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Research Application Summary

Agronomic performance and carotenoid content of Kenyan yellow-fleshed cassava clones

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Abstract

Vitamin A deficiency is common in Kenya, and is common 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 were 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 at Thika. Cassava Mosaic Disease, Cassava Brown Streak Disease (CBSD) and Cassava Green Mite (CGM) severity were 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. 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 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.

Key words: Beta-carotene, cassava, clonal evaluation, Kenya, yield quality

Résumé

La carence en vitamine A est fréquente au Kenya; et est communément observée chez les populations qui dépendent du manioc, comme principal aliment de base. Une évaluation de 324 clones de manioc à chair jaune, développés localement, a été réalisée sur deux sites, Thika et Kiboko, situés respectivement au centre et à l'est du Kenya. Des données ont été recueillies sur les performances agronomiques, la qualité des rendements et la réaction aux contraintes biotiques. Les clones ont présenté de bons résultats pour les caractères évalués. La performance globale était meilleure à Kiboko que Thika. La gravité de la pathologie de la mosaïque du manioc (MM), la striure brune du manioc (SBM) et l'acarien vert du manioc (AVM) ont été notées sur une échelle de 1 à 5. Le score moyen de SBM était de 1,17 à Kiboko et de 1,46 à Thika, la gravité du MM était de 1,15 à Kiboko mais la pathologie n'a pas été observée à Thika. La gravité des dommages de l'AVM était de 1,63 à Kiboko et de 1,97 à Thika. Le poids des racines par plante était respectivement de 3,49 et 2,25 kg à Kiboko et à Thika. La teneur en matière sèche des racines était de 49,86% à Kiboko et de 29,06% à Thika. Le potentiel en cyanogène a été évalué à l'aide du test au pictrate et noté sur une échelle de 1 à 9. Kiboko a obtenu un score de 2,79 contre 4,14 à Thika. Un total de 91 échantillons ont été analysés pour la teneur en caroténoïde en utilisant la méthode de spectrophotométrie. Le site de Kiboko présentait une teneur en caroténoïde moyenne plus élevée (1736,96 µg / 100 g) par rapport à Thika (1105,85 µg / 100 g). La plupart des échantillons analysés (71%) ont enregistré une teneur plus élevée en caroténoïdes chez Kiboko que chez Thika. La teneur en bêta-carotène était plus élevée chez Thika (374,16 µg / 100 g) que chez Kiboko (291,30 µg / 100 g). Des clones prometteurs adaptables aux deux sites ont été identifiés.

Mots clés: bêta-carotène, manioc, évaluation clonale, Kenya, qualité du rendement

Introduction

Cassava (*Manihot esculenta* Crantz) 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. Ever since the crop was introduced in Kenya, it has assumed the role of a subsistence commodity especially as a food security crop during famine periods. Cassava has wide environmental adaptation and modelling studies show that it will remain largely unaffected by climate change (Fermont *et al*., 2009). Cassava is an important crop in eastern Kenya, a region largely characterized by erratic rainfall. The major source of income for communities in the region is crop farming. Cassava plays an important role as a food security crop in the region. However, none of the varieties grown in the region has improved nutritional value, making the population vulnerable to malnutrition. Vegetable sources of vitamin A are often inadequate and inaccessible outside the growing season.

The nutritional quality of cassava can be improved by plant breeders through exploitation of essential genes. In a bid to improve the nutritional value of cassava, beta-carotene was introduced into local cassava germplasm at the International Institute of Tropical Agriculture (IITA) through hybridisation between local white-fleshed parents and high beta-carotene parents from IITA. The seedlings were advanced to a clonal trial comprising of disease free yellow fleshed clones in two sites in Kenya.

Materials and methods

The trial was conducted at two sites of the Kenya Agricultural and Livestock Research Organization (KALRO)-Thika and KALRO-Kiboko in central and eastern, Kenya, respectively. A total of 324 entries were evaluated in an unbalanced lattice square design in two replications. Cuttings were established at a spacing of 0.75 x 1m. Normal agronomic practices for cassava were carried out. The trial was conducted over a period of nine months.

During the vegetative growth stage the clones were evaluated for reaction to Cassava Mosaic Disease (CMD), Cassava Brown Streak Diseases (CBSD) and damage by the cassava green mite (CGM). The CMD, CBSD and CGM severity were 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 were collected at 3 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 and weighed. 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-white, 2-cream, 3-yellow, and 4-orange. The raw root was also tasted and rated on a scale of 1-3 where 1 was sweet, 2 –average and 3 was bitter. At each site the taste rating was carried out by the same individual. A freshly harvested root sample of about 3 kg was obtained from each clone for determination of percentage Dry Matter (DM) by the specific gravity method (Ceballos *et al*., 2012). The picrate 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.

