



Review Article

Genotypic evaluation of twenty-eight high- and low-cyanide cassava in low-land tropics, southeast Nigeria

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ABSTRACT

A two-year field experiment was carried out in a randomized complete block design with two replications in 2015/16 and 2016/17 cropping seasons at the National Root Crops Research Institute, Umudike (05° 29'N; 07° 33'E; 122 m above sea level) in Nigeria. The objectives of the study were to assess growth, disease status and yield responses of twenty-eight (28) newly developed high- and low-cyanide cassava genotypes in low-land humid tropics of Umudike, Nigeria. Plant height, stem girth, canopy diameter, number of leaves/plant, cassava mosaic disease (CMD) and cassava bacterial blight (CBB) incidence and severity as well as bulking rate and fresh root yield varied significantly ($P < 0.05$) amongst the high- and low-cyanide cassava genotypes in both cropping seasons. Also, the results showed that bitter cassava genotypes exhibited greater tolerance to CMD than sweet cassava. However, there was no significant ($P > 0.05$) difference in bulking rate and fresh root yield between the two groups. The Pearson's and Spearman's ranked associations between fresh root yield of the cassava genotypes and other variables analysed across the two cropping seasons were highly significant ($P \leq 0.01$) and positive contrary to the other variables. However, they exhibited different degrees of associations amongst themselves, especially CMD incidence that indicated highly significant and positive association with severity. The principal component analysis across the two cropping seasons indicated eigen-values of the four axes > unity with cumulative variance of 68.98 %. Most of the characters that contributed to the 22.35 % observed variability in principal component (PC1) were CMD incidence and severity, and number of leaves/plant while PC2 also exhibited high vector load from plant attributes such as number of leaves/plant, bulking rate ha^{-1} and canopy diameter. The bi-plot clustering indicated that genotypes (BI-56, NR110439 and B1-29) exhibited strong similarity amongst themselves across the tested variables. The combined fresh root yield sequence of the first ten high yielding genotypes was in the order: NR110439 > TMS010354 > NR110315 > NR 110238 > NR 110228 > NR 060169 > BI-117 > BI-50 > NR110084 > NR 110181. These cassava genotypes were considered to be better endowed genetically, hence their improvement can be encouraged to ensure high and sustainable root yield. A poly-linear and positive regression was recorded between CMD and root yield as well as between CBB and root yield indicating that they affected fresh root yield of high- and low-cyanide cassava genotypes and demands attention also in cassava improvement studies.

1. Introduction

Cassava (*Manihot esculenta* Crantz), which is a crop that is very tolerant to drought and heat stress produces well on marginal soils (Alves, 2002; Calle et al., 2005; Dixon et al., 2008) and serves as a staple food crop in South-eastern Nigeria. Cultivars of cassava are generally classified as bitter (high-) or sweet (low-) cyanide depending on the level

of the two cynogenic glucosides (CG) (linamarin, which accounts for 80 % of CG and lotaustralin) present in the plant parts (Siritunga and Sayre, 2003); such that on enzymatic hydrolysis they release cyanohydrin and free-hydrocyanic acid (HCN) (Cardoso et al., 2005; Njoku and Ano, 2018).

Studies by Maziya-Dixon et al. (2007) and Wobeto et al. (2007) indicated that the presence of CG in the leaves of cassava are relatively

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higher (53–1300 mg kg⁻¹ of dry matter) compared with cassava roots (10–500 cyanide equivalents kg⁻¹ of dry matter). Further works have also shown that hydrogen cyanide (HCN) in cassava ranges from 1 to 2000 mg kg⁻¹ of dry matter (Cardoso et al. 2005) depending on factors such as genetic make-up of the cultivar, plant age, soil and environmental conditions as well as the type of fertilizer applied during production (Iglesias et al., 2002; Rolinda et al., 2008; Gitebo et al., 2009). Also, Riis et al. (2003), Mburu et al. (2012) as well as Njoku and Ano (2018) submitted that the level of HCN in cassava plays a role in plant defense against insect pests, diseases, herbivours and unfavourable biotic conditions in the field.

The food value of cassava is greatly compromised by the level of toxic HCN content in it (Akely et al., 2007; Adepoju et al., 2010). In contrast to sweet (low-cyanide) cassava roots which are processed simply by peeling and boiling or roasting, bitter (high-cyanide) cassava roots demand a more extensive processing method that goes in sequential order: peeling, washing, grating, fermenting, drying or frying, among others to reduce the HCN content to a safe level for human consumption. According to World Health Organization (WHO), the safe level for cyanide in cassava flour is 10 ppm or 10 mg HCN kg⁻¹ (FAO/WHO, 1991; Cardoso et al., 2005).

Among the two main cassava groups, bitter cassava is characterised by its high contents of CG (15–400 mg of HCN per kilogram of fresh weight of roots) while sweet cassava with low cyanide contents will typically contain approximately 15–50 mg HCN per kilogram of fresh weight of roots (Irtwange and Achimba, 2009; Njoku and Ano, 2018). Also, Wilson and Dufour (2002) reported that high-HCN cassava genotypes exhibit more than 100 parts per million (ppm) of cyanogenic equivalents while low-HCN cassava exhibits less than 50 ppm. Bitter cassava cultivars are more drought resistant, exhibits better photosynthetic efficiency (Eke-Okoro, 2000) and gives higher storage root yield than low-cyanide cassava cultivars (Okpara et al., 2014). More so, it is readily available and cheaper, hence demands more scientific attention in cassava crop improvement.

