Phenotypic Evaluation of a Multi-location Cassava Breeding Trial to improve a Genomic Selection Training Population

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ABSTRACT

Thirty white fleshed cassava genotypes derived from crosses between genotypes from West Africa x East Africa and West Africa x Latin America were evaluated in five major agroecological zones in Nigeria during 2012-2013. The trials were established in a randomized complete block design with four replications. The objectives of the study were (i) to evaluate genotype (G), environment (E), and G X E interaction on cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava anthracnose disease (CAD), and cassava green spider mite (CGM) in a multi-location uniform yield trial and (ii) to characterize morphological variation to improve a genomic selection training population that includes these genotypes. Combined analysis of variance showed differences (*P-<- 0.001*) among E for all traits evaluated. Genotypes did not differ significantly in their field reaction to CMD and CBB but showed variation in mild CAD symptoms and more severe, CGM reaction. Environmental variation accounted for 53.09% of the total sum of squares for CMD; 49.53% for CBB; 64.76% for CAD and 59.39% for CGM. The high influence of E demonstrated large differences in disease and pest severity in different locations. Morphological parameters such as levels of branching, angle of branching, height of branching, and plant height varied significantly among genotypes. Genotypes I090488, I090536 and I090590 branched the most, while I090574, I090564 and TMEB 419 (check) branched the least. Our results revealed that genotypes I090506, I090537 and I090609 were either low branching or have a wide angle of branching. This is significant as it helps in suppressing the weed flora, especially spear grass (*Imperata cylindrical*) by forming a dense canopy.

Keywords: cassava breeding, phenotype, genomic selection, training population, genotype x environment interaction.

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz), along with maize, sugarcane and rice, constitute the most important sources of energy in the diet of most tropical countries of the world (Ceballos *et al.,* 2004). The species originated in South America and was domesticated less than 10,000 years ago (Allem, 2002; Olsen and Schaal, 2001). Early European sailors soon recognized the advantages of cassava and carried it to Africa and Asia during the 16th century. Cassava is an important food security crop in the developing world and a major source of food calories for

about two of every five Africans (Nweke *et al.*, 2002). In 2008, Nigeria became the leading cassava producing country in sub-Saharan Africa, producing 44.6 million tonnes of fresh tuberous roots on 3.8 million hectares (FAO, 2011). Globally, cassava is grown in an area of 18.5 million hectares producing 202.6 million tonnes with a productivity of 10.95 t ha^{-1} (FAO, 2005). In Nigeria, more than 70% of cassava production is processed at the village level into *gari*, the principal source of calories for 70 – 80 million Nigerians (Maroya *et al.,* 2012). Chávez *et al.* (2005) noted that about 70 million people derive more than 500 cal/day from food based on cassava roots. Cassava is grown throughout tropical Africa, Asia, and the Americas. Its large, starchy, roots and edible leaves provide food for 800 million people globally, many of whom subsist on it, in part because it is drought tolerant and requires little in terms of inputs (Ceballos *et al.,* 2010). Cassava will continue to assume greater importance with time as a major source of carbohydrate for human consumption in the tropics and subtropics, where the low per capital income will not permit a change in the dietary habits (Akparobi *et al.,* 2006). Cassava is an outcrossing, heterozygous species propagated clonally from stem cuttings. The multiplication rate of cassava through vegetative cuttings is low (Ceballos *et al.,* 2004, 2012). Under good environmental conditions, a cassava plant from a modern clone can easily yield up to 20 cuttings. However, when thousands of clones are handled in a range of environments, a realistic multiplication rate is in the range of 5–10 cuttings per plant. This imposes a critical limitation, because cassava breeding cycles takes several years due to the 12-month cropping cycle and the slow rate of multiplication to have sufficient planting material required for proper multi-locational field trial evaluation. This limits the rate of variety improvement and breeders' ability to respond to new challenges.