In each site root samples were collected for carotenoid quantification. 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 deionized 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 â-carotene in each sample were analyzed in duplicate spectrophotometrically as described by Dixon *et al*. (2008). Results were obtained for 91 samples of which 41 were from Kiboko and 50 from Thika.

Analysis of variance components for each environment was conducted using Restricted Maximum Likely (REML) Procedure using Genstat, 14th Edition. The entries were considered fixed in the model. The mean data obtained for each environment were then used to run a combined analysis of variance for the two sites.

Results

There were significant (P<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. A total of 48 clones in Thika had a root pulp colour score of three or four compared to 35 in Kiboko. Seven of the clones had a root pulp colour score of three across the two sites. The seven best performing clones across sites for root pulp colouration also performed well with respect to other agronomic and qualitative traits. The clones remained free of CMD throughout the evaluation period. They were also CBSD-free at Kiboko whereas in Thika they had a CBSD score of between 1-2. The CGM damage score for the clones ranged from 1-2 across the two sites. The yield and related attributes of the clones were appreciable across the two sites. The clones had an average root count of 5-11 and 10-15 per plant at Thika and Kiboko, respectively. Their root yield (kg/plant) was higher at Kiboko with six of the seven clones having a yield above 5kg/plant. Though their HCN was higher at Thika, it was within the acceptable level of 1-5. Only two of the seven clones had low HCN levels at the site. The % DM was highest in Kiboko (Table 1).

The combined analysis of variance for the two sites showed that the site effect was highly significant (P>0.001) for all the traits evaluated at 3-6 months after planting, and significant entry differences were recorded for CMD and CBSV (Table 2). The interaction between the entries and the sites was significant for CMD. The proportion of the total sum of squares contributed by the entry was higher in CMD (38.9%) compared to CBSD (15.4). The site contributed 6% of total sum of squares for the

CMD, 0.1% for CBSD and 90.5% for CGM.

The site effect was highly significant (P>0.001) for all parameters determined at harvest except HI. The difference between entries were significant (P>0.05) for all the harvest traits determined (Table 3). The factors entry and site contributed different proportions to the total sum of squares of the harvest variables. Generally, the contribution of entry was higher than that of site for all the yield attributes. The entry contributed 34.5%, 37.9%, and 47% to the total sum squares of total biomass, root yield and Harvest index, respectively. The site contributed 8.7% to the total sum of squares of both total biomass and root yield and 0.2% to the harvest index total sum of squares. Entry contributed more to the total sum of squares for two of the three yield quality attributes determined. It contributed 44% to total sum of squares for dry matter, 34% for cyanogenic potential and 45.6 for root pulp colouration. The site contribution to the total sum of squares for the three traits was 1.3%, 36% and 3.3%, respectively.

The total carotenoid content ranged from 165-13612.5µg/100g while the beta-carotene content ranged from 162.5-660 µg/100 g at Kiboko. The mean carotenoid content for the site was 1736.96 µg/100 g and that of the beta-carotene fraction was 291.30 µg/100 g. Thika had a total carotenoid content within the range of 162.5-660µg/100g which was lower than that recorded for Kiboko. The mean carotenoid content was (1105.85µg/100g) which was lower compared to that of Kiboko (1736.9 6µg/100 g). Most of the samples (71%) analyzed recorded a higher carotenoid content in Kiboko than Thika. The beta-carotene fraction was higher in Thika (374.16 µg/100 g) than Kiboko (291.30 µg/100 g). The range of the beta-carotene fraction at the site was 82.5- 2474 µg/100 g. 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 almost 100%. The quantity of the beta-carotene in the four clones ranged from 165-330 µg/100 g. The ranking of the varieties differed among the two sites. Only one clone featured in the best ten clones for the two sites. The combined analysis of variance showed that entry, site and the interaction between the entries within site were significant (P<0.01). The entries' sum of squares contributed the greatest proportion to the total sum of squares compared to site (1.9%) and the interaction between the entries and site (27.83%) (Table 4).