Furthermore, cassava production is limited by factors such as lack of high yielding, disease or pest resistant cultivars as well as inadequate information on the bulking status of the cultivars used by the farmers among other biotic and abiotic factors (Ekanayake, 1998). According to Balagopalan (2002) and Adams et al. (2009) the presence of cassava mosaic disease (*Begomovirus* spp.), caused by a virus and cassava bacterial blight (*Xanthomonas Campestris* PV. *Manihoti*), lead to yield losses in cassava production systems that depend on susceptible cultivars across the region. However, there is paucity of such materials in the low-land humid agro-eco zone of southeast Nigeria. Therefore, the objectives of the study were to evaluate growth, disease status and yield of some twenty-eight (28) newly developed high- and low-cyanide cassava genotypes for their yield potential in low-land humid tropics as well as elucidate the inter-relationships between associated plant attributes.

2. Materials and methods

2.1. Experimental site

Two field experiments were conducted at the National Root Crops Research Institute, Umudike, Nigeria (longitude 07°33' E, latitude 05°29' N and altitude 122 m). Umudike is in the low-land humid tropics of south eastern Nigeria, where a significant amount of cassava (12,167, 984 million tons per annum) on an average of 13.95 t ha⁻¹ is produced on an arable land area of about 1,056,267 hectares (PCU, 2003; Philip et al., 2005). The total area of the agro-eco zone (126,027 sq. klm), which is very suitable for cassava production falls within the coastal region of West Africa spanning towards the Atlantic ocean and shares virtually the same agro-climatic features (hot and humid) with all the countries lying across that axis of the equatorial belt (Okechukwu et al., 2001).

The area had a total annual rainfall and mean daily sunshine hours of 2,388.4 mm and 5.45 hours day⁻¹ (2015/16) and 1,901.8 mm and 5.36

hours day⁻¹ (2016/17) cropping seasons, respectively (Supplementary Table 1). The major rainfall season is from April to July, which is normally interrupted by a short period of dry spell in August while the minor rainfall season is from September to November of each year.

Prior to planting, soil samples were collected from the top soil of the experimental plots with the aid of a soil auger at a depth of 0–25 cm in 2015/16 and 2016/17 cropping seasons and a composite sub-sample collected and subjected to analysis for physico-chemical characteristics of the experimental site. The results showed that the texture of the soil was sandy loam and classified as Ultisol (Paleultstult) (US Soil Classification). In 2015/16 and 2016/17 cropping seasons, soil analysis indicated that pH (soil:water), organic carbon (C) (%), total nitrogen (N) (%) and available phosphorus (P) (mg·kg⁻¹) were 5.8, 1.12, 0.097, 33.4 and 6.1, 1.10, 0.10, 22.7, respectively while exchangeable calcium (Ca²⁺), potassium (K⁺) and magnesium (Mg²⁺) (cmol·kg⁻¹) were 3.20, 9.221, 0.96 and 4.0, 5.30, 1.8, respectively. The results showed that the soil was slightly acidic, with very low N content but high in available P and exchangeable K.

2.2. Cassava genotypes, planting and experimental design

The treatments used in the study were twenty-eight (28) cassava genotypes comprising of nineteen (19), high-cyanide (15–400 mg HCN kg⁻¹ fresh weight of cassava roots) and nine (9), low-cyanide (typically 15–50 mg HCN kg⁻¹ fresh weight of cassava roots) according to Njoku and Ano (2018). Passport details of the genotypes used in the study are shown in Table 1. NR110223 and B1-5, were checks for high- and low-cyanide cassava genotypes, respectively. All the planting materials were sourced from the Genetic Resource Unit, National Root Crops Research Institute, Umudike, Nigeria (NRCRI) cassava breeding lines, which comprised of International Institute of Tropical Agriculture, Ibadan, Nigeria (IITA) and NRCRI origins but domiciled at NRCRI Gene-Bank. However, their pedigree came from white and yellow-fleshed roots parents/germplasm of Program for Emerging Agricultural Research Leaders (PEARL) cassava breeding project.

The experiment was laid out in a complete block design with two replications and the plot size was 5 × 4 m (20 m²). Matured cassava stems that were 12 months old were planted at a spacing of 1 m intra- and inter-row apart on the crest of the ridges that were spaced 1 m from each other which gave a plant population of 10,000 plants·ha⁻¹.

2.3. Cultural practices

The field was cleared, ploughed, harrowed and ridges made with a tractor before it was marked into experimental units. Weeding was done manually with hoe at 3 and 8 weeks after planting (WAP) while slashing was carried out at 6 months after planting (MAP) to achieve a clean farm. The application of fertilizer (N:P:K 20:10:10) was done at the rate of 400 kg ha⁻¹ by ring method immediately after the second weeding regime in all the plots.

2.4. Measurements

Growth data such as plant height, canopy diameter and number of leaves/plant were taken on six sampled plants at 12 MAP. Plant height was measured with the aid of a metre rule from the base of the stem at the root collar to the terminal bud of the main stem while stem girth was measured at the distance of 20 cm above the root neck using vernier calipers.

Plant canopy spread was determined by measuring the diameter covered by the plant's canopy perpendicularly and parallel to the ridge with the aid of a metre rule and the mean obtained considered in the result. The number of leaves/plant was obtained by counting the total number of leaves on the plant.

Table 1

Description of the cassava genotypes used in the study.

Serial Number	Cassava genotype	Origin	Cyanide potential in storage root	Serial Number	Cassava genotype	Origin	Cyanide potential in storage root
1	NR 110238	NRCRI	High	15	NR 110228	NRCRI	High
2	NR 110315	NRCRI	High	16	NR 110439	NRCRI	High
3	NR 110181	NRCRI	High	17	COB 4-100	NRCRI	High
4	B1-78	NRCRI	High	18	NR 050080	NRCRI	High
6	B4-6	NRCRI	High	20	B1-5	NRCRI	Low
7	TMS 950211	IITA	High	21	TMS 961708	IITA	Low
8	NR 090142	NRCRI	High	22	NR 110178	NRCRI	Low
9	NR 060169	NRCRI	High	23	B1-117	NRCRI	Low
10	TMS 010354	IITA	High	24	B1-50	NRCRI	Low
11	NR 050667	NRCRI	High	25	NR 100486	NRCRI	Low
12	B1-56	NRCRI	High	26	NR 100449	NRCRI	Low
13	NR 060251	NRCRI	High	27	COB 5-53	NRCRI	Low
14	NR 110223	NRCRI	High	28	B1-29	NRCRI	Low

NRCRI, National Root Crops Research Institute, Umudike, Nigeria. IITA, International Institute of Tropical Agriculture, Ibadan, Nigeria.

