa but varied significantly from the other locations. Cassava from Zaria had the most VC, followed by Ibadan; both significantly different from each other. The content of VC ranged between 20.5 mg/100 g (Mokwa) and 29.4 mg/100 g (Zaria).

Zaria had the lowest average value (4.6 mg/100 g) for CNP, while Ubiaja and Ibadan had the highest (5.4 mg/100 g). CNP values differed significantly amongst some of the locations. Higher CNP values suggest that end-users of cassava produced in Ubiaja, for instance, must pay considerably more attention to proper processing before consumption to prevent toxicity effects. Cassava varieties in Ibadan had the lowest TC content that varied significantly from the other locations, all of which had no significant differences. The largest TC (5.4 µg/g) content was observed in Ubiaja and Zaria, indicating that cassava produced from these locations will find greater use in combating VAD. In conclusion, Zaria had the best combination of average values of desired chemical properties, i.e., the lowest CNP (the most important), second highest DM, and highest VC and TC.

The PCA results of various genotypes' chemical properties across the four locations are presented in Table 6. The PCA was applied to compress data by decreasing the dimensionality of the dataset obtained (Bartholomew, 2010). Eigenvalues ≥0.8 were selected as the logical cutoff to identify the axis most important in explaining the dataset's variation. The first three axes were set, and the three accounted for about 89.10% of the total variation. Luchese, Spada, & Tessaro (2017) clarified the influence of starch content on the properties of corn and cassava starch-based films using PCA to form three response groups, a trend similar to this study. In the current study, PC1 accounted for total variations (42.7%), while PC2 and PC3 accounted for 28.6 and 17.8%, respectively. The eigenvectors of ≥0.4 were selected as the cutoff point for all the parameters loaded on the selected three PCA. Thus, DM, VC and TC were loaded on PC1, DM and CNP on PC2, and VC and TC on PC3. DM and VC were significantly ($P<0.05$) and positively correlated with PC1, while TC was significantly ($P<0.05$) and negatively correlated with PC1. Also, DM and CNP were significantly correlated with PC2, and PC3 was significantly ($P<0.05$) and positively correlated with TC and VC. It implies that TC is negatively correlated with DM but positively correlated with VC and explained the low dry matter generally observed for yellow-fleshed genotypes. A similar trend for PC1 was reported by Adebowale, Sanni, & Onitilo (2008) and Nwabueze & Trinitas (2009), where moisture, sugar, and starch accounted for 83% of the variation observed in fufu and tapioca samples' chemical composition.

Table 5. Mean value of chemical properties of yellow-fleshed cassava genotypes by location

Location	DM (g/100 g)	VC (mg/100 g)	CNP (mg/100 g)	TC (µg/g)
Ubiaja	31.2 ^{a}	21.6 ^{c}	21.7 ^{a}	5.4 ^{a}
Zaria	26.5 ^{b}	29.4 ^{a}	7.9 ^{c}	5.4 ^{a}
Ibadan	22.5 ^{c}	26.2 ^{b}	11.2 ^{b}	4.6 ^{b}
Mokwa	20.5 ^{d}	20.5 ^{c}	9.3 ^{bc}	5.3 ^{a}
Pr> F(Model)	***	***	***	***
Pr> F(Location)	***	***	***	***

Remarks: DM = Dry matter; VC = Vitamin C; CNP = Cyanide Potential; TC = Total Carotenoids; *** = Significant at P ≤ 0.001; Means with the same letter within the same column are not significantly different

Table 6. Principal components analysis (PCA) of the chemical properties of yellow-fleshed cassava across four locations

	PC1	PC2	PC3
DM	0.6***	0.4*	-
VC	0.6***	-	0.5**
CNP	-	0.9***	-
TC	-0.5***	-	0.8**
Eigen value	1.7	1.1	0.7
Cumulative percentage	42.7	71.3	89.1

Remarks: DM = Dry matter; VC = Vitamin C; CNP = Cyanide Potential; TC = Total Carotenoids; Cut-off point of eigen vector = 0.4; *, **, *** = Significant at P≤0.05, P≤0.01 and P≤0.001

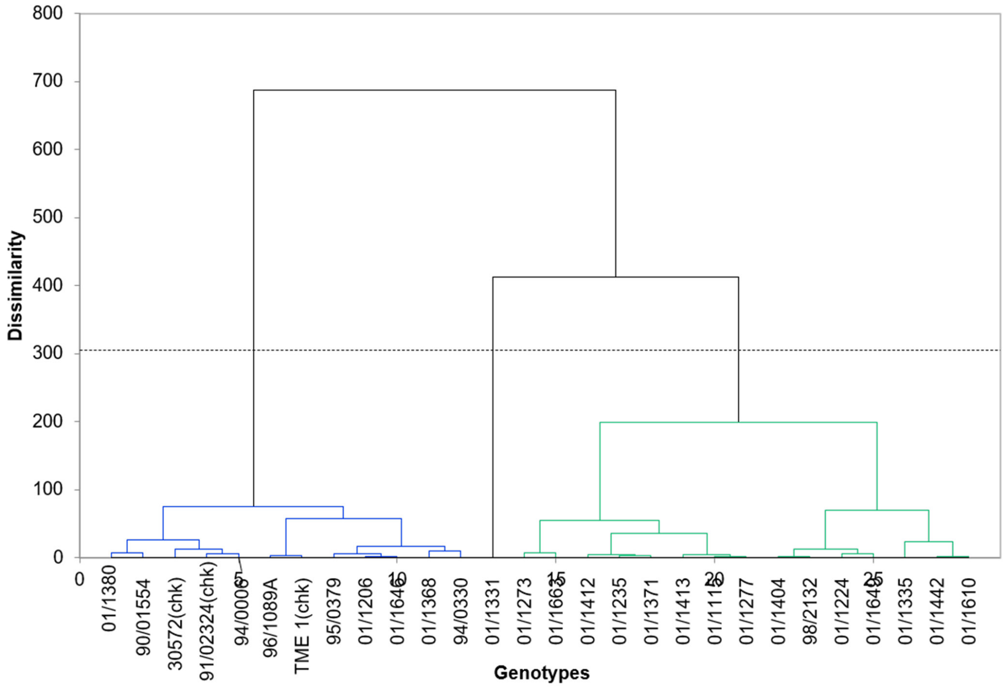

Fig. 1. Dendrogram (Ward's minimum variance clusters) showing the relationship among different cassava genotypes across environments