Shifting from phenotype-based selection to genotype-based selection has the potential to overcome the lengthy cassava breeding cycles. Genomic selection (GS) is a new plant breeding method that seeks to predict phenotypes from a high-density genotypic data alone, using a statistical model based on both phenotypic and genotypic information from a training population. In cassava, the application of genomic selection will offer the opportunity to accelerate the selection cycles, increase the number per unit time of crosses and selections, increase the number of seedlings that could be accurately evaluated, reduce the time for release of improved varieties, increase the genetic gains per unit time and efficiency in cassava breeding. This dramatic increase in efficiency will allow cassava breeders to meet the unprecedented demands of crop improvement. The need to implement genomic selection in cassava breeding programme (at the seedling stage) becomes imperative. This study was, therefore, designed with the following objectives: (i) to evaluate genotype (G), environment

(E), and G X E interaction on cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava anthracnose disease (CAD), and cassava green spider mite (CGM) in a multi-location uniform yield trial and (ii) to characterize morphological variation to improve a genomic selection training population that includes these genotypes.

MATERIALS AND METHODS

Experimental locations: The study was conducted at five experimental locations of the International Institute of Tropical Agriculture (IITA), Nigeria during the 2012-2013 cropping season. The agroecological characteristics of the locations, Abuja, Ibadan, Ikenne, Mokwa, and Ubiaja, which represent the major cassava growing agroecologies in the country, are shown in Table 1.

Genotypes: Thirty white-fleshed cassava genotypes including two checks were compared in an advanced multi-location yield trial. The test genotypes were mostly derived from crosses between elite genotypes from West Africa crossed to genotypes introduced from either East Africa or Latin America (Table 2). The checks were elite white root landraces, TMEB419 (CMD resistant and preferred for starch production) and TMEB693 (CMD resistant with excellent poundability for food uses).

Cultivation, design and field management: The cassava genotypes were grown under rain-fed conditions in a randomized complete block design (RCBD) with four replications in each location. Planting was done during the months June – July in each location, coinciding with the onset of rains using disease-free 25cm stem cuttings having at least four nodes. The Ibadan location was a uniform yield trial with 6-row plots of 7 plants/row at 0.8m spacing between plants on a plot size of 6m x 5.6m (row by column) giving plant population of 42 plants/plot. The ridges 0.3m high and 6m long were spaced 1m apart. The other locations were planted as advanced yield trials with the same spacing but a single 10m row per plot. Neither fertilizers nor herbicides were applied during the course of the experiment and weeding was done manually as necessary.

Data collection: Data were collected at various crop growth stages on morphological parameters, cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava anthracnose disease (CAD), and cassava green spider mite (CGM).

The morphometric parameters evaluated at periodic intervals included sprouting ability (proportion of germinated plants) at one month-after-planting (MAP), vigour at 1-MAP, leaf

retention at 6-MAP, height to the first apical branch (ground level to the base of the first crown-forming branch) at harvest (12-MAP), angle of first apical branch (between the vertical line of the main stem and the first branch) at harvest (12-MAP), levels of branching (number of branch whorls) at harvest (12-MAP), and plant height at harvest (12-MAP). Reaction of genotypes to CMD was taken at 1-3-and 6-MAP, 3-and 6-MAP for CBB, and 6- and 9-MAP for both CAD and CGM using a scale of 1-5, where 1= no symptoms and 5= very severe symptoms (IITA, 1990). An average CMD, CBB, CAD and CGM severity was calculated based on all ratings taken for a particular disease or pest.

Statistical analysis: Data collected were statistically analysed using analysis of variance with the GLM procedure of SAS (version 9.3) to determine the significance of the main effects and interactions. Where the ANOVA test indicated significant differences, treatment means were separated using Student-Newman-Keuls Test (SNK).

RESULTS AND DISCUSSION: Morphological parameters such as plant height, sprouting, height to first branching (whorls), angle of branching, levels of branching, and vigour varied significantly among genotypes (Table 3). Genotypes I090488, I090536, and I090590 branched the most, while genotypes I090564 and I090574 branched the least. Some genotypes evaluated in this study (I090506, I090537, I090578, and I090609) were either low branching or have a wide angle of branching when compared to the checks. This is significant as it helps in suppressing the weed flora, especially spear grass (*Imperata cylindrica*) by forming a dense canopy. All genotypes used in this study had leaf retention below average except I090546 with leaf retention above average and genotypes I090564 and I090482 with average leaf retention. However, these differences were not statistically significant at $P<0.05$ (Table 3). Leaf retention is implicated in drought tolerance and may present an additional opportunity to increase cassava yield. Across locations, genotypes had mean sprouting of 93% showing excellent plant establishment, with genotypes I090536 and I090509 having the highest sprouting (99%) while, genotype I090564 had the lowest value (78%). Genotypes I090510 and I090581 had the highest mean vigour of 6.60 with genotypes I090564 and I090616 recording the lowest mean vigour of 5.40 and 5.30, respectively. The overall mean vigour across locations was 6.03. The mean severity of CMD, CBB, CAD and CGM across locations (Figure 1) revealed that genotypes did not differ significantly in their field reaction to CMD and CBB but showed mild CAD symptoms, with CGM as the most severe biotic stress across locations. The combined analyses of variance using the GLM procedure of SAS indicated that environment (E) effect showed highly significant mean squares ($p<0.001$) for