2.5. Plant disease assessment

The incidence and severity of cassava mosaic disease (CMD) and cassava bacterial blight (CBB) were assessed at 9 MAP. Disease incidence, expressed in percentage was determined by counting the number of infected plants in the experimental plots and estimated as the number of infected plants over the total number of plants in each plot (Fargette et al., 1994; Fauquet and Fargette, 1990). The sampled units were whole cassava plants by visual method. All the cassava plants in each experimental plot were used to ensure a reliable mean estimate.

Disease severity which referred to the degree of symptom expression was assessed visually using the scale 1–5 according to the procedure outlined by Hahn et al. (1980). The overall objective of the study determined the method of disease severity assessment carried out. The study related disease severity to the root yield of the cassava genotypes hence assessment was on each whole plant. Detailed below is the symptom description scale:

2.5.1. The cassava mosaic disease (CMD) symptom scale of 1–5 (symptom description scale)

- (1). Unaffected shoots, no symptoms.
- (2). Mild chlorosis, mild distortions at bases of most leaves, while the remaining parts of the leaves and leaflets appear green and healthy.
- (3). Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets.
- (4). Severe mosaic distortion of two thirds of most leaves and general reduction of leaf size and stunting of shoots.
- (5). Very severe mosaic symptoms on all leaves, distortion, twisting, mis-shapen and severe leaf reductions of most leaves accompanied by severe stunting of plants (Hahn et al., 1980).

2.5.2. The cassava bacteria blight (CBB) symptom scale of 1–5 (symptom description scale)

- (1). Unaffected shoots, no symptoms.
- (2). Angular leaf spotting only.
- (3). Wilting, angular leaf-spot enlarged, leaf blight, defoliation and gum exudates on stem/petioles.
- (4). Wilting, severe blighting, defoliation, intense gum exudation and shoot die-back.
- (5). Wilting and very severe blighting, defoliation and intense gum exudation, abortive lateral shoot formation, stunting and complete die-back (Hahn et al., 1980).

2.6. Fresh root yield ($\text{Mt}\cdot\text{ha}^{-1}$)

The fresh root yields ($\text{Mt}\cdot\text{ha}^{-1}$) of cassava genotypes obtained from

the net plots were extrapolated per hectare while bulking rate was calculated as the ratio of crop yield to duration of time spent by the crop in the field before harvest with the formula:

$$\text{BR} = [\text{Weight of root yield at harvest/Period of root bulking}] (\text{kg}\cdot\text{day}^{-1} \text{ ha}^{-1}) \text{ (Allen and Scott, 1980).}$$

Cyanide levels in the roots of cassava were measured following the procedure outlined by Cooke (1978) as modified by (Onwuka, 2005) in which cassava roots were peeled, properly washed and diced. The flours from the diced samples were thoroughly mixed and used to determine the residual cyanide levels in the cassava genotypes using the alkaline picrate method (Onwuka, 2005) whereby five grams of each samples were dissolved in 50 mL distilled water and allowed to stay overnight. The samples were filtered and the filtrates used for the cyanide determination. To 1 mL of the aqueous extract, 4 mL of alkaline picrate (obtained by dissolving 1 g of picrate and 5 g of sodium carbonate (Na_2CO_3) in 200 mL of distilled water) was added and incubated in a water bath at a temperature of 50 °C for 5 minutes. The formation of a dark red colour was read spectro-photometrically at 490 nm against a reagent blank which contained 1 mL of distilled water and 4 mL of alkaline picrate solution.

A series of serial dilutions were made from potassium cyanide (KCN) (in water, acidified with HCL) corresponding to the concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 $\mu\text{g mL}^{-1}$. The resulting solution was further diluted with 10 mL of water to give a final concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 $\mu\text{g mL}^{-1}$ and the cyanide content of the flours was extrapolated from the standard calibration.

The amount of cyanide in 100 g sample was computed using the formula:

$$\text{Cyanide (mg kg}^{-1}\text{)} = (\mu\text{g mL}^{-1} \text{ of cyanide} \times \text{final volume (mL)} \times 10) / \text{Sample wt, where:}$$

$\mu\text{g mL}^{-1}$ cyanide is obtained from the standard calibration,

Final volume - volume of sample measured from filtered extract,

Sample weight - weight of sample extracted.

2.7. Statistical procedures

The data collected were subjected to analyses of variance using the GenStat Discovery edition 4.23 (2007) to estimate genotype effect on the crop characters measured while mean separation was performed with F-tests (LSD) at $P \leq 0.05$ according to Obi (2002). The following linear model was used for statistical analysis:

$$X_{ijk} = \mu + G_i + B_j + E_{ijk}, \text{ where,}$$

X_{ijk} = Individual observation, μ = Genotype mean, G_i = Effect of cassava genotype, B_j = Effect of block, E_{ijk} = Experimental error.

Combined Pearson and Spearman's ranked correlation coefficients of cassava fresh root yield to other plant attributes were determined to assess the inter-relationships among them using the PROC CORR of SAS (SAS Institute Inc, 2007) and the significance tested by referring to the standard table following the procedure of Snedecor and Cochran (1980)

with $n - 2$ degrees of freedom, where n is the total number of observations.

To identify attributes responsible for high root yield among the genotypes, principle component analysis (PCA) was performed using the main values of the two replications of the attributes according to [Iezzoni and Pritts \(1991\)](#). The PCA according to [Suzan et al. \(1975\)](#) removes the inter-correlation that may exist between variables by transforming the original variables into smaller hypothetical components.

