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Agronomic Performance and Carotenoid Content of Kenyan Yellow-Fleshed Cassava Clones

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Abstract

Vitamin A deficiency is common in Kenya; it has been reported among populations that depend on cassava as their main staple. Clonal evaluation of 324 locally developed yellow-fleshed cassava clones was carried out in two sites, Thika and Kiboko located in central and eastern Kenya respectively. Data was collected on agronomic performance, yield quality and reaction to biotic constraints. The clones rated well for the evaluated traits. The overall performance was better in Kiboko than Thika. Cassava Mosaic Disease, Cassava Brown Streak Disease (CBSD) and Cassava Green Mite (CGM) severity was scored on a scale of 1-5. The mean CBSD score was 1.17 in Kiboko and 1.46 in Thika, CMD severity was 1.15 in Kiboko but the disease was not observed at Thika. The CGM damage severity was 1.63 in Kiboko and 1.97 in Thika. The weight of roots per plant was 3.49 and 2.25 kg at Kiboko and Thika respectively. The dry, matter content of the roots was 49.86% at Kiboko and 29.06 at Thika. The cyanogenic potential was assessed using the pictrate test and rated on a scale of 1-9 and Kiboko had a score of 2.79 compared to 4.14 in Thika. A total of 91 samples were analyzed for carotenoid content using the spectrophotometry method. The Kiboko site had a higher mean carotenoid content (1736.96 µg/100 g) compared to Thika (1105.85 µg/100 g). Most of the samples (71%) analyzed had recorded a higher carotenoid content in Kiboko than Thika. The beta-carotene content was higher in Thika (374.16 µg/100 g) than Kiboko (291.30 µg/100 g). Promising clones adaptable to the two sites were identified.

Keywords: clonal evaluation, carotenoid, cassava, Kenya

1. Introduction

Cassava is an important source of dietary calories to an estimated 40% of the African population. It is also a source of income for resource poor households. Cassava roots can be used as a raw material in production of animal feeds, industrial starch and ethanol (El-Sharkawy, 1993). In addition, it has a high productivity and low labour requirements compared to other crops. Ever since the crop was introduced in Kenya in the 15 century, it has assumed the role of a subsistence commodity especially as a food security crop during famine periods. Dry areas of Eastern regions of Kenya generally depend on cassava as a staple food. It is predicted that cassava will remain largely unaffected by climate change (Jarvis et al., 2012).

The eastern Kenya region is largely characterized by erratic rainfall, on average adequate rainfall is experienced once in three years. The source of income is food crop farming and vegetable sources of vitamin A are often inadequate and inaccessible outside the growing season. Cassava plays an important role as a food security crop in the region. However, none of the cassava varieties grown in the region has improved nutritional value.

Conventional breeding was carried out to biofortify local germplasm for beta-carotene, a precursor of vitamin A. This was intended to help reduce cases of vitamin A deficiency reported particularly among children and mothers. Kenya fits within the World Health Organisation (WHO) severe sub-clinical deficiency cut-off with vitamin A deficiency prevalence of seventy percent among pre-school children (Munene et al., 2003; UNICEF, 2004).

The nutritional quality of cassava can be improved by plant breeders through exploitation of essential genes in both conventional and biotechnology approaches. The new varieties have a reasonable chance of acceptance. The nutritional trait is fixed by vegetative propagation of the crop and is effectively maintained and monitored.

Evaluation of biofortified clones is important to select for qualitative and quantitative attributes preferred by the producers and consumers of the crop. Common root quality and yield characteristics considered are cyanogenic potential, dry matter content and harvest index (Ojulong et al., 2008). Important constraint is also considered. The most important diseases of cassava in Kenya are the Cassava Brown Streak Disease (CBSD) and the Cassava Mosaic Disease (CMD) while the Cassava Green Mite (CGM) is the most important pest. Evaluation of newly bred cassava genotypes was therefore necessary to ascertain not only the agronomic performance but also yield and levels of vitamin A.

2. Materials and Method

2.1 Clonal Establishment

The trial was conducted at two sites KARI-Thika and KARI-Kiboko in central and eastern Kenya respectively in the 2011/2012 season. The kiboko site is located at an altitude of 975 m a.s.l. It experiences two rainy seasons and the soils are ferric luvisols. It is a high disease and pest pressure site. It is at close proximity to Kibwezi, where vitamin A deficiency is prevalent (Talsma et al., 2013). The altitude at Thika is 1548m a.s.l and the soils are acrisols.