all the disease traits evaluated (Table 4). Variation due to environment effects were larger than other sources of variation, a reflection of the differences in climate and soil types in which the genotypes were grown. This observation agrees with the earlier findings of (Ngeve, 1999). The high influence of E was demonstrated by large differences in disease and pest severity in different locations. The genotype (G) effect was significant for CAD ($p<0.001$) and CGM ($p<0.01$). Significant interaction between genotypes and environments was observed at $(p<0.001)$ for CMD, CBB and CGM.

CONCLUSION: High variability existed among improved cassava genotypes for morphological parameters such as plant height, height to first branching, and angle of branching. Our results revealed that genotypes respond differently to biotic stress across different environments. However, improved cassava genotypes showed high level tolerance to prevalent diseases and pests across Nigeria.

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Table 1. Agroecological characteristics of five experimental locations where the trials were grown.

Location	Agroecological zone	Soil type	Position	Altitude (masl)	Mean annual rainfall (mm)	Wet season	Temp. min/max (˚C)
Abuja	Southern Guinea-Savanna	Ferric luvisols	9˚16′N 7˚20′E	300	1221	Mar-Oct	20-33 ˚C
Ibadan	Forest-savanna transition	Ferric luvisols	7˚38′N 3˚89′E	210	1320	Mar-Nov	20-35 ˚C
Ikenne	Rainforest	Eutric nitosols	6˚86′N 3˚71′E	44	1515	Mar-Oct	22-33 ˚C
Mokwa	Southern Guinea savanna	Ferric luvisols	9˚28′N 5˚05′E	195	2627	Apr-Nov	18-37 ˚C
Ubiaja	Humid forest	Dystric nitosols	6˚65′N 6˚38′E	287	1186	Mar-Dec	20-33 ˚C

masl= metres above sea level.

Table 2. Genotypes evaluated and their pedigrees

Genotype	Pedigree	Genotype	Pedigree
	West Africa		**West Africa x Latin America**
I090454	94/0561 (4X) X 06/0813	I090482	91934 X CM6119-5
I090536	96/1569 S1	I090485	91934 X CM6119-5
I090537	96/1569 S1	I090486	91934 X CM6119-5
TMEB 419 (CHK)		I090488	97/2205 X FECULA
TMEB 693 (CHK)		I090546	91/02324 X M.COL 1468
	West Africa x East Africa	I090564	96/1089A X CM6921-3
I090498	97/2205 X MAUNJILI	I090574	96/1632 X CM 5306-8
I090504	97/2205 X MKONDEZI	I090576	96/1632 X CM 5306-8
I090506	97/2205 X MKONDEZI	I090578	96/1632 X CM 6921-3
I090508	97/2205 X MKONDEZI	I090581	96/1632 X M. BRA 1045
I090509	97/2205 X MKONDEZI	I090590	97/2205 X M. COL 1468
I090510	97/2205 X MKONDEZI	I090597	97/4763 X CM7514-8
I090516	97/3200 X KIBAHA	I090609	98/0581 X M. COL 1468
I090520	97/4763 X KALESO	I090616	M98/0068 X CM6921-3
I090521	97/4763 X MAUNJILI		
I090522	97/4763 X MAUNJILI		
I090523	97/4763 X MAUNJILI		

Table 3. Mean morphological performance of 30 white-fleshed cassava genotypes evaluated at 5 locations in 2012-2013.