3. Results

The results from the descriptive statistics for bitter (High cyanide) and sweet (Low cyanide) cassava genotypes ([Table 2](#)) indicated that in 2015/2016 cropping season all the variables assessed (plant height, stem girth, number of leaves/plant, canopy diameter, cassava mosaic disease (CMD) incidence, cassava mosaic disease (CMD) severity, cassava bacterial blight (CBB) incidence, cassava bacterial blight (CBB) severity, bulking rate and fresh root yield) did not exhibit any significant ($P > 0.05$) difference between the two groups of cassava genotypes. The trend was the same in 2016/2017 cropping season except variables such as stem girth, CMD incidence, CMD severity and CBB incidence which showed significant ($P < 0.05$) variations between bitter and sweet cassava genotypes. Similar non-significant trend was also recorded in the combined analysis across the two cropping seasons except CMD severity at the sampled date. Bitter (High cyanide) cassava genotype had bigger stem girth, higher CMD incidence and severity rate at the sampled date

but exhibited lower CBB incidence relative to sweet (Low cyanide) cassava genotype. Furthermore, in the combined analysis, bitter cassava genotypes indicated higher CMD severity rate, which was higher by 17.24 per cent relative to sweet cassava genotypes.

The results from the year and combined analysis of variance ([Supplementary Table 2](#)) indicated that in both cropping seasons and across both years, cassava genotypes significantly ($P < 0.05$) affected plant height and stem girth. Among the high- and low-cyanide cassava genotypes, plant height ranged from 95.0 to 220.0 cm and 128.33–191.67 cm in 2015/16 and 2016/17 cropping seasons, respectively while the mean across both cropping seasons ranged from 126.1 (NR 110315) to 197.9 cm (B1-56). Also, the results showed that NR 060169 and NR 110238 had the biggest stem girth in 2015/16 and 2016/17 cropping seasons, respectively compared with other genotypes while the combined analysis indicated that NR 110238 exhibited the biggest stem girth relative to the other cassava genotypes evaluated.

The individual cropping seasons and combined analysis of variance indicated significant ($P < 0.05$) variations in number of leaves/plant and canopy diameter among the twenty-eight cassava genotypes ([Supplementary Table 3](#)). The number of leaves/plant ranged from 28.0 to 340.0 (2015/16) and 106.33 to 347.0 (2016/17) with B1-5 cassava genotype exhibiting the highest number of leaves/plant in both cropping seasons. However, combined analysis indicated that NR 50667 had the least number of leaves/plant while NR060251 gave the highest number of leaves/plant compared to the other genotypes. In both cropping seasons, NR110238 closely followed by NR110315 cassava genotypes exhibited

Table 2

Descriptive statistics for plant height, stem girth, canopy diameter, number of leaves/plant, canopy diameter, cassava mosaic disease incidence and severity (9 MAP), cassava bacterial blight incidence and severity (9 MAP), bulking rate and fresh root yield of bitter (high-cyanide) and sweet (low-cyanide) cassava genotypes in 2015/16, 2016/17 and combined cropping seasons.

Variables	Descriptive statistics	2015/2016		2016/2017		Combined	
		Bitter cassava (High cyanide)	Sweet cassava (Low cyanide)	Bitter cassava (High cyanide)	Sweet cassava (Low cyanide)	Bitter cassava (High cyanide)	Sweet cassava (Low cyanide)
Plant height (cm)	Mean $\pm$ St.dv.	158.24 $\pm$ 1.75	164.44 $\pm$ 7.86	157.93 $\pm$ 12.00	160.93 $\pm$ 2.88	158.09 $\pm$ 7.00	162.69 $\pm$ 5.24
	<i>P</i> value	0.387		0.724		0.164	
	Sig.	ns		ns		ns	
Stem girth (cm)	Mean $\pm$ St.dv.	2.12 $\pm$ 0.74	2.20 $\pm$ 0.09	1.99 $\pm$ 0.10	1.85 $\pm$ 0.11	2.05 $\pm$ 0.10	2.02 $\pm$ 0.22
	<i>P</i> value	0.105		0.034		0.845	
	Sig.	ns		*		ns	
No. leaves/plant	Mean $\pm$ St.dv.	122.11 $\pm$ 1.49	165.0 $\pm$ 49.89	203.37 $\pm$ 11.44	204.89 $\pm$ 8.90	162.74 $\pm$ 47.4	185.03 $\pm$ 37.2
	<i>P</i> value	0.446		0.933		0.517	
	Sig.	ns		ns		ns	
Canopy diameter (cm)	Mean $\pm$ St.dv.	115.26 $\pm$ 16.0	118.94 $\pm$ 3.06	136.94 $\pm$ 3.04	141.91 $\pm$ 7.41	126.10 $\pm$ 15.7	130.43 $\pm$ 14.0
	<i>P</i> value	0.830		0.623		0.715	
	Sig.	ns		ns		ns	
Cassava mosaic disease incidence (%)	Mean $\pm$ St.dv.	22.53 $\pm$ 3.28	19.28 $\pm$ 1.65	31.82 $\pm$ 3.76	20.39 $\pm$ 6.21	27.17 $\pm$ 6.09	19.83 $\pm$ 3.76
	<i>P</i> value	0.216		0.096		0.114	
	Sig.	ns		*		ns	
Cassava mosaic disease severity	Mean $\pm$ St.dv.	1.36 $\pm$ 0.041	1.32 $\pm$ 0.060	1.54 $\pm$ 0.05	1.07 $\pm$ 0.02	1.45 $\pm$ 0.11	1.20 $\pm$ 0.15
	<i>P</i> value	0.731		0.024		0.058	
	Sig.	ns		*		*	
Cassava bacterial blight incidence (%)	Mean $\pm$ St.dv.	61.39 $\pm$ 6.36	58.33 $\pm$ 3.93	58.97 $\pm$ 9.04	67.50 $\pm$ 9.82	60.18 $\pm$ 6.54	62.92 $\pm$ 8.08
	<i>P</i> value	0.747		0.041		0.578	
	Sig.	ns		*		ns	
Cassava bacterial blight severity	Mean $\pm$ St.dv.	2.21 $\pm$ 0.31	1.97 $\pm$ 0.46	2.14 $\pm$ 0.09	2.18 $\pm$ 0.05	2.18 $\pm$ 0.19	2.08 $\pm$ 0.30
	<i>P</i> value	0.271		0.325		0.565	
	Sig.	ns		ns		Ns	
Bulking rate (kg ⁻¹ .day ⁻¹ ha ⁻¹)	Mean $\pm$ St.dv.	842.54 $\pm$ 214.30	971.99 $\pm$ 182.99	979.28 $\pm$ 23.421	828.24 $\pm$ 28.150	910.91 $\pm$ 147.39	900.12 $\pm$ 135.33
	<i>P</i> value	0.108		0.151		0.908	
	Sig.	ns		ns		ns	
Fresh root yield (Mt·ha ⁻¹)	Mean $\pm$ St.dv.	10.11 $\pm$ 2.57	11.66 $\pm$ 2.20	11.75 $\pm$ 0.28	9.94 $\pm$ 0.34	10.93 $\pm$ 1.77	10.80 $\pm$ 1.62
	<i>P</i> value	0.108		0.151		0.908	
	Sig.	ns		ns		ns	