A total of 324 entries were evaluated in an unbalanced lattice square design in two replications. The entries were cloned from a seedling population that had resulted from crosses among local white fleshed parents and yellow fleshed parents from International Institute of Tropical Agriculture (IITA). All the coloured and disease free entries from the seedling evaluation were advanced to the Clonal Evaluation Trial (CET). Cuttings were established at a spacing of 1 × 0.75 m. Normal agronomic practices for cassava were carried out. The trial was conducted over a period of nine months from August 2011 to May 2012.

2.2 Vegetative Stage and Harvest Data Collection

During the vegetative growth stage the clones were evaluated for reaction to CMD, CBSD and damage by the CGM. The CMD, CBSD and CGM severity was rated on a scale of 1-5 where one was a healthy plant and 5 was total destruction of the plant by the biotic stresses. Data on disease and pest severity was collected at three and six months after planting and at nine months just before harvesting. At harvest, a tally of the roots per plant was obtained. The roots were then grouped into the marketable and the unmarketable categories. The root yield (kg/plant) was determined by weighing the fresh roots. The shoot weight (kg/plant) was also obtained and the Harvest Index (HI) determined as a ratio of the root weight (kg) to the total biomass (kg). A longitudinal section of a root sample from each entry was obtained and the pulp colour scored on a scale of 1-4 where 1. The pulp colour was also rated using the harvest plus colour chart. The raw root was also tasted and rated on a scale of 1-3 where, 1 was sweet, 2 was average and 3 was bitter. At each site the taste rating was carried out by the same individual. A root sample of about 3kg was obtained from each clone for determination of percentage dry matter by the specific gravity method (Kawano et al., 1987).

Finally, the pictrate test was used to assess the cyanogenic potential of the harvested clones. The cyanide levels were recorded on a scale of 1-9. The HCN level of 1-4 was ranked low, 5-6 medium and 7-9 as high.

2.3 Carotenoid Quantification

Freshly harvested root samples were collected from plants within each of the root pulp colour scores and sent to the laboratory for carotenoid quantification by spectrophotometry. Two roots were harvested from each of the sampled plants in the early morning and care was taken to avoid physical damage. The roots were then transported to the laboratory in dark paper bags for carotenoid analysis. Three intact fresh storage roots were picked from each sample, peeled and washed in deionised water. The roots were then cut in four opposite longitudinal sections which were then combined and homogenized. Aliquots of the homogenized materials were weighed and stored at -18 ^{o}C until the time of analysis. Total carotenoids and β-carotene in each sample was analyzed in duplicate spectrophotometrically as described by method No. 44 of the International Federation of Fruit Juice Products (IFFJP, 1972). Results were obtained for 91 samples of which 41 were from Kiboko and 50 from Thika.

The combined analysis of variance of the collected data was carried out using the General Linear Model (GLM) on GENSTAT 14. The magnitude of the levels of sources of variation and their interactions were determined.

3. Results

There were significant ($P \leq 0.001$) differences across sites for all the variables determined in the study, except number of non-marketable roots. The cassava mosaic disease symptoms were not observed at Thika. The site also recorded the highest cyanogenic potential (Table 1). The entries with the highest root yield per plant were

05/0078 × 07/0520 and 07/0534 × 990183 at Kiboko and Thika respectively. The entries with the highest dry matter content were 05/0047 × 07/0751 at Kiboko and 05/0078 × 0752 at Thika.

Table 1. Summary of biotic stresses and yield attributes in yellow-fleshed cassava clones at Kiboko and Thika sites, Kenya

Variable	Kiboko site			Thika site		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
CBSD	1	2	1.17	1	2	1.46
CMD	1	3	1.15	1	1	1
CGM	1	3	1.63	1	2	1.97
Yield (kg/plant)	1.5	16.3	3.49	0.5	11.5	2.25
Mkt	0.5	16.3	3.44	0.25	10.1	2.2
TBM	0.1	42.1	10.24	0.5	29.53	7.07
HI	6	79.49	33.57	2.44	88.89	34.73
DM	19	49.86	35.92	16.3	45.25	29.06
HCN	2	4	2.79	2	7	4.14
Pulp colour score	2	4	2.13	2	4	2.23

Note. CBSD: cassava brown streak disease; CMD: cassava mosaic disease; CGM: cassava green mite; Mkt: number of marketable roots per plant; TBM: total biomass (kg) per plant; HI: harvest index, DM: % dry matter; HCN: cyanogenic potential.