Genotype	Plant height (cm)		Sprout (%)		Branching height (cm)		Level of branching		Vigour		Angle of branching		Leaf retention	
I090454	216.3	bcde	98.5	a	85.7	cdef	2.3	abcde	6.4	ab	105.1	a	2.3	a
I090482	182.4	defgh	92.0	abc	62.2	def	2.7	abcd	5.6	bcde	107.5	a	3.0	a
I090485	195.9	cdefg	90.5	abcd	70.7	cdef	2.8	abc	5.7	bcde	102.0	a	2.3	a
I090486	139.1	gh	85.5	cd	46.3	f	2.2	abcde	5.7	bcde	95.9	a	2.3	a
I090488	186.4	defgh	84.0	d	74.3	cdef	3.3	a	5.5	cde	100.9	a	2.3	a
I090498	177.9	defgh	93.5	ab	63.9	def	3.0	ab	5.8	abcde	107.8	a	2.0	a
I090504	129.3	h	94.0	ab	45.5	f	1.9	abcde	6.2	abcd	100.1	a	1.8	a
I090506	148.2	fgh	95.0	ab	71.6	cdef	2.6	abcd	6.2	abcd	110.1	a	2.3	a
I090508	182.0	defgh	96.5	ab	.	.	.	.	5.8	abcde	.	.	2.8	a
I090509	205.6	cdef	99.0	a	84.0	cdef	2.9	ab	6.3	abc	97.8	a	2.5	a
I090510	237.4	bcd	97.0	ab	111.7	abc	2.3	abcde	6.6	a	95.6	a	1.8	a
I090516	194.0	cdefg	95.5	ab	77.6	cdef	1.9	abcde	6.2	abcd	97.0	a	2.5	a
I090520	251.7	abc	94.0	ab	122.6	ab	1.4	cde	6.2	abcd	55.6	b	2.0	a
I090521	201.9	cdef	97.5	ab	85.3	cdef	2.3	abcde	6.3	abc	96.8	a	1.8	a
I090522	190.5	defg	96.5	ab	78.9	cdef	2.8	abc	5.9	abcde	102.8	a	2.0	a
I090523	171.8	efgh	96.0	ab	73.8	cdef	3.0	ab	6.3	abc	101.3	a	1.8	a
I090536	172.2	efgh	99.0	a	50.4	ef	3.3	a	6.4	ab	101.9	a	2.3	a
I090537	191.5	defg	97.5	ab	70.7	cdef	2.7	abcd	6.3	abc	110.3	a	2.0	a
I090546	201.4	cdef	84.5	d	92.8	abcd	1.7	bcde	6.0	abcde	103.3	a	3.3	a
I090564	283.4	a	77.5	e	102.2	abcd	1.3	de	5.4	de	104.5	a	3.0	a
I090574	263.9	ab	95.5	ab	109.7	abc	1.1	e	5.9	abcde	92.2	a	2.0	a
I090576	181.0	defgh	95.5	ab	88.1	bcde	2.0	abcde	6.0	abcde	100.8	a	2.0	a
I090578	171.7	efgh	92.5	abc	66.7	def	1.8	bcde	5.7	bcde	109.9	a	2.5	a
I090581	231.1	bcde	89.0	bcd	110.3	abc	2.0	abcde	6.6	a	101.9	a	2.3	a
I090590	221.6	bcde	93.5	ab	68.7	def	3.3	a	5.9	abcde	106.7	a	1.8	a
I090597	205.0	cdef	91.5	abc	100.3	abcd	2.1	abcde	6.0	abcde	107.8	a	2.0	a
I090609	204.5	cdef	95.5	ab	85.0	cdef	2.1	abcde	6.1	abcd	112.0	a	2.5	a
I090616	181.9	defgh	92.0	abc	76.2	cdef	1.7	bcde	5.3	e	97.6	a	2.0	a
TMEB 419[c]	185.9	defgh	92.5	abc	132.6	a	1.4	cde	6.3	abc	97.2	a	2.0	a
TMEB 693[c]	221.3	bcde	97.0	ab	105.7	abcd	2.5	abcd	6.4	ab	97.4	a	2.0	a
Mean	**197.6**		**93**		**81.46**		**2.34**		**6.03**		**101.63**		**2.2**	
SE	**3.7**		**0.01**		**2.33**		**0.08**		**0.05**		**1.37**		**0.1**	
CV (%)	**12.3**		**8.18**		**18.27**		**21.09**		**12.16**		**10.33**		**28.7**	

[C]=genotypes used as checks.