<0.05 (*), not significant (ns).

the widest canopy diameter relative to the other genotypes evaluated in the study. The canopy of the other genotypes varied significantly amongst themselves. The trend was the same in the combined analysis with canopy diameter ranging from 104.2 cm (NR060169) to 186.70 cm (NR110238).

The results from the analysis of variance (Supplementary Table 4) showed that the twenty-eight high- and low-cyanide cassava genotypes tested were significantly affected by CMD and CBB incidence and severity except CMD severity in 2015/16 and CBB incidence and severity in 2016/17 cropping seasons. NR060251 cassava genotype exhibited the highest CMD incidence with 51 % disease incidence level while NR060169 had the lowest in 2015/16 contrary to TMS 350211 that had the highest (87 %) but non-significant ($P > 0.05$) CMD incidence level in 2016/17 cropping season. Among the cassava genotypes, TMS 950211 and NR100486 recorded the highest CBB incidence while NR060169 and NR110178 had the highest CBB severity in 2015/16 and 2016/17 cropping seasons, respectively. However, disease incidence and severity across all the genotypes were generally low, which may be due to the genetic make-up relating to disease resistance developed by the tested genotypes.

The individual year and combined analysis of variance (Supplementary Table 5) of bulking rate and fresh root yield indicated significant ($P < 0.05$) variations amongst the tested cassava genotypes. Bulking rate of the cassava genotypes ranged from 7.53 to 62.05 $\text{kg}^{-1} \cdot \text{day}^{-1} \text{ ha}^{-1}$ (2015/16), 14.73 to 51.44 $\text{kg}^{-1} \cdot \text{day}^{-1} \text{ ha}^{-1}$ (2016/17) and 14.1 (NR100449) to 49.5 (NR110439) $\text{kg}^{-1} \cdot \text{day}^{-1} \text{ ha}^{-1}$ (combined). The highest root bulking was recorded under NR050667 closely followed by TMS010354 cassava genotype (2015/16) and NR110315 closely followed by NR100297 cassava genotype (2016/17) cropping seasons, relative to other genotypes. The highest significant fresh root yield in 2015/16 cropping season was TMS010354 followed by NR050667 cassava genotype while the least yielder was the high-cyanide cassava genotype (NR110223), which also was recorded as having the least bulking ability compared to the other genotypes. However, the trend was not the same in 2016/17 cropping season where BI-5 was the highest fresh root yielder closely followed by NR110238 while NR050080 had the lowest fresh root yield compared to the other cassava genotypes evaluated in the study.

The combined Pearson's correlation analysis across the two cropping seasons (Supplementary Table 6) showed that fresh root yield exhibited positive but non-significant ($P \geq 0.05$) correlation with all the variables evaluated except bulking rate. The results further showed that highly significant ($P \leq 0.01$) and positive relationships were obtained between stem girth and canopy diameter as well as cassava mosaic disease (CMD) incidence and CMD severity with correlation coefficients (r) of 0.54 and 0.86, respectively. Across the two cropping seasons, a positive and significant correlation was recorded between stem girth and bulking rate ($r = 0.42$), and canopy diameter and bulking rate ($r = 0.38$). All the other variables exhibited different degrees of correlation amongst themselves. Across the two cropping seasons, combined Spearman's ranked correlation analysis, which indicated the strength and direction of the associations showed positive and highly significant ($P \leq 0.01$) association between fresh root yield and bulking rate, while the other variables showed positive but non-significant association with fresh root yield. Also, the relationship between stem girth and bulking rate ($r = 0.45$), cassava bacterial blight (CBB) incidence and canopy diameter ($r = 0.40$), as well as CMD incidence and number of leaves/plant ($r = 0.44$) were positive and significant while CMD severity exhibited highly significant ($P \leq 0.01$) and positive relationship with CMD incidence.

The individual and combined correlation analysis across two years of nineteen high- and nine low-cyanide cassava genotypes (Supplementary Table 7) indicated that the correlation coefficients between fresh root yield of high- and low-cyanide cassava genotypes with bulking rate were highly significant ($P \leq 0.01$) and positive. In contrast to low-cyanide cassava genotypes, fresh root yield of high-cyanide cassava genotypes exhibited significant and positive correlation with stem girth as well as number of leaves per plant.