A total of 48 clones in Thika had a root pulp colour score of three or four compared to 35 clones in Kiboko. Seven of the clones had a root pulp colour score of three across the two sites while 167 clones had a score of 2 in both sites. The seven best performing clones across sites for beta carotene also performed well with respect to other agronomic and qualitative traits (Table 2). The clones remained free of CMD throughout the evaluation period. The DM ranged from 23.83-40.56 and the cyanogenic potential was in the range of 2-5.

Table 2. Agronomic performance and qualitative traits of clones maintaining the root pulp colour score across Kiboko and Thika sites, Kenya

Entry	Family	Site	RTC	RTY	CBSD	CMD	CGM	Taste	DM	HCN
12	01/1412 × 820058	Kiboko	11	5.7	1	1	1.5	1	37.05	2
		Thika	5.25	1.65	2	1	2	3	33.91	3
41	05/0045 × 07/0752	Kiboko	9.25	5.7	1	1	1	1	29.6	2
		Thika	7.25	2.08	1.5	1	2	1	32.55	3
56	05/0055 × 01/1412	Kiboko	8	5.23	1	1	1	1	40.36	4
		Thika	8.67	3.87	1.5	1	2	2	32.33	4
73	05/0059 × 01/1412	Kiboko	9	10.4	1.5	1	1.5	1	40.56	4
		Thika	10.25	3.68	1.5	1	2	2	32.13	4.5
236	07/0752 × 05/0078	Kiboko	11.25	8.1	1	1	1.5	1	39.45	3
		Thika	9	1.55	1.5	1	2	1	25.13	4.5
254	820058 × 01/1412	Kiboko	10.75	5.43	1	1	2	1	34.37	3
		Thika	6	4.1	1	1	2	1	23.83	5
260	990132 × 07/0520	Kiboko	5	3.23	1	1	2	1	32.01	3
		Thika	7.5	2.78	1	1	2	2	28.44	4

Note. RTC: root count per plant; RTY: root yield (kg/plant); CBSD: cassava brown streak disease severity; CMD: cassava mosaic disease severity: DM: % dry matter content; HCN: cyanogenic potential.

The sites were highly significant ($P \geq 0.001$) for all the traits evaluated except HI (Table 3). The entries were significant for all traits except CGM. The site × entry interaction was significant for CMD and DM.

Table 3. Mean square values for quantitative and qualitative traits from the combined ANOVA for the clonal evaluation at Kiboko and Thika sites, Kenya

Source of Variation	d.f.	CMD	CBSV	CGM	Root yield	TBM	HI	DM	HCN	PLP	Taste
Site	1	4.35***	0.03***	23.31***	7.86***	63.72***	184.27	313.03***	307.22***	8.2406***	0.44***
Entry	287	0.13***	0.01*	0.16	0.12*	0.86*	160.45*	37.72*	1.04*	0.4069*	0.02*
Rep	1	0.45*	0.11	1.76***	2.39***	23.49***	118.07***	21.96***	0.29*	3.5695***	0.17***
Block	17	0.08	0.10	0.13	0.1	1.52*	167.36***	76.19***	1.04*	0.4837*	0.107
Site × Entry	145	0.097*	0.07	0.12	0.88	0.74	76.32	30.84***	0.80*	0.273	0.02
Entry × Rep	205	0.12*	0.01	0.13	0.06	1.93*	80.09	31.56*	0.43	0.3244	0.03*
Error	56	0.06	0.03	0.14	0.09	0.7	59.24	17.85	0.49	0.2455	0.01
CV		22.67	1.54	21.01	23.17	29.73	21.70	12.96	20.32	24.73	30.07

Note. df: degrees of freedom; CMD: cassava mosaic disease; CBSV: cassava brown streak disease; CGM: cassava green mite; TBM: total biomass per plant; HI: harvest index; DM: % dry matter; HCN: cyanogenic potential; PLP: pulp colour rating.

The Kiboko site had a higher mean carotenoid content (1736.96 µg/100 g) compared to Thika (1105.85 µg/100 g). Most of the samples (71%) analysed had recorded a higher carotenoid content in Kiboko than Thika. The beta-carotene content was higher in Thika (374.16 µg/100 g) than Kiboko (291.30 µg/100 g). The total carotenoid content ranged from 165-13612.5 µg/100 g and 162.5-660 µg/100 g in Kiboko and Thika respectively. The range of the beta-carotene fraction was 162.5-660 µg/100 g in Kiboko and 2474-82.5660 µg/100 g in Thika. High levels of total carotenoid did not translate to high levels of beta-carotene among the clones at the two sites. Though all the clones with high carotenoid content had coloured flesh, no correlation was observed between flesh colour and carotenoid content (Table 4). There were differences observed in carotenoid content among clones in the same root pulp colour score. The average proportion of beta-carotene to total carotenoid was 42.27%. Twelve clones had undetectable levels of beta-carotene fraction while four clones had a beta-carotene fraction of 100%. The quantity of the beta-carotene in the four clones ranged from 165-330 µg/100 g. The combined analysis of variance showed that there was no significant difference in total carotenoid and beta-carotene among the clones.