Table 1. Yield and related attributes of clones with high and stable root pulp colour score at Thika and Kiboko

Entry	Family	Kiboko					Thika				
		RTC	RTY	Taste	DM	HCN	RTC	RTY	Taste	DM	HCN
12	01/1412 x 820058	11	5.7	1	37.1	2	5.25	1.65	3	33.9	3
41	05/0045 x 07/0752	9.25	5.7	1	29.6	2	7.25	2.08	1	32.6	3
56	05/0055 x 01/1412	8	5.23	1	40.4	4	8.67	3.87	2	32.3	4
73	05/0059 x 01/1412	9	10.4	1	40.6	4	10.3	3.68	2	32.1	4.5
236	07/0752 x 05/0078	11.3	8.1	1	39.5	3	9	1.55	1	25.1	4.5
254	820058 x 01/1412	10.75	5.43	1	34.4	3	6	4.1	1	23.8	5
260	990132 x 07/0520	5	3.23	1	32.0	3	7.5	2.78	2	28.4	4

RTC - root count per plant, RTY - root yield (kg/plant), DM-% dry matter content and HCN - cyanogenic potential

Table 2. Mean square values for quantitative and qualitative traits from the combined ANOVA for the clonal evaluation at Kiboko and Thika

Source of variation	DF	CMD	CBSV	CGM
Site	1	4.4***	0.03***	23.31***
Entry	287	0.1***	0.01*	0.16
Rep	1	0.5*	0.11	1.76
Block	17	0.1	0.1	0.13
Site x Entry	145	0.1*	0.07	0.12
Entry x Rep	205	0.1*	0.01	0.13
Error	56	0.06	0.03	0.14
CV		22.7	1.5	21

*, ***-significant at P<0.05and P<0.001, DF-degrees of freedom, CMD-Cassava Mosaic Disease, CBSV-Cassava Brown Streak, CGM-Cassava Green Mite

Table 3. Mean square values for yield and related attributes from the combined ANOVA for the clonal evaluation at Kiboko and Thika

Source of variation	Df	Root yield	TBM	HI	DM	HCN	PLP	Taste
Site	1	7.86***	63.72***	184.3	313***	307.2***	8.2***	0.4***
Entry	287	0.12*	0.86*	160.5*	37.7*	1.0*	0.4*	0.02*
Rep	1	2.39***	23.49***	118.1***	21***	0.3*	3.6***	0.2***
Block	17	0.1	1.52*	167.4***	76.2***	1.0*	0.5*	0.2
Site x Entry	145	0.88	0.74	76.3	30.8***	0.8*	0.3	0.02
Entry x Rep	205	0.06	1.93*	80.1	31.6*	0.4	0.3	0.03*
Error	56	0.09	0.7	59.2	17.9	0.5	0.2	0.01
CV		23.2	29.7	21.7	13.0	20.3	24.7	30.1

*,***-significant at P<0.05 and P<0.001, df-degrees of freedom, TBM-Total biomass per plant, HI-Harvest index, DM-% Dry matter, HCN-Cyanogenic potential, PLP-Pulp colour rating

The clones performed well in terms of response to biotic stresses across the two sites. The severity levels recorded were low for CMD, CBSD and CGM. Though both sites had a high prevalence of CBSD, CMD was absent in Thika perhaps due to the isolation of the site from other cassava fields. The agronomic performance was better in Kiboko than Thika. Although Thika is characterized by fertile soils, than Kiboko, it has lower temperatures and higher rainfall that may have reduced the cassava performance. Overall, the yield quality was good as was characterized by high DM content, and sweet taste of the raw roots. The yield was also within that realized in the research environment. The roots produced at both sites were within the marketable range an indicative of good bulking ability of the clones. The mean % dry matter content at the two sites showed that the clones had a high dry matter content despite reports that cassava biofortified for beta-carotene tends to be low on dry matter (Njoku *et al.*, 2015).In cassava % dry matter content above 30% is considered high and breeding programmes aim for values of between 35-40%. Normally, % dry matter content for cassava is 10-57% though majority of genotypes fall within the range of 20-40%.

Table 4. Mean square values and proportion of total sum of squares from the combined analysis of variance for total carotenoid content at Kiboko and Thika

Source	df	ms	%ss
Entry	66	4.95**	70.03
Site	1	8.87**	1.90
Entry x Site	25	5.20**	27.83
Error	4	0.28	
CV	29.7		

**significant at P<0.01, df-degrees of freedom, ms-mean square, ss-sum of squares, CV-coefficient of variation

The clones appeared safe in terms of hydrocyanide content with Kiboko having clones within the low range. In Thika, the mean hydrocyanide level was low but there were two clones with values of seven which is considered moderate. 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 and has low HCN levels of <60mg HCN/ kg whereas bitter clones have high cyanide levels of about 138-208mg HCN/kg (Oke, 1994). Taste has an influence on adoption of cassava varieties. The HCN trait is an important consideration especially where cassava is processed by boiling and it is recommended that varieties for release should have a maximum HCN content of not more than 150 ppm. Thus this study identified clones that are likely to perform well in regard to these traits, an attribute that improves their acceptability among cassava consumers.

The total carotenoid content in the analyzed samples did not infer a high proportion of beta carotene. Previous studies have shown that a high carotenoid concentration does not indicate a high beta-carotene content (Chavez *et al*., 2005; Alcides Oliveira *et al*., 2010). The correlation between root pulp colouration and carotenoid content was weak upon quantification of the carotenoids. This could be attributed to genotypes, weather conditions and sample handling.