The combined principal component analysis (Supplementary Table 8) performed over the two cropping seasons (2015/16 and 2016/17) to identify variables that determined the root yield of the 28 newly developed cassava genotypes indicated that all four principal axes (PC1, PC2, PC3, and PC4) had eigen-values up to unity indicating cumulative variance of 68.98 %. Principal components (PC1), (PC2), (PC3) and (PC4) with eigen-values of 2.235, 2.166, 1.296 and 1.201, respectively accounted for total variability observed among the 28 cassava genotypes. In PC1, the characters that contributed most of the 22.35 % observed variability among the cassava genotypes were cassava mosaic disease (CMD) incidence at 9 MAP and cassava mosaic disease (CMD) severity at 9 MAP with vector loading of 0.34931 and 0.34179, respectively. PC2 also had the highest vector load from the same plant attributes as well as from number of leaves/plant, bulking rate ha^{-1} and canopy diameter. Except number of leaves/plant, canopy diameter and cassava bacterial blight (CBB) incidence at 9 MAP in PC3, the other principal components did not influence fresh root yield of the 28 cassava genotypes. In PC4, only bulking rate and plant height influenced fresh root yield of cassava contrary to the other variables. The cumulative variance of 68.98 % by the four axes with eigen-values > 1 showed that identified attributes within the axes exhibited strong influence on the phenotype of the newly developed cassava genotypes and could be an effective selection tool among the genotypes.

The bi-plot clustering (Fig. 1) of the twenty-eight (28) cassava genotypes and the ten plant attributes were carried out to show the relationships between and among the cassava genotypes studied. The principal component bi-plot exhibited the pooled performances of the genotypes across the two (2015/16 and 2016/17) cropping seasons. Genotypes 12 (BI-56), 16 (NR110439) and 28 (BI-29) indicated strong similarity among themselves across the tested attributes. The trend was the same in genotypes 22 (NR110178), and 23 (BI-117). The similarity exhibited by the genotypes may be due to duplication. However, appropriate morphological and molecular characterization should be carried out to achieve correct inference on the strength and source of the closeness of these genotypes. The observed weak similarities recorded between genotypes 6 (B4-4), 18 (NR050080), 21 (TMS961708), 25 (NR100486) and 26 (NR100449), and genotypes 5 (NR110084), 8 (NR090142), 9 (NR0660169), 11 (NR050667) and 15 (NR110228), which showed the relative distance amongst themselves are good indicators of the existence of exploitable variability observed among the

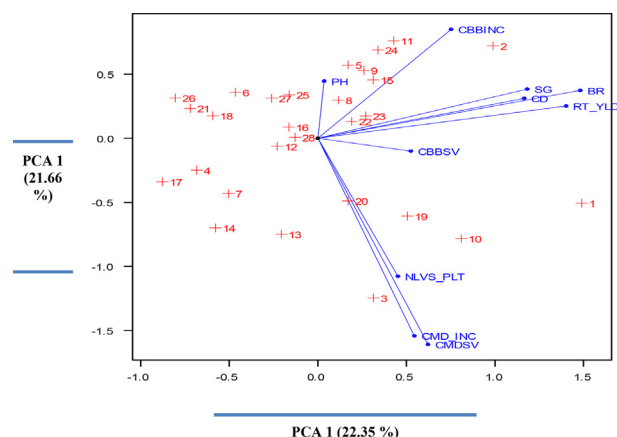


Fig. 1. Principal component bi-plot of PCA 1 against PCA 2 indicating the clustering of the twenty-eight high- and low-cyanide cassava genotypes and the ten attributes measured across the two (2015/16 and 2016/17) cropping seasons. Trait-standardized PCA. PH. Plant height (cm), SG. stem girth (cm), CD. canopy diameter (cm), NLVS PLT. number of leaves/plant, BR. bulking rate ($\text{kg}^{-1} \cdot \text{day}^{-1} \text{ ha}^{-1}$), RT YLD. fresh root yield ($\text{Mt} \cdot \text{ha}^{-1}$), CBBINC. cassava bacterial blight incidence (%), CBBVS. cassava bacterial blight severity (Scale 1–5), CMDINC. cassava mosaic disease incidence (%), CMDSV. cassava mosaic disease severity (Scale 1–5).

twenty-eight newly developed cassava genotypes. The bi-plot further showed that cassava genotypes such as 17 (COB4-100), 4 (B1-78), 14 (NR110223), 7 (TMS950211), 13 (NR060251), 3 (NR110181), 20 (B1-5), 10 (TMS010354), 19 (NR100297) and 1 (NR110238) and 2 (NR110315) were completely distant from others, hence have a good environment for wider variability that can be exploited for the great benefit of heterosis in plant breeding programmes. Furthermore, the bi-plot exhibited wide variability between most of the cassava genotypes, though a number of genotypes showed strong similarities. Moreso, the angle between the plant attributes determined the strength of the correlation between them, which implied that the smaller the angle between the plant attributes, the stronger the positive or negative correlation between them. The findings indicated strong association between stem girth and canopy diameter, and between bulking rate and fresh root yield, an indication that bulking rate impacted on root yield; as well as between CMD incidence and CMD severity. These are good attributes that could be further studied in enhancing yield of the newly developed cassava genotypes before being released to farmers in the agro-eco region of southeast Nigeria.

The regression analysis (Fig. 2) between CMD severity (a) and fresh root yield of cassava indicated the relationship as poly-linear, positive (Fig. 2a and b), which implied that fresh root yield of the twenty-eight cassava genotypes decreased as the scale of CMD severity increased in the two cropping seasons. Again, the relationship between CBB and fresh

root yield of cassava was also poly-linear and positive (Fig. 2c and d), indicating slight increase in fresh root yield as CBB severity increased in 2015/16 while in 2016/17 cropping season, fresh root yield of cassava was depressed with increased CBB severity up to 2.6 (on a 1–5 CBB severity score scale) and then increased with further increase in CBB severity. This implied that the genotypes exhibited strong CBB resistant characteristics, which is desirable in cassava breeding programmes aimed at improved fresh root yields. The relationships between fresh root yield of cassava and CMD severity as well as CBB severity in both cropping seasons were positive but very weak as indicated by the coefficients of determination (R^2), which measured how well the regression lines represented the whole data. The R^2 obtained in all the regression graphs had very low values.