Fig. 1 and Table 7 present the cluster analysis of the genotypes evaluated. It is an analysis that organizes multivariate data into subgroups (Everitt, Landau, Leese, & Stahl, 2011). Cluster analysis grouped the genotypes into three (designated as classes/clusters 1, 2, and 3) based on all the chemical properties tested. In contrast, Nwabueze (2009) presented a dendrogram analyzing 'fufu' flour from 43 cassava varieties with six classes based on the similarity of their flours' proximate compositions. Of the 28 genotypes assessed, class 1 has 15 (53.6%), class 2 has 12 (42.9%), and class 3 has one (3.57%). Classification into each category is based on similarity in the genotypes' chemical properties across all environments. Thus, cluster 1 could be described as genotypes with high TC, Cluster 2 as high DM and VC with low CNP and TC, and cluster 3 as high CNP and high TC. All check genotypes (white-fleshed) were observed to fall under the same class (class 2). It was also observed that the only genotype in class 3 (01/1331) had the highest value for CNP (Table 4). However, average TC values of genotypes of class 1 were compared favorably to that of class 3 but with different CNP levels. These results imply that breeders can choose genotypes with superior performance from each group or cluster.

Table 7. Cluster analysis (and mean values) of yellow-fleshed cassava across all locations

Class	1	2	3
	01/1115	01/1206	01/1331
	01/1224	01/1368	
	01/1235	01/1380	
	01/1273	01/1646	
	01/1277	30572(chk)	
	01/1335	90/01554	
	01/1371	91/02324(chk)	
	01/1404	94/0006	
	01/1412	94/0330	
	01/1413	95/0379	
	01/1442	96/1089A	
	01/1610	TME 1(chk)	
	01/1649		
	01/1663		
	98/2132		
DM (g/100 g)	22.75	28.53	20.9
VC (mg/100 g)	21.46	28.53	20.1
CNP (mg/100 g)	12.25	11.1	33.1
TC (µg/g)	6.42	3.53	6.6

Remarks: DM = Dry matter; VC = Vitamin C; CNP = Cyanide Potential ; TC = Total Carotenoids (TC)

Table 8. Pearson correlation coefficients (PCC) of chemical properties of yellow-fleshed cassava genotypes across four locations

Variables	CNP	DM	VC	TC
CNP	1.000			
DM	0.209*	1.000		
VC	-0.128 ns	0.450 ***	1.000	
TC	0.128 ns	-0.301 ***	-0.304 ***	1.000

Remarks: *, **, *** = Significant at P≤0.05, P≤0.01 and P≤0.001; ns = not significant at P>0.05

Table 8 presents Pearson's correlation coefficients (PCC) analysis results, an analysis done to measure the linear correlation between chemical properties assessed and reflecting their strength and direction (Kozak, Krzanowski, & Tartanus, 2012). Discussions presented in Adeboye, Bamgbose, Adebo, Okafor, & Azeez (2019)) have guided the discussion in Table 8. There is a positive linear relationship between DM and CNP (r=0.209), VC and DM (r=0.450), and TC and CNP (r=0.128) at all three levels of significance tested (P≤0.001, P≤0.01, and P≤0.05). It indicates that it is easier to draw a connection between VC and DM contents; VC directly relates to the genotypes' DM content across all four breeding locations. This trend is similar for TC/CNP and DM/CNP. The correlation between DM and CNP is expected because higher CNP values are observed with higher DM values. A negatively linear correlation existed between VC and CNP (r= –0.128), TC and DM (r= –0.301), and TC and VC (r= –0.304). VC had the strongest correlation with DM (i.e., the most positive; r= 0.450), while TC and VC had the weakest correlation (i.e., the most negative; r= –0.304). It indicates that we cannot have varieties with high TC and VC values concurrently across the four locations. The same PCC magnitude was observed between VC and CNP and between TC and CNP, indicating that linear relationships exist in each case negatively (r= –0.128) and positively (r= 0.128) respectively. The implication of this finding for breeding is that the properties with a positive correlation could be bred together, while those with negative correlation will be difficult (Adeboye, Bamgbose, Adebo, Okafor, & Azeez, 2019).

CONCLUSION

Based on these research findings, genotype 95/0379 is recommended for advancement in the breeding program, as it had the best profile for all chemical properties tested, i.e. low CNP and high values of DM, VC, and TC. Genotypes from the Zaria location had the best combination of average values of studied chemical properties, i.e. the lowest CNP (the most important), second highest DM, and highest VC and TC. This research has been able to group genotypes based on similarities. Thus, it is an index of suitability for planting across the four environments. It is suggested that breeders consider the findings herein to select genotype(s) to be produced. Three analytical variables (DM, VC, and TC) were seen to be loaded heavily on PC1, DM, and CNP on PC2, and VC and TC on PC3. The only significant negative loading was TC on PC1.

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