Seven clones had stable root pulp colour scores across sites. This indicates there is a possibility of selecting clones adaptable for multiple locations early in the breeding programme. The seven clones also remained CMD free during the clonal evaluation which marked their second year of evaluation, an indication they could be tolerant to the disease. This is more so since the seedling evaluation had been carried out in a high disease pressure site (Kiboko) which also featured in the clonal evaluation.

The significant interaction between entries and sites implied that there were entries that were specifically adapted to a particular site. Cassava is adapted to a wide range of environments and shows strong Genetic x Environment (GxE) effect. In cassava, GxE interaction has been reported on traits like yield, DM, disease severity and carotenoid content.

The entries differed significantly for all the biotic stresses and yield attributes evaluated except cassava green mite indicating high variability among entries for these traits. The effect of sites were also significant for all the traits evaluated except harvest index. However, the interaction between entries and site was significant for %dry matter, cyanogenic potential, cassava mosaic disease and total carotenoid content implying that some genotypes were specifically adapted to a particular site. This shows that significant genetic gains for these traits may be achieved through judicious selection among these entries in a breeding programme.

The entries contributed more to the total sum of squares for total biomass, root yield, harvest index, % dry matter and root pulp colouration. The same traits were significantly influenced by site save for harvest index. This indicates these traits may be gene influenced by both genotype and environment. Harvest index has been shown to be highly influenced by genetic effects which could explain its deviation from the rest of the traits.

Carotenoid content in cassava is a stable trait that is greatly influenced by the genotype with limited environmental effects (Maroya *et al*., 2012) Similarly, in this study, the impact of entry was highest (70%) for total carotenoid content compared to other traits. This suggests qualitative nature of the trait and effects of a few genes. The high genotype and low environment effects observed in this study are similar to 73.7% observed early in development of Nigerian yellow-fleshed genotypes (Ssemakula *et al*., 2007). However, as improvement for the trait continued, the genotypic effects were reduced to 34.4% (Maroya *et al*., 2012). The genotypic effects in this study contributed 45.6% to the total sum of squares which was closer to 34.4% reported in advanced stages of selection. This indicates that selection for beta-carotene in early selection cycles can be based on root pulp colouration as opposed to carotenoid quantification. This will help reduce the time required for carotenoid quantification and associated cost. Furthermore, cassava root pulp colouration is positively correlated to carotenoid content and the trait can be used for visual selection of clones for carotenoid content. As the selection for the trait advances, most of the clones retained are those within the yellow pulp category as they accumulate more carotenoids than the cream. There exists a wide variability within the yellow clones and the accumulation of carotenoids in this category is quantitative and polygenic (Morillo-Coronado, 2009). Therefore, carotenoid content among clones in this root pulp colour is likely to be influenced by both genotype and environment. This will lead to a reduction in genetic effects and an increase in environmental effects unlike in the case cream coloured clones whose carotenoid trait is under the influence of a few genes.

The site effect was significant for all the traits except harvest index. The interaction between entries and sites was significant for % dry matter, cyanogenic potential, cassava mosaic disease and total carotenoids. This suggests specific adaptation of entries in different sites. Selection efficiency for these traits can be improved by selecting genotypes that are stable across a number of sites through multi-locational evaluation. This is especially so since cassava demonstrates high GxE interactions that necessitates multi-locational evaluation.

This study suggests potential for improvement of local germplasm with promising agronomic potential for carotenoid content. The most important quality attributes are dry matter content and cyanogenic potential. Carotenoid content is inversely linked to dry matter content and a positive correlation has also been reported between carotenoid content and cyanogenic potential. However, high carotenoid clones with appreciable DM content and low HCN. These could be tested further in multi-location trials for possible adoption if they meet the consumer preference. A sensory evaluation study carried out in Eastern Kenya 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).

Conclusion

Conventional breeding approach can be employed to develop clones high in carotenoid content and with appreciable agronomic attributes. This will require careful selection of parents to have a good background for other traits and then introduce the carotenoid trait into such clones. There is however, a likelihood of carotenoid content being influenced by the environment to some extent requiring multiplication testing to identify stable closer due to the smaller environmental compared to genetic effects. Cassava agronomic characterists, and response to biotic stresses is influenced by genotype and environment interaction. This implies that cultivation and use of biofortified cassava will benefit from multi-locational evaluation just like the selection for white coloured germplasm. In early evaluation stages for carotenoid improved cassava, root pulp colouration can be used to assess stability of genotypes across environments without carotenoid quantification. Quantification can be done in later stages when the population under evaluation reduces.

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