4. Discussion

The studies showed slight significant variations between bitter (high cyanide content) and sweet (low cyanide content) cassava genotypes especially on disease status. Bitter cassava exhibited higher tolerance to CMD due to the presence of the cyanogenic glycosides while sweet cassava was more susceptible to CBB resulting in moderate bulking rate and fresh root yield that showed non-significant difference between the two groups. The findings contradicted previous studies by Wilson and Dufour (2002) and Okpara et al. (2014) in which they asserted that bitter cassava

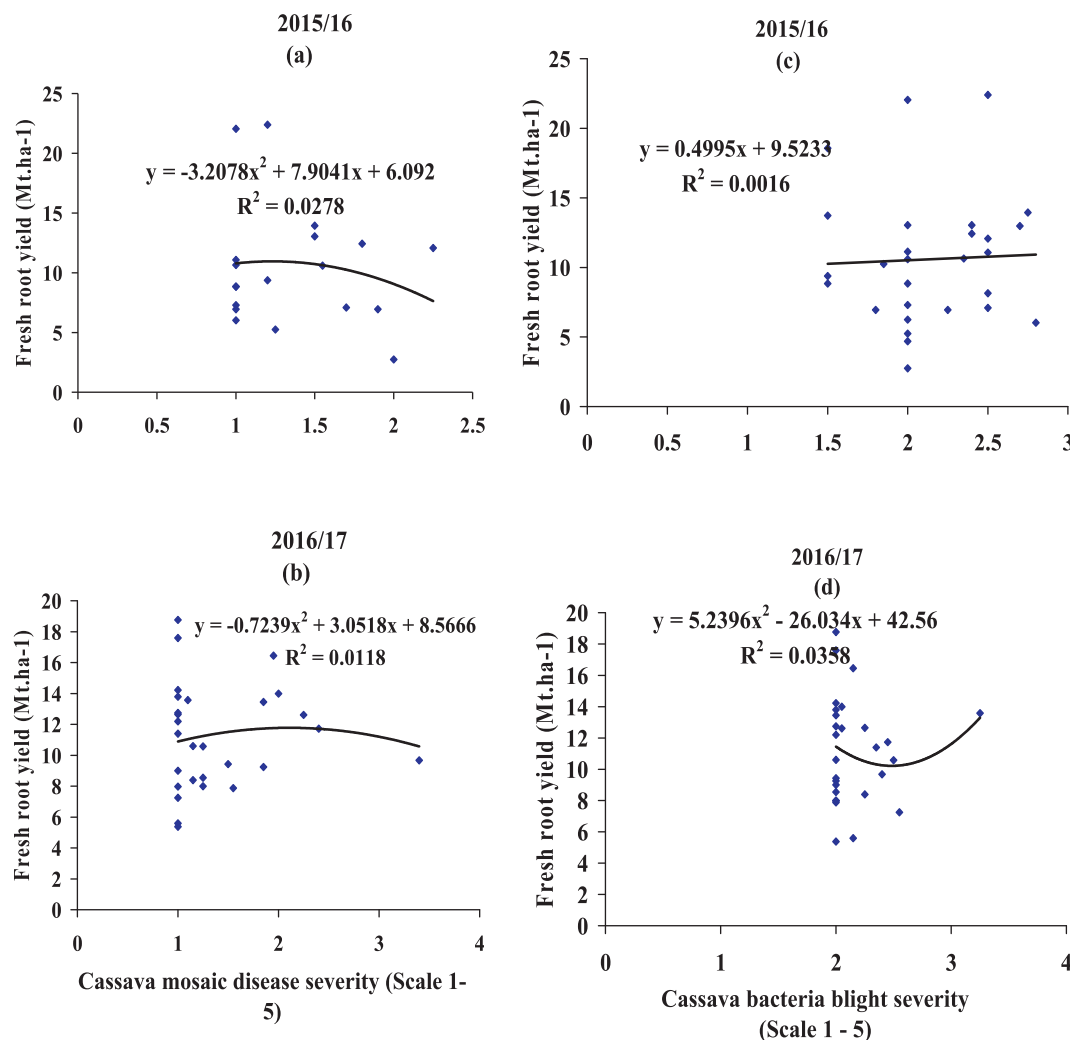


Fig. 2. (A) Relationship between cassava mosaic disease severity (b) and fresh root yield ($\text{Mt} \cdot \text{ha}^{-1}$) of twenty-eight high- and low-cyanide cassava genotypes with quadratic regression lines, and relationship between cassava bacteria blight incidence (c), severity (d) and fresh root yield ($\text{Mt} \cdot \text{ha}^{-1}$) of twenty-eight high- and low-cyanide cassava genotypes with quadratic regression lines.

cultivars yielded more than sweet (low cyanide content) cultivars on the plausible inference that bitter cultivars had the tendency to exhibit stronger resistance to diseases and pests than sweet cultivars due to the high presence of cyanogenic glycosides thereby resulting in higher root yields. Also, Valle et al. (2004) reported that bitter cassava genotypes are potential breeding materials for crosses with sweet cassava genotypes especially when the aim is to develop genotypes with higher concentration of total carotenoids because products of such crossings generate genotypes with low cyanide contents.