Table 4. Total carotenoid and beta-carotene content of the best ten yellow-fleshed clones and the respective beta-carotene content at Kiboko and Thika sites, Kenya

Kiboko				Thika			
Entry	Root pulp colour score	Total carotenoids (µg/100gm)	Beta-carotene (µg/100gm)	Entry	Root pulp colour score	Total carotenoids (µg/100gm)	Beta-carotene (µg/100gm)
44	4	13612.5	165	3	2	7382.5	825
148	2	10642.5	660	85	2	6817.5	330
150	2	9900	165	104	3	6105	2475
159	3	3465	330	109	3	4125	165
248	2	2145	165	115	2	2475	
285	3	1650	248	174	2	1897.5	495
300	2	1567.5	165	205	2	1650	330
320	2	1485	495	281	4	1650	
322	2	1485	165	302	2	1492.5	412.5
324	4	1402.5	165	320	2	1237.5	330

4. Discussion

The evaluated clones ranked highly in agronomic and yield quality attributes. The pest and disease severity was low. The yield quality was also good as characterized by high DM content, HCN levels and sweet taste of the raw roots. Though both sites had a high prevalence of CBSD, CMD was absent in Thika due to the isolation of the site from other cassava fields. The agronomic performance was better in Kiboko than Thika. Thika is

characterized by fertile soils, lower temperatures and higher rainfall than Kiboko that lend to competition among clones and reduced performance.

The sites had a strong effect on all the variables evaluated in the study. Cassava is adapted to a wide range of environments and shows strong Genetic × Environment (G×E) effect (Kvitschal et al., 2007). G×E interaction has been reported on yield, DM, disease severity and carotenoid content. The clones also had strong influences. The greatest influence of the clones was on CMD but there was no influence on CGM. Therefore, performance of the clones will vary depending on the environment in which they are established.

Cassava root pulp colouration is positively correlated to carotenoid content and the trait can be used for visual selection of clones for carotenoid content (Iglesias et al., 1997; Chávez et al., 2005). The sites and clones were significant for the trait. This is indicative of the variability in carotenoid content among the clones and the influence of the environment in expression of the trait.

The clones total carotenoid contents did not infer a high proportion of beta carotene. Previous studies have shown that a high carotenoid concentration does not necessarily indicate a high beta-carotene content (Alcides Oliveira et al., 2010; Chavez et al., 2005). The correlation between root pulp colouration and carotenoid content was not observed upon quantification of the carotenoids. This could be attributed to maturation stage, weather conditions and sample handling. The sampling was carried out at nine months after planting whereas carotenoid content reduction occurs in cassava ten months. Degradation of the samples may have occurred during refrigeration or due to autoxidation or photodegradation during analysis.

The seven clones that had stable root pulp colour scores across sites indicate that it is possible to select clones adaptable for multiple locations early in the breeding program. They remained CMD free during the clonal evaluation which marked their second year of evaluation an indication they could be tolerant to the disease. The seedling evaluation had been carried out in a high disease pressure site (Kiboko) which also featured in the clonal evaluation. The HCN of the raw roots was also low except for the two clones in Thika that had moderate HCN levels. Cassava is mainly consumed after some form of processing which leads to a significant reduction in cyanide content. Previous studies have shown that most yellow fleshed cassava have high HCN levels and low DM content (Chavez et al., 2005). Dry matter content and HCN have an influence on the taste of cassava. High DM cassava is sweet while a bitter taste is associated with high HCN cassava. Taste has an influence on adoption of cassava varieties (Nestel et al., 2006). This study identified clones that perform well in these traits, an attribute that improves their acceptability among cassava consumers.

Improvement of local germplasm for carotenoid produced clones with high agronomic potential. The identified clones have high potential for adoption as they meet the consumer preference. A sensory evaluation study showed a preference for yellow-fleshed cassava by school children and their guardians based on attractiveness of the colour, texture and taste (Talsma et al., 2013).

In conclusion, the adaptation and carotenoid content of locally developed yellow-fleshed cassava varies with environment. However, stable clones can be identified early in the breeding program upon evaluation in multiple sites.

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