Means with the same letter in a column are not significantly different at 95% level.

Table 4. Combined analysis of variance of 30 white-fleshed cassava genotypes evaluated at five locations in Nigeria in 2012-2013 for reaction to biotic stresses.

Source	DF	CMDS		CBBS		CADS		CGMS	
Genotype	29	0.032	ns	0.447	ns	0.428	***	0.590	**
Environment (E)	4	2.719	***	25.03	***	45.510	***	41.537	***
G x E	116	0.030	***	0.290	***	0.130	ns	0.302	***
Rep (Env)	15	0.004	ns	0.230	*	0.939	***	0.365	***
Error	435	0.012		0.120		0.113		0.126	
CV (%)		10.189		20.305		23.813		12.708	
R^2		0.746		0.743		0.814		0.807	

*, ** and *** indicate significant level at $P<0.05$, 0.01 and 0.001; ns = not significant. CMDS= Cassava Mosaic Disease Severity; CBBS= Cassava Bacterial Blight Severity; CADS= Cassava Anthracnose Disease Severity; CGMS= Cassava Green Mite Severity.

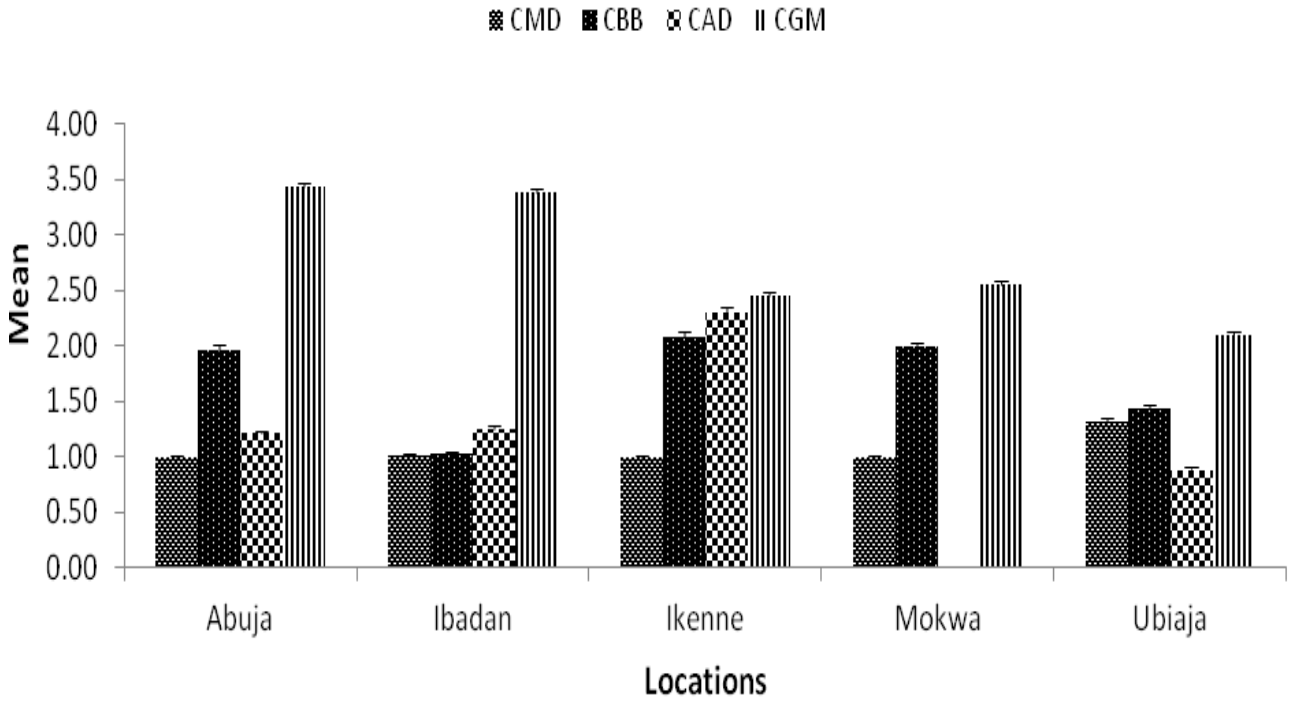

Figure 1. Severity of CMD, CBB, CAD and CGM at five locations in Nigeria in 2012-2013.

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