The twenty-eight high- and low cyanide cassava genotypes exhibited significant variations in growth variables such as plant height, stem girth, number of leaves/plant and canopy diameter in both individual years and combined analysis. These variables, which were good indicators of growth showed considerable agronomic characteristics of the cassava genotypes evaluated in the study and the findings were in consonance with previous studies by Lenis et al. (2006), Ojulong, et al. (2007), Ojulong, et al. (2010), El-Sharkawy (2012), Parkes et al. (2013) and Odedina et al. (2015) who submitted that these variables exact strong influence on root yield of cassava. Further more, large canopy diameter enhances increased solar interception and photosynthesis due to larger surface exposure thereby leading to increased fresh leaf, stem and root yield of cassava. The findings further corroborate similar studies by El-Sharkawy (2006), Egesi et al. (2007) as well as Baafi and Safo-Kantanka (2008) in which they reported the benefits of large plant canopy to increased crop yield due to better competitive performance of the cassava crop to growth resources, especially via interception of sufficient solar radiation, which the crop enjoys.

The results on the effect of cassava mosaic disease (CMD) and cassava bacterial blight (CBB) incidence and severity on the twenty-eight cassava genotypes were similar to the findings recorded by Ogbe et al. (2003) in Nigeria, Sseruwagi et al. (2004) in Central Africa, Owor et al. (2004) in Uganda, Legg et al. (2006) in East and Central Africa as well as Ntawurhunga et al. (2007) in the Democratic Republic of Congo in which they asserted that cassava exhibits significant variability to CMD and CBB incidence, severity and even epidemiology depending on location, seasonal environment and genetic characteristics of the genotypes. The findings from this study indicated medium to low disease incidence and severity due to the disease-resistance exhibited by the newly developed cassava genotypes across both cropping seasons.

Bulking rate and fresh root yield in individual year (2015/16 and 2016/17, and combined across both seasons) varied significantly among the cassava genotypes. The increase in these variables could be attributed primarily to the genetic constituents of the cassava genotypes and their responses to growth resources in the cropping seasons. The findings corroborate previous works by Manu-Aduening et al. (2006) in Ghana, Eze and Ugwoke (2010) in Nigeria, Polthanee et al. (2014) in Thailand, Odedina et al. (2015), Danquah et al. (2016), as well as Chipeta et al. (2016) in which they reported the essence of developing early bulking high yielding cassava genotypes to ensure quick financial returns and food security in Sub-Sahara Africa.

The two types of correlation analysis indicated not only the linear but also direction and strength of the inter-relationships between the variables studied. The correlation findings from the study were in consonance with similar studies by Oliveira et al. (2014) who reported medium to high magnitude relationship between morphological and genetic characters of cassava. Also, Munyahalia et al. (2017) reported a strong relationship between number of leaves/plant and fresh root yield in cassava. Furthermore, Legg et al. (2004), Ntawurhunga et al. (2007) and Muengula-Manyi et al. (2012) in their various works reported strong and positive correlation between CMD and CBB disease incidence and severity depending on season. Further correlation results based on cyanide levels of the genotypes from the study corroborate similar works by Ntawurhunga and Dixon (2010), Okpara et al. (2014) and Rao et al. (2017) on a number of broad-based cassava genotypes in which they reported significant correlation between fresh root yield and a number of growth (plant height, stem girth, canopy diameter, number of

leaves/plant, leaf area) and yield (number of roots/plant, root diameter, root length, root weight) attributes irrespective of the level of cyanide content in the genotypes evaluated indicating the importance of these attributes in cassava crop improvement programs. However, the present results indicated that significant association between fresh root yield and some growth attributes were recorded under high-cyanide cassava genotypes contrary to low-cyanide cassava genotypes.

The results from the combined principal component analysis were similar to the findings reported by Varma and Rai (1993), Jombart et al. (2010) on cassava and Afuape et al. (2011) on sweetpotato in their previous studies on root crops in which they indicated that principal components such as plant height, stem girth, number of leaves/plant, bulking rate, and some yield attributes as well as disease incidence and severity could serve as important indices during breeding and selection in cassava root crop improvement in SSA. Also, Fermont et al. (2009) submitted that there is great need to bridge the yield gap in cassava production by studying these attributes properly in respect to crop improvement studies to ensure appropriate food security in the region.

5. Conclusion

The disease tolerant level between bitter and sweet cassava genotypes was slightly significant. The findings showed that bitter cassava was less susceptible to CMD, perhaps due to high presence of cyanogenic glycosides than sweet cassava. However, there was no significant difference in their bulking ability and root yield. Therefore, these newly developed cassava genotypes especially the high-yielding, disease tolerant promising ones among them in the two groups can be subjected to further investigation aimed at improving root yield. Furthermore, the twenty-eight high- and low-cyanide cassava genotypes exhibited significant growth and yield attributes as well as tolerance to incidence and severity of CMD and CBB in both cropping seasons. Also the variables showed different degrees of inter-relationships amongst themselves, especially between fresh root yield and bulking rate, and between CMD incidence and severity, which were highly significant and positive. The inter-relationships of agronomic factors in determining cassava fresh root yield on the basis of cyanide levels in cassava genotypes require additional research to fully understand the implications of cyanide contents in improving cassava yield. The principal components that affected root yield indicated eigen-values that were above unity with cumulative variance of 68.98 % in which CMD incidence and severity, number of leaves/plant, bulking rate ha^{-1} and canopy diameter were the principal components that contributed to yield. Further more, significant genotypic variability existed for number of stem girth, leaves/plant, canopy diameter, CMD and CBB tolerance as well as bulking rate of fresh root; therefore, they can be incorporated as indices in crop improvement when selecting high yielding and disease-resilient cassava genotypes for release to farmers in the humid agro-ecological zone of Nigeria. Ten high- and low-cyanide cassava genotypes exhibited high yielding characteristics in this sequential order: NR110439 > TMS010354 > NR110315 > NR 110238 > NR 110228 > NR 060169 > BI-117 > BI-50 > NR110084 > NR 110181. Hence, they are considered to be better endowed genetically and demands systematic improvement to ensure high and sustainable root yield prior to their release for on-farm studies.

Declarations

Author contribution statement

Emmanuel Ukaobasi Mbah, Damian Ndubisi Njoku, Michael A. Gore: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Blessing Chinwoke Nwankwo: Performed the experiments;

Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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